CMLS, Cell. Mol. Life Sci. 60 (2003) 241–250
1420-682X/03/020241-10

CMLS Cellular and Molecular Life Sciences

O-fucose modifications of epidermal growth factor-like repeats and thrombospondin type 1 repeats: unusual modifications in unusual places

L. Shao and R. S. Haltiwanger *

Department of Biochemistry and Cell Biology, Institute for Cell and Developmental Biology, State University of New York at Stony Brook, Stony Brook, New York 11794-5215 (USA), Fax + 1 631 632 8575, e-mail: Rhaltiwanger@ms.cc.sunysb.edu

Abstract. Recent discoveries revealing that carbohydrate modifications play critical roles in a wide variety of biological processes have brought wide recognition to the field of glycobiology. Growing attention has focused on the function of unusual *O*-linked carbohydrate modifications such as *O*-fucose. *O*-fucose modifications have been described in several different protein contexts, including epidermal growth factor-like repeats and thrombospondin type 1 repeats. The *O*-fucose modifications on thrombospondin type 1 repeats have only recently been described, but the site of modification occurs in a region proposed to play a role in cell adhesion. *O*-fucose modifications on epidermal growth factor-like repeats have been described as important players in several signal transduction systems. For instance, Notch, a cell-surface signaling receptor required for many developmental events, bears multiple *O*-fucose saccharides on the epidermal growth factor-like repeat of its extracellular domain. The *O*-fucose moieties serve as a substrate for the β1,3 *N*-acetylglucosaminyltransferase activity of Fringe, a known modifier of Notch function. The alteration of *O*-fucose structures by Fringe influences the ability of Notch ligands to activate the receptor and provides a means to regulate Notch signaling. Thus, *O*-fucose and Fringe provide a clear example of how carbohydrate modifications can have direct functional consequences on the proteins they modify.

Key words. *O*-fucose; EGF repeat; TSR; thrombospondin; Notch; Fringe.

Introduction

Carbohydrate modifications play numerous roles in the biology of the proteins they modify, including assisting in folding, protection from degradation, modulation of activity and participating in binding interactions with other proteins. Unusual carbohydrate modifications appear to play more specific roles in biology [1]. Examples include the mannose 6-phophate for targeting lysosomal enzymes to lysosomes [2] and sialyl Lewis x for recruiting leukocytes to sites of inflammation [3]. Historically, research has focussed on the more abundant forms of glycosylation, including N-linked glycosylation, mucin-type *O*-linked glycosylation and glycosaminoglycans. The study of other less abundant forms of *O*-linked modifications, such as *O*-GlcNAc [4], *O*-mannose [5], *O*-fucose [6] and *O*-glucose [6], has only recently begun to reveal specific functions for these unusual structures. Although the *O*-linked carbohydrate modifications on epidermal growth factor-like (EGF) repeats (*O*-fucose and *O*-glucose) were only initially described in the early 1990s [6], they have generated a great deal of excitement in the past few years. In particular, *O*-fucose modifications on EGF repeats are known to play significant roles in several signal transduction pathways. For instance, *O*-fucose on urinary-type plasminogen activator (uPA) was shown to be required for activation of the uPA receptor, a mitogenic receptor capable of inducing a wide variety of kinase cascades [7]. Recently, the presence of an *O*-fucose on the EGF repeat of Cripto was demonstrated to be essential for Cripto to mediate Nodal-dependent signaling [8, 9]. Cripto is an essential cofactor for Nodal, a transforming growth factor β

* Corresponding author.

family member that plays an essential role in establishment of polarity in early vertebrate embryos [10]. These discoveries draw attention to the study of *O*-linked carbohydrates and their function in biology. Here we will review the *O*-fucose modification, the recent discovery of *O*-fucose modifications in a different sequence context (thrombospondin type 1 repeats or TSRs) and the involvement of *O*-fucose modifications in the Notch signaling pathway.

O-fucose modifications of EGF repeats

O-fucose consists of the sugar L-fucose covalently attached to protein via the hydroxyl group of either serine or threonine. This unique sugar-protein linkage was first observed in mammalian systems almost 30 years ago as the amino acid fucoside, Glc-β1,3-Fuc-α1-*O*-Thr, isolated from human urine [11]. This finding was surprising since fucose is typically thought of as a terminal modification in mammalian systems. Later, Klinger and co-workers found similar structures (Glc-β1,3-Fuc-α1-*O*-Ser/Thr, Fuc-α1-*O*-Ser/Thr) on proteins in extracts of rat tissue [12]. The first protein identified bearing an *O*-fucose was uPA [13]. Identification of *O*-fucose on several other serum glycoproteins, including tissue-type plasminogen activator (tPA) [14] and coagulation factors VII [15], IX [16] and XII [17], followed. Interestingly, each of these proteins is modified with the monosaccharide *O*-fucose except for factor IX, which bears *O*-fucose elongated into a tetrasaccharide with the structure Sia-α2,6-Galβ1,4-GlcNAc-β1,3-Fuc-α1-*O*-Ser [16].

Analysis of the sites of *O*-fucose modification on these proteins demonstrated they occur at consensus sites within EGF repeats. EGF repeats are a common structural motif found in numerous secreted and cell-surface proteins in metazoans [18]. They are typically 30–40 amino acids in length and are often involved in mediating protein-protein interactions. The defining characteristics of an EGF repeat are six conserved cysteine residues participating in three disulfide bridges, maintaining the EGF repeat in a distinct three-dimensional fold. Other than the conserved cysteine residues, the sequences of different EGF repeats are highly diverse.

By comparing the sequence contexts surrounding the fucosylated residues in each EGF repeat, a consensus sequence for the *O*-fucose modification was proposed: $C^2XXGG\underline{S/T}C^3$ (where C^2 and C^3 are the 2nd and 3rd conserved cysteines of the EGF repeat, S/T is the modified residue and X can be any amino acid) [6]. We and others have used this consensus sequence to successfully predict the presence of *O*-fucose on the EGF repeats of proteins such as Notch, Serrate/Jagged, Delta and Cripto [8, 9, 19, 20]. However, more detailed analysis of *O*-fucose modification sites on Notch, Serrate/Jagged and Delta has revealed that *O*-fucose occurs on a broader set of sites than predicted by the consensus site. We have recently proposed a broader consensus site to account for these observations: $C^2X_{3-5}\underline{S/T}C^3$ [20, 87]. Further work needs to be done to narrow this new consensus site before it can be used effectively to identify proteins modified with *O*-fucose.

