Glycosyltransferase activity of Fringe modulates Notch–Delta interactions

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Ligands that are capable of activating Notch family receptors are broadly expressed in animal development, but their activity is tightly regulated to allow formation of tissue boundaries[1]. Members of the *fringe* gene family have been implicated in limiting Notch activation during boundary formation[2–8], but the mechanism of Fringe function has not been determined. Here we present evidence that Fringe acts in the Golgi as a glycosyltransferase enzyme that modifies the epidermal growth factor (EGF) modules of Notch and alters the ability of Notch to bind its ligand Delta. Fringe catalyses the addition of *N*-acetylglucosamine to fucose, which is consistent with a role in the elongation of *O*-linked fucose *O*-glycosylation that is associated with EGF repeats. We suggest that cell-type-specific modification of glycosylation may provide a general mechanism to regulate ligand–receptor interactions *in vivo*.

In the developing *Drosophila* wing, asymmetric activation of Notch by the dorsally expressed ligand Serrate and the ventrally expressed ligand Delta is required to induce Wingless and Vestigial expression and establish a signalling centre at the dorsal–ventral boundary[9–13]. Fringe is expressed in dorsal cells and contributes to making them more sensitive to Delta and less sensitive to Serrate[2,4,14]. One means by which Fringe might change the sensitivity of dorsal cells to Notch ligands is by modulating ligand–receptor interaction. Alternatively, Fringe might act indirectly to influence cellular signalling responses to a given level of ligand binding. To distinguish between these possibilities, we measured the effect of Fringe on Notch–Delta binding.

We expressed the extracellular domain of Delta as a secreted alkaline phosphatase fusion protein for use in a ligand-binding assay (Delta–AP; Fig 1a). To measure interaction of Delta–AP with transiently transfected *Drosophila* SL2 cells, bound Delta–AP was quantified using an enzymatic assay for alkaline phosphatase activity. Expression levels of the transfected proteins were monitored by immunoblot analysis in parallel to the binding assays. SL2 cells expressing Notch alone or Fringe alone bound Delta–AP at a very low level (Fig 1b). Co-expression of Notch and Fringe resulted in a large increase in the quantity of bound Delta–AP (Fig 1b), even when the level of Notch expression was lower than in the cells expressing Notch alone. The level of proteolytic processing required for formation of a functional receptor was not increased by co-expression of Fringe. This suggests that Fringe activity may increase the ability of Notch-expressing cells to bind Delta.

Although Fringe and its vertebrate homologues can be found as secreted proteins (Fig 2b; refs 2, 15), genetic analysis has suggested that Fringe acts cell-autonomously in the wing disc[11,14]. These apparently contradictory observations prompted us to ask whether secreted Fringe can affect Notch–Delta binding. We compared Delta–AP binding to cells co-expressing Notch and Fringe with its binding to Notch-expressing cells that were co-cultured with Fringe-expressing cells for two days before the binding assay (Fig. 1b). Co-cultured cells bound Delta–AP at background levels. Thus, Fringe does not appear to influence the ability of Notch to

bind Delta when provided as an extracellular protein, but does act when co-expressed with Notch.

The requirement for co-expression of Fringe and Notch could be explained if Fringe exerts its activity within the Notch-expressing cell. Fringe and Brainiac have been suggested to show similarity to bacterial glycosyltransferase enzymes[16], and several mammalian glycosyltransferases related to Brainiac have been characterized[17]. If Fringe functions as a glycosyltransferase enzyme, it should act in the Golgi. To test this possibility, we prepared a Golgi-tethered version of Fringe in which the first 40 amino acids (including the predicted signal peptide) were replaced by the first 121 amino acids of the Golgi-resident glycosyltransferase GalNAc-T3 (Fig 2a; ref. 18). The resulting fusion protein includes the transmembrane domain of GalNAc-T3, which functions as a Golgi-retention signal[19]. Immunoprecipitation from transfected SL2 cells showed that Fringe–GT was expressed at a comparable level to wild-type Fringe, but was not
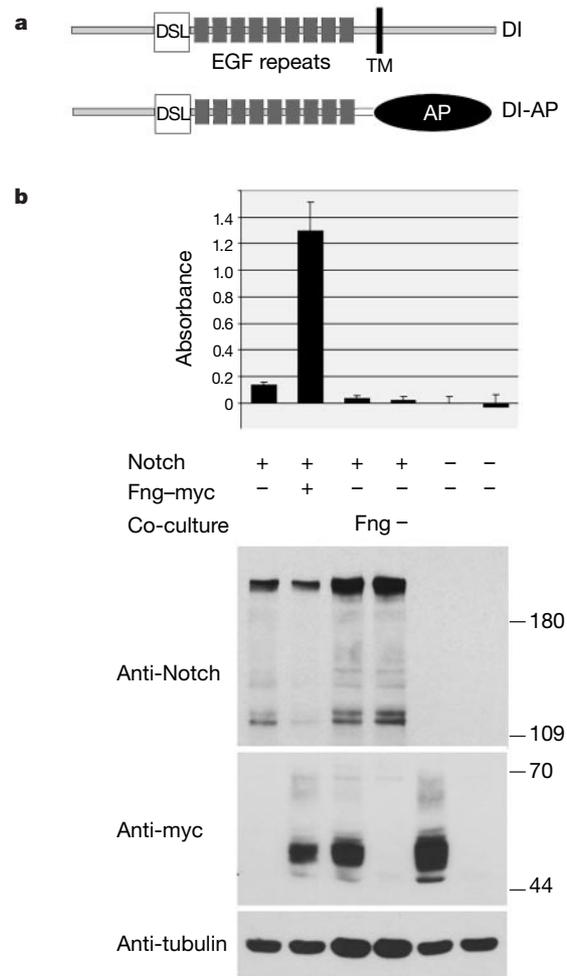