O-fucose modifications on thrombospondin type 1 repeats

O-fucose modifications have been found in two sequence contexts other than EGF repeats. The proteinase inhibitor PMP-C from *Locusta migratoria* bears an *O*-fucose [21]. The PMP-C structure bears some resemblance to that of an EGF repeat, with three disulfide bridges and several β sheets, but the sequence surrounding the *O*-fucose site is quite different [22]. Little is known about the *O*-fucose on PMP-C other than that it causes a slight stabilization of the peptide it modifies. More recently, Hofsteenge and co-workers identified *O*-fucose modifications on human thrombospondin-1 [23]. Thrombospondin-1 is a large, multidomain extracellular matrix glycoprotein that influences a wide variety of processes, including platelet aggregation, fibrin clot formation, cell-cell contact, activation of extracellular proteases and angiogenesis [24]. Interestingly, thrombospondin-1 is modified with a disaccharide form of *O*-fucose, glucose-fucose. This modification is believed to be the same as the Glcβ1,3Fuc disaccharide originally reported by Hallgren and co-workers [11].

The *O*-fucose modifications on thrombospondin-1 map to the thrombospondin type 1 repeats (TSR). Like EGF repeats, TSRs are found in many extracellular proteins. Each TSR is about 60 amino acids long and is characterized by conserved Cys, Trp, Ser and Arg residues [24]. Thrombospondin-1 contains three TSRs, all three of which are modified with *O*-fucose. Analysis of other proteins containing TSRs (properdin, F-spondin) revealed that they are also modified with *O*-fucose [25]. Comparison of the sequences surrounding the *O*-fucose site on the TSRs revealed a putative consensus sequence for modification: W $X_5CX_{2/3}\underline{S/T}CX_2G$ (where S/T is the *O*-fucose site) [23, 25]. The consensus site corresponds to a region of the TSR proposed to play a role in binding to cells [24]. For example, the peptide CSV$\underline{T}$CG from within the consensus site (where T is the predicted *O*-fucose site) has been shown to interact with heparin sulfate proteoglycans and CD36 [24]. The binding studies were performed with unglycosylated synthetic peptides, suggesting that the presence or absence of the *O*-fucose could modulate these interactions. Database comparisons show that the majority of proteins containing TSRs con-

tain this consensus site (63 out of 88 [23]), suggesting this modification will be widespread.

The *O*-fucose site on TSRs is in close proximity to sites for another unusual form of glycosylation: C-mannosylation [23]. This consists of an α-mannopyranosyl residue attached to the C-2 atom of the side chain of Trp. Sites for C-mannosylation occur both within and outside of TSRs, and initial analysis suggests C-mannosylation and *O*-fucosylation are not dependent upon one another [25]. For a review on C-mannosylation, see [26].

O-fucose glycosylation pathways

The presence of *O*-fucose in different contexts suggests the presence of at least two separate *O*-fucose glycosylation pathways (fig. 1). *O*-fucose on EGF repeats can be elongated to the tetrasaccharide Sia-α2,3/6-Gal-β1,4-GlcNAc-β1,3-Fuc, whereas the *O*-fucose on TSRs is believed to be elongated to Glcβ1,3Fuc. No reports of elongated *O*-fucose on PMP-C have been reported. Several of the enzymes responsible for synthesis of these structures have been identified. The enzyme responsible for addition of the fucose onto EGF repeats (GDP-fucose: protein *O*-fucosyltransferase 1, or *O*-FucT-1) was originally identified and purified from Chinese hamster ovary (CHO) cells [27, 28] and has recently been cloned [29]. Homologues of the *O*-FucT-1 gene sequence are found in human, mouse, *Drosophila* and *Caenorhabditis elegans*, and the gene is widely expressed in mammalian tissues [29]. *O*-FucT-1 fucosylates properly folded EGF repeats containing the *O*-fucose consensus site [27–29]. *O*-fucose on EGF repeats can be further elongated by Fringe, a fucose-specific β1,3-*N*-acetylglucosaminyltransferase, which is described in more detail below. The GlcNAc-β1,3-Fuc is elongated by β4GalT1 [30] and either α2,3- or α-2,6-sialyltransferases [19] to form the tetrasaccharide (fig. 1).

Less is known about synthesis of the *O*-fucose glycans on TSRs, but preliminary data from our laboratory suggests that a separate protein *O*-fucosyltransferase (tentatively identified as *O*-FucT-2) exists that is responsible for addition of *O*-fucose to TSRs [Y. Luo and R. S. Haltiwanger, unpublished]. The *O*-fucose on TSRs is believed to be elongated by a fucose-specific β1,3-glucosyltransferase, required for synthesis of the Glc-β1,3-Fuc disaccharide (fig.1). An enzymatic activity capable of forming the Glc-β1,3-Fuc linkage has been identified and characterized in extracts of CHO cells by Moloney and co-workers [31]. Interestingly, this β1,3-glucosyltransferase activity will not transfer glucose to *O*-fucose attached to an EGF repeat, consistent with the observation that the Glc-Fuc disaccharide is found only on TSRs [D. J. Moloney and Haltiwanger, unpublished]. No information is available concerning the biosynthesis of the *O*-fucose on PMP-C.

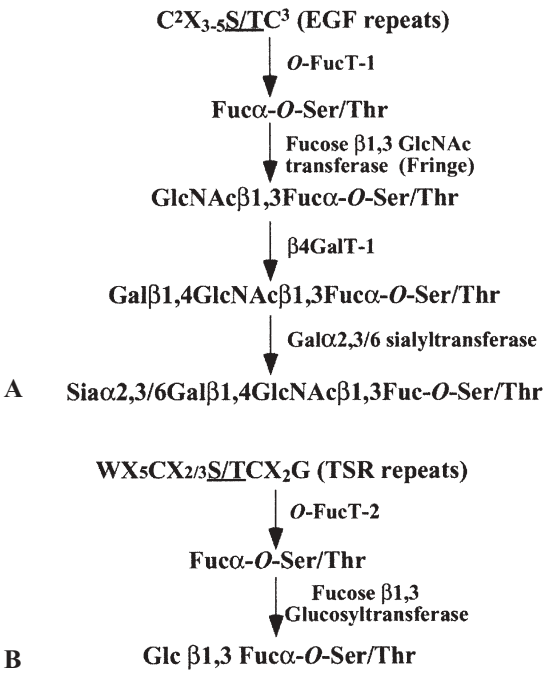

Figure 1. *O*-Fucosylation pathways. The pathways for *O*-fucosylation of EGF repeats (*A*) and TSR repeats (*B*) are shown. No pathway is shown for *O*-fucosylation of PMP-C from *Locusta migratoria,* because no information is available on the enzyme responsible for fucosylation of PMP-C. The four enzymes involved in modifying EGF repeats are *O*-FucT-1 [29], fucose-β1,3-GlcNAc transferase (Fringe) [42, 74], β4GalT-1 [30] and Galα 2,3/6 sialyltransferases. The enzymes involved in modifying the TSR repeats are *O*-FucT-2 [proposed, Y. Luo and R. S. Haltiwanger, unpublished observation] and fucose-β1,3-glucosyltransferase [31].

Role of *O*-fucose modifications in Notch signaling

The Notch signaling pathway

The *Notch* locus was first identified as a sex-linked lethal mutation in 1915 during one of the original *Drosophila* mutant screens [32]. Loss of *Notch* function leads to embryonic lethality for males and notched wings for females, from which the locus derives its name. The gene encoding *Drosophila Notch* was identified in 1985 [33] and shown to encode a large, cell-surface signaling receptor. Homologues have been identified in all metazoans, with four Notches in mammals. Notch signaling plays an important role in determining cell fates during many phases of invertebrate and vertebrate development [34]. In humans, defects in the Notch pathway result in a variety of serious diseases, including T-cell leukemia, cerebral autosomal dominant arteriopathy with subcortical infarcts (CADASIL), Alagille syndrome and spondylocostal dysostosis [35–38]. T-cell leukemia results from a constitutively active form of Notch1 caused by truncation of the Notch1 gene (called *TAN-1)* [35]. CADASIL is a hereditary adult-onset neurological disorder causing stoke and dementia [39] and results from point mutations

in the extracellular domain of Notch 3 [36]. Alagille syndrome is an autosomal dominant disorder with developmental abnormalities in many systems, including liver, skeleton, kidney, eye, heart and face. It is associated with choleostasis, cardiac disease, ocular and skeletal malfunction and a characteristic facial phenotype. Alagille syndrome appears to be caused by defects in Jagged1, one of the ligands known to activate Notch signaling (see below) [37]. Spondylocostal dysostosis is a group of vertebral mal-segmentation syndromes with reduced stature resulting from axial skeletal defects [38]. Recent evidence indicates that segmentation of the embryonic body relies on a molecular oscillator called the segmentation clock, which requires Notch signaling for its proper functioning [40]. Mutations in the Notch ligand Delta-like 3 (DLL3) interrupt the segmentation clock resulting in this syndrome.

The Notch protein is a large cell-surface receptor (>300 kDa) with approximately one-third of its mass intracellular and two-thirds extracellular. Notch is synthesized as a single polypeptide chain, but becomes proteolytically processed into a heterodimer during maturation in the Golgi apparatus by a furin-like convertase [41]. In the mature protein, the extracellular domain (ECD) remains tethered to the transmembrane/intracellular domain. This heterodimer is believed to be the active form of Notch in most signaling events. The ECD consists largely of 36 tandem EGF repeats (fig. 2) [33], many of which contain potential *O*-fucose modification sites [19]. Several of these sites are conserved across species, suggesting biological importance. Comparison of all Notch sequences currently in the database reveals five evolutionarily conserved *O*-fucose sites (EGF repeats 3, 20, 24, 26, 31) (fig. 2). Using the broader consensus site for *O*-fucose modification [20], another eight evolutionarily conserved sites (EGF repeats 2, 5, 8, 9, 12, 21, 27, 30) can be identified (fig. 2). We have shown that Notch1 is modified with *O*-fucose [19] and analysis of Notch in cells defective for synthesis of GDP-fucose suggests that *O*-fucosylation of Notch is required for proper Notch function [30, 42].

Notch becomes activated upon binding to its ligands, members of the Delta, Serrate/Jagged family (for an excellent review on Notch activation, see Mumm and Kopan [43]). Notch ligands are also cell-surface, transmembrane proteins. Thus, Notch can only be activated by ligand expressed on an adjacent cell. *Drosophila* expresses one Delta and one Serrate, whereas mammals express multiple homologues of each (Serrate homologues are termed Jagged in mammals). Ligand binding induces proteolysis of the Notch ECD by the cell-surface metalloprotease TACE (TNF-α-converting enzyme) [44]. Loss of the ECD induces a further proteolytic event catalyzed by presenilin-dependent γ-secretase activity [45]. This last cleavage occurs within the membrane, releasing the Notch intracellular domain (NICD) from the membrane into the cytoplasm. Upon release, the NICD translocates to the nucleus where it interacts with members of the CSL (CBF1/Suppressor of hairless/Lag-1) family of transcriptional regulators, converting them from repressors to activators of transcription. The effects of Notch are mediated by activation of a variety of downstream gene products [43]. The activation of a signaling cascade by proteolysis has been seen not only in Notch, but several other systems such as amyloid precursor protein (APP) and sterol regulatory-element binding proteins (SREBP) pathway, and has been recently termed regulated intramembrane proteolysis (RIP) [46, 47].

Fringe is a modulator of Notch function

The importance of the Notch pathway is revealed by the numerous levels at which it is regulated (see [48] and [49] for excellent reviews on Notch regulation). A variety of proteins interact with the NICD, regulating its degradation or ability to bind to other proteins. For instance, several proteins related to E3 ubiquitin ligase, such as Sel-10, Neuralized and Deltex, have been shown to be involved in regulation of Notch signaling [50–53]. Notch is also regulated through its ECD. Notch ligands can serve both as activators and inhibitors of Notch signaling depending on whether they are expressed on cells adja-

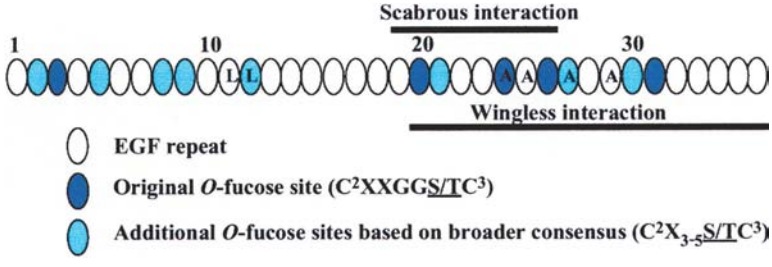

Figure 2. Functional EGF repeats in the Notch ECD overlap with *O*-fucose modification sites. The 36 tandem EGF repeats of the Notch ECD are shown, and EGF repeats with predicted *O*-fucose modification sites (both based on the original and broader consensus sites) are indicated. EGF repeats responsible for ligand binding are indicated with an 'L' [82], and EGF repeats containing Abruptex mutations are indicated with an 'A' [84]. Regions involved in binding to Wingless [58] and Scabrous [56] are also indicated.

cent to Notch-expressing cells (activators) or in the same cells as Notch (inhibitors) [54]. Recent reports suggest that the inhibitory effect of ligand, termed cell-autonomous inhibition, may result from cis interactions between Notch and ligand in the same cell causing a sequestering of Notch within the cell [55].

Besides the ligands, several other proteins, such as Scabrous and Wingless, have also been observed to bind to the ECD of Notch and affect its function (see fig. 2). Scabrous binds to Notch through EGF repeats 19–26 [56], and overexpression of Scabrous downregulates Notch signaling [57]. Wingless binds directly to Notch EGF repeats 19–36 and has been reported to induce a distinct signaling pathway [58]. Finally, Fringe is known to regulate Notch by covalently modifying the ECD of Notch. Fringe activity inhibits Serrate-dependent Notch signaling, while potentiating Delta-dependent signaling [59, 60].

The *Fringe* gene was first identified in a *Drosophila* mutant screen for genes involved in boundary formation [61]. Proper *Drosophila* wing development is dependent on the localization of *Wingless* (an essential gene for wing formation) expression at a border between the dorsal and ventral compartments of the wing imaginal disc (the structure from which the wing will develop), and *Wingless* expression is induced by Notch activation. *Fringe* was initially identified as an essential player in the formation of this boundary [61]. Subsequent work demonstrated that *Fringe* performs this function by modulating the Notch signaling pathway [59, 60]. Although Notch and its ligands are expressed throughout the imaginal disc, Notch is only activated at the dorsal-ventral boundary. Fringe acts by restricting Notch activation to this boundary (fig. 3). Both Serrate and Delta are expressed in all wing cells during early wing development. Later, their expression becomes restricted with Serrate expressed in dorsal cells and Delta expressed in ventral cells. Fringe is expressed dorsally, where it inhibits signaling from Serrate, preventing Notch activation in the dorsal compartment. Similarly, Notch is not activated by Delta in ventral cells due to the absence of Fringe. Notch becomes activated only in the border cells where Fringe is present for Delta to signal, or Fringe is absent for Serrate to signal (fig. 3). Notch activation induces increased expression of the ligands, causing a positive feedback loop where Notch becomes strongly activated in a border two cell-layers thick. Activation of Notch also induces expression of Wingless. Fringe plays a similar role in other

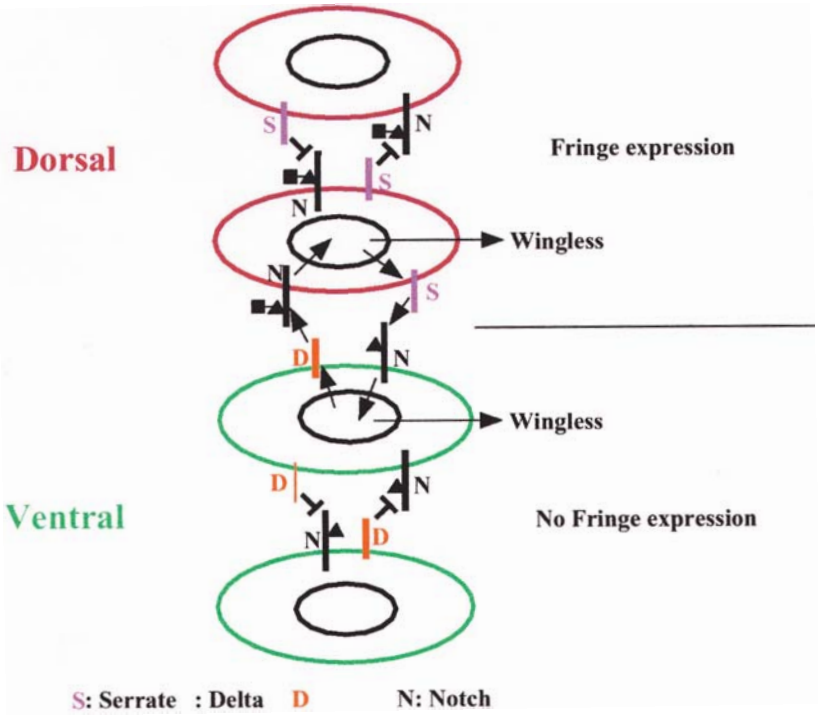

Figure 3. Signal interactions at the dorsal and ventral compartment border in developing *Drosophila* wing. The boundary between the dorsal and ventral compartments of the wing imaginal disc is shown. Notch is expressed in all cells; Serrate and Fringe are expressed on the dorsal side and Delta on the ventral side. Notch is modified with *O*-fucose in all cells (*O*-fucose represented by triangles), and *O*-fucose is elongated by Fringe with a β1,3-GlcNAc in ventral cells (β1,3-GlcNAc represented by squares). For simplicity, the further elongation of GlcNAc is not shown. Arrows indicate activation, and crossed lines indicate a lack of productive interaction. In dorsal cells where Fringe is expressed, Serrate cannot activate Notch, but Delta can. Conversely, in ventral cells where Fringe is not expressed, Serrate can activate Notch, but Delta cannot. This results in activation of Notch only in the cells at the dorsal-ventral border. Notch activation stimulates expression of the ligands (Delta and Serrate) as well as Wingless. Adapted from [59, 60].

tissues in *Drosophila,* including eyes and leg segmentation and growth [62].

Three *Fringe*-related genes have been identified in mammalian systems: *Lunatic Fringe* (*Lfng*), *Radical Fringe* (*Rfng*) and *Manic Fringe* (*Mfng*) [63, 64]. Many features in the *Fringe* genes' sequence are conserved among species, such as distribution of cysteine residues, potential N-glycosylation sites and an internal proteolytic processing site [63]. The conserved proteolytic processing site among different Fringes suggests that Fringe may be synthesized as an inactive precursor that requires proteolytic processing to transform into an active form. Amino acid comparison of the three proteins suggests that they are evolutionarily conserved within the mature protein region. They all appear to enter the secretory pathway, but they differ in their efficiency of secretion and in their requirement for posttranslational proteolytic processing [63]. Misexpression assays in *Drosophila* wing imaginal discs using the mammalian Fringe proteins mimics several of the effects seen by *Drosophila* Fringe, suggesting they all modulate Notch function in this context [63, 64]. Studies from several laboratories have demonstrated the importance of Fringe function in vertebrate systems. By analogy to *Drosophila* imaginal disc, the apical ectodermal ridge (AER) has been proposed to control wing bud outgrowth and patterning in developing chicks [65]. In the chick embryo, the positioning of AER is dependent on Rfng [66]. Misexpression of Rfng can induce the formation of an ectopic ridge at the boundary between cells expressing and not expressing Rfng [67]. Recent studies have also shown that Lfng is an essential gene in mice [68, 69]. The Lfng mutants in mice fail to form boundaries between individual somites, the initial segmental unit of the vertebrate trunk. In contrast, Rfng-deficient mice do not show any obvious phenotypes, and Lfng/Rfng double homozygous mutant mice show no additional phenotypes beyond those seen in the Lfng mutants [70, 71]. Mfng mutants have not yet been generated. These results indicate that some functional redundancy may exist between Mfng and Rfng. Interestingly, no limb bud phenotypes have been observed in any of the mouse Fringe ablations to date, suggesting that Fringe may not play a role in limb bud development in mice. Although there may be some redundancy in function, Lfng, Mfng and Rfng show different expression profiles during mouse embryogenesis [63, 64]. In addition, misexpression of the mammalian Fringe proteins in *Drosophila* shows some subtle differences, suggesting each Fringe functions somewhat differently [63, 64].

Fringe is a glycosyltransferase that modifies Notch

Recent work in several laboratories has revealed a mechanism by which Fringe modulates Notch function. Earlier work had shown that Fringe must be expressed in the same cell as Notch to exert its effects on Notch function, suggesting that Fringe might be an enzyme capable of modifying the Notch protein in the secretory pathway [59, 60]. Two conserved regions in the Fringe protein sequence, including a putative DxD motif [72], share similarity with a group of bacterial glycosyltransferases, raising the possibility that Fringe might function by altering the sugar structures on Notch [73]. In order to investigate whether Fringe could alter the *O*-linked sugar structures on Notch, mammalian Fringe was transfected into CHO cells to analyze changes in the *O*-fucose structures on Notch [42]. Results showed that expression of Mfng resulted in an increased amount of elongated *O*-fucose on Notch [42]. Detailed study of the elongated *O*-fucose structures showed Mfng-induced increases in a disaccharide (GlcNAc-β1,3-Fuc), a trisaccharide (Gal-β1,4-GlcNAc-β1,3-Fuc) and a tetrasaccharide (Sia-α2,3-Gal-β1,4-GlcNAc-β1,3-Fuc). The fact that all three multisaccharide structures increased when Mfng was expressed suggested Fringe could be a GlcNAc transferase. In vitro assays using purified Fringe proteins (*Drosophila* Fringe, mouse Lfng, mouse Mfng) verified that Fringe is a fucose-specific β1,3-*N*-acetylglucosaminyltransferase, showing a marked preference for fucose linked to a properly folded EGF repeat [42, 74]. Thus, Fringe is a glycosyltransferase capable of modifying the *O*-fucose structures on Notch. To determine whether Fringe only modifies *O*-fucose on Notch, or whether it will modify *O*-fucose in other sequence contexts, we examined whether Notch ligands can also be modified by Fringe [20]. Our results indicated that *O*-fucose on mammalian Delta1 and Jagged 1, as well as *Drosophila* Delta and Serrate, are modified by Fringe. These results indicate that Fringe is capable of modifying *O*-fucose in a variety of sequence contexts.

Further support for the involvement of glycans in the Fringe-dependent regulation of Notch has come from the identification of the *Fringe connection* gene in *Drosophila* [75, 76]. *Fringe connection* encodes a nucleotide sugar transporter capable of transporting several nucleotide sugars, including UDP-glucuronic acid, UDP-GlcNAc and UDP-xylose. Mutants in *Fringe connection* show phenotypes resembling *Wingless* and *Notch* mutants. Fringe-dependent Notch function is disrupted in *Fringe connection* mutants, consistent with an essential role for glycosylation in Fringe function.

To demonstrate that the β1,3-*N*-acetylglucosaminyltransferase activity is responsible for Fringe-mediated changes in Notch function, a variety of in vivo studies have been performed. Mutants of *Drosophila Fringe* were made where residues in the DxD motif, predicted to be in the catalytic site of the enzyme, were made. These mutants were enzymatically inactive in in vitro assays for β1,3-*N*-acetylglucosaminyltransferase activity [42, 74]. Analysis

of the mutants in vivo in ectopic expression assays demonstrated that they were unable to alter Notch function in contexts where wild-type Fringe does [42, 74, 77]. A similar mutation made in mouse *Mfng* inactivated in vitro β1,3-*N*-acetylglucosaminyltransferase activity and the ability of Mfng to modulate Notch function in cell-based signaling assays [30]. These results indicate that Fringe must catalyze the transfer of GlcNAc to fucose in order to modulate Notch activity.

To further examine the type of fucose needed as an acceptor for the GlcNAc, Fringe-mediated inhibition of Jagged1-dependent Notch activation was measured in glycosylation mutants of CHO cells. Both Mfng and Lfng were capable of inhibiting Jagged1-dependent Notch activation in both wild type and Lec1-CHO cells [42]. Since Lec1-CHO cells do not synthesize complex-type *N*-glycans, these results indicated that Fringe does not recognize fucose on *N*-glycans. In contrast, Lec13-CHO cells, which are defective in GDP-fucose biosynthesis [78], showed a decrease in Jagged1-dependent Notch activation and no inhibition by Fringe. The effect in Lec13-CHO cells could be at least partially reversed by either addition of fucose to the media (taking advantage of the salvage pathway for GDP-fucose biosynthesis) [42] or by transfection of GDP-mannose 4,6-dehydratase (the enzyme missing in Lec13-CHO cells) [30]. These results indicated that fucose plays an essential role in ligand-dependent activation of Notch and inhibition by Fringe. Since *N*-glycans are not involved, these data strongly suggest the *O*-fucose modifications are essential for proper Notch function.

The fact that the GlcNAc added to *O*-fucose via Fringe can be further elongated into a tetrasaccharide with galactose and sialic acid raised the question of how much of the tetrasaccharide is required to affect Notch signaling. Chen and co-workers examined this question by performing cell-based Notch signaling assays in a series of CHO cell glycosylation mutants [30]. Both Mfng and Lfng inhibited Jagged1-dependent Notch signaling in cells with decreased sialylation (Lec2-CHO), but not in cells with defects in addition of galactose (Lec8-CHO and Lec20-CHO). Further analysis showed β4GalT-1 to be the galactosyltransferase responsible for modification of the GlcNAc. Thus, at least a trisaccharide is required for Fringe-dependent inhibition of Jagged1-induced Notch activation.

Potential models for how elongated *O*-fucose affects Notch function

Although it is now clear that Fringe modulates Notch function by altering *O*-fucose structures, the mechanism by which changes in *O*-fucose structure modulates Notch signaling is not yet clear. Several working models have been proposed and are summarized here (see [42], [79] for more details). In the simplest model, an elongated *O*-fucose structure on Notch could alter its ability to bind ligands. Several reports have suggested that ligand binding is altered by Fringe [74, 80, 81]. Different EGF repeats on Notch are responsible for different interactions in its signaling pathway. EGF repeats 11 and 12 of Notch are both necessary and sufficient for interaction with Delta and Serrate [82] (fig. 2). Recent work in our laboratory has demonstrated that EGF 12 is modified with *O*-fucose and can be further elongated by Mfng [87]. This raises the interesting possibility that the inhibition of Serrate/Jagged signaling by Fringe may be caused by a decrease in binding to Notch due to steric blocking of the binding site by the elongated sugar. Nonetheless, this would not explain how Fringe increases signaling from Delta. An increase in binding would require that Delta have a lectin-like activity or that an accessory protein with lectin activity assist in Delta binding. Other possibilities must also be considered. Besides the ligand-binding region, other parts of the Notch extracellular domain are also known to influence ligand-dependent Notch activation. For instance, EGF repeats 24–26 of Notch influence signaling from Delta and Serrate [83]. As mentioned before, proteins such as Scabrous and Wingless bind to different regions of Notch and modulate its activity. Scabrous binds to sites within EGF 19–26 [56], and Wingless to a site within EGF 19–36 [58]. Thus, Fringe modification of EGF repeats in these regions could also influence Notch signaling. Preliminary data from our laboratory confirms that Fringe does modify *O*-fucose on many of these EGF repeats [87].

In addition to a direct affect on ligand binding, the inhibition of Notch by ligand expressed in the same cell (cis interaction) provides another model for how changes in *O*-fucose structure could modulate Notch. The Notch Abruptex (Ax) mutations are gain-of-function missense mutations (fig. 2) that result in hyperactivatable forms of Notch [84, 85], and some of the Ax mutants appear to be refractory to Fringe [85]. The hyperactivation is believed to be caused by abrogation of cell autonomous inhibition by ligands [55, 85]. Ax mutations are located within EGF repeats 24–29 [84], overlapping with several *O*-fucose modification sites that can be elongated by Fringe [87] (fig. 2). Thus, Fringe modification on EGF 24–29 could regulate Notch signaling by affecting cell-autonomous inhibition of Notch. The fact that both *O*-fucose and Fringe also modify Notch ligands raises the possibility that alterations on ligands may contribute to modulation of Notch activa-tion [20].

Altering the modifications on ligands is another potential means of modulating cis interactions between Notch and its ligands. Determination of which of these models, or others, are involved in Notch regulation is an area of continuing research.

Conclusions and future directions

Modulation of Notch function by alterations in *O*-fucose structures is yet another example of how unusual carbohydrate structures play a specific role in biology. In combination with reports of *O*-fucose modulating uPA-uPA receptor signaling [7] and Cripto/Nodal signaling [8, 9], carbohydrate modifications of EGF repeats appear to provide an additional layer of control to signaling pathways. The presence of *O*-fucose glycosylation sites in EGF repeats of several other proteins suggests it may play similar roles in other signaling pathways. The recent cloning of protein *O*-fucosyltransferase I (*O*-FucT-1) [29] is making possible genetic ablation experiments that should provide evidence for which of these modifications is functionally important. In addition to *O*-fucose, EGF repeats bear *O*-glucose modifications at a putative consensus site between the first and second conserved cysteines: $C^1X\underline{S}XPC^2$ [6]. Although no function for this modification is yet known, Notch bears *O*-glucose modifications, and many of the *O*-glucose sites on Notch are evolutionarily conserved [19]. We have recently identified and initially characterized the enzyme responsible for addition of *O*-glucose to EGF repeats [86]. The finding of *O*-fucose modifications on a region of TSRs involved in protein-protein interactions suggests that form of *O*-fucose may also regulate function. These observations suggest we are just beginning to understand the many and varied ways that carbohydrate modifications are involved in biology.

Acknowledgements. The authors would like to thank Yi Luo, Kelvin Luther, Raajit Rampal and Nadia Rana for helpful comments and discussions. Original work was supported by NIH grant GM61126.

1 Varki A. (1993) Biological roles of oligosaccharides: all of the theories are correct. Glycobiology **3:** 97–130
2 Kornfeld S. (1990) Lysosomal enzyme targeting. Biochem. Soc. Trans. **18:** 367–374
3 McEver R. P., Moore K. L. and Cummings R. D. (1995) Leukocyte trafficking mediated by selectin-carbohydrate interactions. J. Biol. Chem. **270:** 11025–11028
4 Comer F. I. and Hart G. W. (2000) *O*-Glycosylation of nuclear and cytosolic proteins. Dynamic Interplay Between *O*-GlcNAc and *O*-phosphate. J. Biol. Chem. **275:** 29179–29182
5 Endo T. (1999) *O*-mannosyl glycans in mammals. Biochim. Biophys. Acta **1473:** 237–246
6 Harris R. J. and Spellman M. W. (1993) *O*-linked fucose and other post-translational modifications unique to EGF modules. Glycobiology **3:** 219–224
7 Rabbani S. A., Mazar A. P., Bernier S. M., Haq M., Bolivar I., Henkin J. et al. (1992) Structural requirements for the growth factor activity of the amino-terminal domain of urokinase. J. Biol. Chem. **267:** 14151–14156
8 Schiffer S. G., Foley S., Kaffashan A., Hronowski X., Zichittella A., Yeo C. Y. et al. (2001) Fucosylation of Cripto is required for its ability to facilitate Nodal signaling. J. Biol. Chem. **276:** 37769–37778
9 Yan Y. T., Liu J. J., Luo Y. E. C., Haltiwanger R. S., Abate-Shen C. and Shen M. M. (2002) Dual roles of Cripto as a ligand and coreceptor in the nodal signaling pathway. Mol. Cell. Biol. **22:** 4439–4449
10 Schier A. F. and Shen M. M. (2000) Nodal signalling in vertebrate development. Nature **403:** 385–389
11 Hallgren P., Lundblad A. and Svensson S. (1975) A new type of carbohydrate-protein linkage in a glycopeptide from normal human urine. J. Biol. Chem. **250:** 5312–5314
12 Klinger M. M., Laine R. A. and Steiner S. M. (1981) Characterization of novel amino acid fucosides. J. Biol. Chem. **256:** 7932–7935
13 Kentzer E. J., Buko A. M., Menon G. and Sarin V. K. (1990) Carbohydrate composition and presence of a fucose-protein linkage in recombinant human pro-urokinase. Biochem. Biophys. Res. Commun. **171:** 401–406
14 Harris R. J., Leonard C. K., Guzzetta A. W. and Spellman M. W. (1991) Tissue plasminogen activator has an *O*-linked fucose attached to threonine-61 in the epidermal growth factor domain. Biochemistry **30:** 2311–2314
15 Bjoern S., Foster D. C., Thim L., Wiberg F. C., Christensen M., Komiyama Y. et al. (1991) Human plasma and recombinant factor VII: characterization of *O*-glycosylation at serine residues 52 and 60 and effects of site-directed mutagenesis of serine 52 to alanine. J. Biol. Chem. **266:** 11051–11057
16 Nishimura H., Takao T., Hase S., Shimonishi Y. and Iwanaga S. (1992) Human factor IX has a tetrasaccharide *O*-glycosidically linked to serine 61 through the fucose residue. J. Biol. Chem. **267:** 17520–17525
17 Harris R. J., Ling V. T. and Spellman M. W. (1992) *O*-linked fucose is present in the first epidermal growth factor domain of factor XII but protein C. J. Biol. Chem. **267:** 5102–5107
18 Campbell I. D. and Bork P. (1993) Epidermal growth factor-like modules. Curr. Opin. Struct. Biol. **3:** 385–392
19 Moloney D. J., Shair L., Lu F. M., Xia J., Locke R., Matta K. L. et al. (2000) Mammalian Notch1 is modified with two unusual forms of *O*-linked glycosylation found on epidermal growth factor-like modules. J. Biol. Chem. **275:** 9604–9611
20 Panin V. M., Shao L., Lei L., Moloney D. J., Irvine K. D. and Haltiwanger R. S. (2002) Notch ligands are substrates for EGF protein *O*-fucosyltransferase and Fringe. J. Biol. Chem. **272:** 29945–29952
21 Nakakura N., Hietter H., Van Dorsselaer A. and Luu B. (1992) Isolation and structural determination of three peptides from the insect *Locusta migratoria*. Identification of a deoxyhexose-linked peptide. Eur. J. Biochem. **204:** 147–153
22 Mer G., Hietter H. and Lefévre J. F. (1996) Stabilization of proteins by glycosylation examined by NMR analysis of a fucosylated proteinase inhibitor. Nat. Struct. Biol. **3:** 45–53
23 Hofsteenge J., Huwiler K. G., Macek B., Hess D., Lawler J., Mosher D. F. et al. (2001) C-mannosylation and *O*-fucosylation of the thrombospondin type 1 module. J. Biol. Chem. **276:** 6485–6498
24 Adams J. C. and Tucker R. P. (2000) The thrombospondin type 1 repeat (TSR) superfamily: diverse proteins with related roles in neuronal development. Develop. Dynamics **218:** 280–299
25 Gonzalez De Peredo A., Klein D., Macek B., Hess D., Peter-Katalinic J. and Hofsteenge J. (2002) C-mannosylation and *O*-fucosylation of thrombospondin type 1 repeats. Mol. Cell. Proteomics **1:** 11–18
26 Furmanek A. and Hofsteenge J. (2000) Protein C-mannosylation: facts and questions. Acta Biochim. Pol. **47:** 781–789
27 Wang Y., Lee G. F., Kelley R. F. and Spellman M. W. (1996) Identification of a GDP-Lfucose: polypeptide fucosyltransferase and enzymatic addition of *O*-linked fucose to EGF domains. Glycobiology **6:** 837–842
28 Wang Y. and Spellman M. W. (1996) Identification and purification of a GDP-fucose: polypeptide fucosyltransferase. EGF domain *O*-fucosylation. Glycobiology **6:** 759

29 Wang Y., Shao L., Shi S., Harris R. J., Spellman M. W., Stanley P. et al. (2001) Modification of epidermal growth factor-like repeats with *O*-fucose: molecular cloning of a novel GDP-fucose: protein *O*-fucosyltransferase. J. Biol. Chem. **276:** 40338–40345

30 Chen J., Moloney D. J. and Stanley P. (2001) Fringe modulation of Jagged1-induced Notch signaling requires the action of β4Galactosyltransferase-1. Proc. Natl. Acad. Sci. USA **98:** 13716–13721

31 Moloney D. J. and Haltiwanger R. S. (1999) The *O*-linked fucose glycosylation pathway: identification and characteriztion of a UDP-glucose: *O*-fucose β1,3-glucosyltransferase. Glycobiology **9:** 679–687

32 Moor O. L. (1919) Genetics **4:** 252

33 Wharton K. A., Johansen K. M., Xu T. and Artavanis-Tsakonas S. (1985) Nucleotide sequence from the neurogenic locus notch implies a gene product that shares homology with proteins containing EGF-like repeats. Cell **43:** 567–581

34 Artavanis-Tsakonas S., Rand M. D. and Lake R. J. (1999) Notch signaling: cell fate control and signal integration in development. Science **284:** 770–776

35 Ellisen L. W., Bird J., West D. C., Soreng A. L., Reynolds T. C., Smith S. D. et al. (1991) TAN-1, the human homolog of the *Drosophila* Notch gene, is broken by chromosomal translocations in T lymphoblastic neoplasms. Cell **66:** 649–661

36 Joutel A., Corpechot C., Ducros A., Vahedi K., Chabriat H., Mouton P. et al. (1996) *Notch3* mutations in CADASIL, a hereditary adult-onset condition causing stroke and dementia. Nature **383:** 707–710

37 Artavanis-Tsakonas S. (1997) Alagille syndrome: a notch up for the Notch receptor. Nat. Genet. **16:** 212–213

38 Bulman M. P., Kusumi K., Frayling T. M., McKeown C., Garrett C., Lander E. S. et al. (2000) Mutations in the human delta homologue, DLL3, cause axial skeletal defects in spondylocostal dysostosis. Nat. Genet. **24:** 438–441

39 Ruchoux M. M. and Maurage C. A. (1997) CADASIL: cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. J. Neuropathol. Exp. Neurol. **56:** 947– 964

40 Pourquie O. and Kusumi K. (2001) When body segmentation goes wrong. Clin. Genet. **60:** 409–416

41 Logeat F., Bessia C., Brou C., LeBail O., Jarriault S., Seidah N. G. et al. (1998) The Notch1 receptor is cleaved constitutively by a furin-like convertase. Proc. Natl. Acad. Sci. USA **95:** 8108–8112

42 Moloney D. J., Panin V. M., Johnston S. H., Chen J., Shao L., Wilson R. et al. (2000) Fringe is a glycosyltransferase that modifies Notch. Nature **406:** 369–375

43 Mumm J. S. and Kopan R. (2000) Notch signaling: from the outside in. Dev. Biol. **228:** 151–165

44 Brou C., Logeat F., Gupta N., Bessia C., LeBail O., Doedens J. R. et al. (2000) A novel proteolytic cleavage involved in Notch signaling: the role of the disintegrin-metalloprotease TACE. Mol. Cell **5:** 207–216

45 Mumm J. S., Schroeter E. H., Saxena M. T., Griesemer A., Tian X., Pan D. J. et al. (2000) A ligand-induced extracellular cleavage regulates gamma-secretaselike proteolytic activation of Notch1. Mol. Cell **5:** 197–206

46 Ebinu J. O. and Yankner B. A. (2002) A RIP tide in neuronal signal transduction. Neuron **34:** 499–502

47 Brown M. S., Ye J., Rawson R. B. and Goldstein J. L. (2000) Regulated intramembrane proteolysis: a control mechanism conserved from bacteria to humans. Cell **100:** 391–398

48 Panin V. M. and Irvine K. D. (1998) Modulators of Notch signaling. Sem. Cell Dev. Biol. **9:** 609–617

49 Baron M., Aslam H., Flasza M., Fostier M., Higgs J. E., Mazaleyrat S. L. et al. (2002) Multiple levels of Notch signal regulation (review). Mol. Membr. Biol. **19:** 27–38

50 Hubbard E. J., Wu G., Kitajewski J. and Greenwald I. (1997) sel-10, a negative regulator of lin-12 activity in *Caenorhabditis elegans*, encodes a member of the CDC4 family of proteins. Genes Dev. **11:** 3182–3193

51 Gupta-Rossi N., Le Bail O., Gonen H., Brou C., Logeat F., Six E. et al. (2001) Functional interaction between SEL-10, an F-box protein, and the nuclear form of activated Notch1 receptor. J. Biol. Chem. **276:** 34371–34378

52 Yeh E., Zhou L., Rudzik N. and Boulianne G. L. (2000) Neuralized functions cell autonomously to regulate *Drosophila* sense organ development. EMBO J. **19:** 4827–4837

53 Xu T. and Artavanis-Tsakonas S. (1990) deltex, a locus interacting with the neurogenic genes, Notch, Delta and mastermind in *Drosophila melanogaster*. Genetics **126:** 665–677

54 Jacobsen T. L., Brennan K., Arias A. M. and Muskavitch M. A. (1998) Cis-interactions between Delta and Notch modulate neurogenic signalling in *Drosophila*. Development **125:** 4531–4540

55 Sakamoto K., Ohara O., Takagi M., Takeda S. and Katsube K. (2002) Intracellular cellautonomous association of Notch and its ligands: a novel mechanism of Notch signal modification. Dev. Biol. **241:** 313–326

56 Powell P. A., Wesley C., Spencer S. and Cagan R. L. (2001) Scabrous complexes with Notch to mediate boundary formation. Nature **409:** 626–630

57 Lee E. C., Yu S. Y. and Baker N. E. (2000) The scabrous protein can act as an extracellular antagonist of notch signaling in the *Drosophila* wing. Curr. Biol. **10:** 931–934

58 Wesley C. S. (1999) Notch and wingless regulate expression of cuticle patterning genes. Mol. Cell. Biol. **19:** 5743–5758

59 Panin V. M., Papayannopoulos V., Wilson R. and Irvine K. D. (1997) Fringe modulates notch ligand interactions. Nature **387:** 908–912

60 Fleming R. J., Gu Y. and Hukriede N. A. (1997) *Serrate*-mediated activation of *Notch* is specifically blocked by the product of the gene *fringe* in the dorsal compartment of the *Drosophila* wing imaginal disc. Development **124:** 2973–2981

61 Irvine K. D. and Wieschaus E. (1994) Fringe, a boundary-specific signaling molecule, mediates interactions between dorsal and ventral cells during *Drosophila* wing development. Cell **79:** 595–606

62 Irvine K. D. (1999) Fringe, Notch and making developmental boundaries. Curr.Opin.Genet.Dev. **9:** 434–441

63 Johnston S. H., Rauskolb C., Wilson R., Prabhakaran B., Irvine K. D. and Vogt T. F. (1997) A family of mammalian Fringe genes implicated in boundary determination and the Notch pathway. Development **124:** 2245–2254

64 Cohen B., Bashirullah A., Dagnino L., Campbell C., Fisher W. W., Leow C. C. et al. (1997) Fringe boundaries coincide with Notch-dependent patterning centres in mammals and alter Notch-dependent development in *Drosophila*. Nat. Genet. **16:** 283–288

65 Wolpert L. (1998) Pattern formation in epithelial development: the vertebrate limb and feather bud spacing. Philos. Trans. R. Soc. Lond. B Biol. Sci. **353:** 871–875

66 Laufer E., Dahn R., Orozco O. E., Yeo C. Y., Pisenti J., Henrique D. et al. (1997) Expression of Radical fringe in limb-bud ectoderm regulates apical ectodermal ridge formation. Nature **386:** 366–373

67 Rodriguez-Esteban C., Schwabe J. W., De La Pena J., Foys B., Eshelman B. and Belmonte J. C. (1997) Radical fringe positions the apical ectodermal ridge at the dorsoventral boundary of the vertebrate limb. Nature **386:** 360–366

68 Zhang N. and Gridley T. (1998) Defects in somite formation in lunatic fringe-deficient mice. Nature **394:** 374–377

69 Evrard Y. A., Lun Y., Aulehla A., Gan L. and Johnson R. L. (1998) Lunatic fringe is an essential mediator of somite segmentation and patterning. Nature **394:** 377–381

70 Zhang N., Norton C. R. and Gridley T. (2002) Segmentation defects of Notch pathway mutants and absence of a synergistic phenotype in lunatic fringe/radical fringe double mutant mice. Genesis **33:** 21–28

71 Moran J. L., Levorse J. M. and Vogt T. F. (1999) Limbs move beyond the radical fringe. Nature **399:** 742–743
72 Wiggins C. A. R. and Munro S. (1998) Activity of the yeast *MNN1* α-1,3-mannosyltransferase requires a motif conserved in many other families of glycosyltransferases. Proc. Natl. Acad. Sci. USA **95:** 7945–7950
73 Yuan Y. P., Schultz J., Mlodzik M. and Bork P. (1997) Secreted fringe-like signaling molecules may be glycosyltransferases. Cell **88:** 9–11
74 Bruckner K., Perez L., Clausen H. and Cohen S. (2000) Glycosyltransferase activity of Fringe modulates Notch-Delta interactions. Nature **406:** 411–415
75 Goto S., Taniguchi M., Muraoka M., Toyoda H., Sado Y., Kawakita M. et al. (2001) UDP-sugar transporter implicated in glycosylation and processing of Notch. Nat. Cell Biol. **3:** 816–822
76 Selva E. M., Hong K., Baeg G. H., Beverley S. M., Turco S. J., Perrimon N. et al. (2001) Dual role of the fringe connection gene in both heparan sulphate and fringedependent signalling events. Nat. Cell Biol. **3:** 809–815
77 Munro S. and Freeman M. (2000) The notch signalling regulator fringe acts in the Golgi apparatus and requires the glycosyltransferase signature motif DXD. Curr. Biol. **10:** 813–820
78 Ripka J., Adamany A. and Stanley P. (1986) Two Chinese hamster ovary glycosylation mutants affected in the conversion of GDP-mannose to GDP-fucose. Arch. Biochem. Biophys. **249:** 533–545
79 Haltiwanger R. S. and Stanley P. (2002) Modulation of receptor signaling by glycosylation: Finge is an *O*-fucose-β1,3-*N*-acetylglucosaminyltransferase. Biochem. Biophys. Acta **1573:** 328–335
80 Shimizu K., Chiba S., Saito T., Kumano K., Takahashi T. and Hirai H. (2001) Manic fringe and lunatic fringe modify different sites of the notch2 extracellular region, resulting in different signaling modulation. J. Biol. Chem. **276:** 25753–25758
81 Hicks C., Johnston S. H., DiSibio G., Collazo A., Vogt T. F. and Weinmaster G. (2000) Jagged1 and Delta1 mediated Notch signaling is differentially regulated by Lunatic Fringe. Nat. Cell Biol. **2:** 515–520
82 Rebay I., Fleming R. J., Fehon R. G., Cherbas L., Cherbas P. and Artavanis-Tsakonas S. (1991) Specific EGF repeats of Notch mediate interactions with delta and serrate: implications for Notch as a multifunctional receptor. Cell **67:** 687–699
83 Lawrence N., Klein T., Brennan K. and Martinez Arias A. (2000) Structural requirements for notch signalling with delta and serrate during the development and patterning of the wing disc of Drosophila. Development **127:** 3185–3195
84 Kelley M. R., Kidd S., Deutsch W. A. and Young M. W. (1987) Mutations altering the structure of epidermal growth factor-like coding sequences of the *Drosophila Notch* Locus. Cell **51:** 539–548
85 De Celis J. F. and Bray S. J. (2000) The Abruptex domain of Notch regulates negative interactions between Notch, its ligands and Fringe. Development **127:** 1291–1302
86 Shao L., Luo Y., Moloney D. J. and Haltiwanger R. S. (2002) *O*-Glycosylation of EGF repeats: identification and initial characterization of a UDP-glucose: protein *O*-glucosyltransferase. Glycobiology **12:** 763–770
87 Shao L., Moloney D. J. and Haltiwanger R. S. (2003) *Fringe* modifies *O*-fucose on mouse Notch I at EGR repeats within the ligand-binding site and the Abruptex region. J. Biol. Chem. in press