Figure 1 Fringe increases binding of Delta to Notch. **a**, Representation of Delta (DI) and the secreted Delta–alkaline phosphatase fusion protein. TM, transmembrane domain. DSL, Delta Serrate Lag domain. **b**, Upper panel, Delta–AP binding to control and transfected SL2 cells. Cells were transfected with constructs to direct expression of Notch or Fringe–myc as indicated. Cells transfected with empty vector were used as a control. Co-culture indicates that cells transfected with Notch were grown as a mixed culture with cells independently transfected to express Fringe–myc or with cells transfected with empty vector. AP activity is shown in absorbance units. Means ± s.d. of duplicate binding assays are shown. Lower panels, immunoblots of cell extracts prepared in parallel to those used in the binding assay and probed with anti-Notch, anti-myc and anti-Tubulin. Two forms of Notch are recognized by antibody 9C6. The upper band is the unprocessed form of Notch that does not reach the cell surface[24]; the lower band is the C-terminal portion of the proteolytically processed form, reflecting production of the mature cell-surface protein.

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secreted at detectable levels (Fig. 2b). Immunofluorescent labelling of transfected cells with antibody to a Golgi-resident protein and with anti-myc to visualize Fringe–GT–myc showed that the proteins co-localize (Fig. 2c). Together, these observations confirm that the transmembrane tether provided by GalNAc-T3 is effective in SL2 cells.

In binding experiments, co-expression of Fringe–GT was sufficient to stimulate Delta–AP binding to Notch and was almost as effective as wild-type Fringe (Fig. 2d). This suggests that Fringe–GT has comparable activity to wild-type Fringe. Fringe–GT was also found to be functional *in vivo*, despite not being secreted. When expressed under *patched[GAL4]* control Fringe–GT had no effect in the dorsal compartment where endogenous Fringe is expressed, but interrupted the endogenous Wingless stripe at the dorsal–ventral boundary and induced ectopic expression of Wingless in the ventral compartment (Fig. 2e, f). These effects are comparable to those caused by expression of wild-type Fringe–myc (Fig. 2g), and suggest that the Golgi-resident form of Fringe has full biological activity. Many Golgi glycosyltransferase enzymes are also found as secreted soluble enzymes, although the function of the secreted forms is unknown. Therefore, the secretion of Fringe proteins may not be of functional significance to their roles as modifiers of Notch activity.

A D-x-D amino-acid motif found in many glycosyltransferases is required for catalytic activity and appears to be involved in coordination of a divalent metal ion in the binding of the donor nucleotide sugar[16,20,21]. In Fringe, this motif may correspond to residues D236–238. If Fringe acts as a glycosyltransferase, replacing residues 236–238 with asparagine (Fringe-NNN) should destroy enzymatic activity but have a minimal effect on overall protein structure. Consistent with this possibility, co-expression of the Fringe-NNN mutant with Notch did not increase Delta–AP

binding above background levels (Fig. 3a). Furthermore, ectopic expression of Fringe-NNN under *patched[GAL4]* control did not cause Notch activation in the ventral compartment in the wing imaginal disc (not shown). These observations suggest that Fringe-NNN is inactive *in vivo*.

To determine whether Fringe has intrinsic glycosyltransferase activity, we produced wild-type Fringe and Fringe-NNN by baculovirus infection of insect cells. Microsomal fractions enriched for Golgi membranes were partially solubilized and assayed for the ability of the expressed proteins to catalyse the transfer of ^{14}C-labelled UDP donor sugars onto acceptor sugars. We tested a variety of different donor–acceptor combinations (Fig. 3b). The highest level of activity was observed with wild-type Fringe microsome lysate and the combination of UDP-*N*-acetylglucosamine (GlcNAc) and fucose (18-fold over the background level observed with Fringe-NNN). Fringe showed no significant activity with other donor–acceptor combinations.

Fucose is commonly found as an unsubstituted terminal sugar residue in *N*- and *O*-linked oligosaccharide chains of glycoproteins and in glycosphingolipids of eukaryotic cells. In contrast, addition of *O*-linked fucose directly to proteins is a rare type of glycosylation that is found in association with the cysteine-rich consensus sequence C-x-x-G-G-S/T-C (ref. 22). This consensus sequence is found in EGF modules, including a subset of those in Notch, Serrate, Delta, and in their nematode and vertebrate homologues (ref. 23; Fig. 4a). Our results raise the possibility that Fringe functions by elongating *O*-linked fucose residues in the EGF repeats of Notch through the addition of GlcNAc.

To determine whether Fringe acts through the EGF repeats of Notch, we expressed Notch as a fusion protein in which all sequences following the EGF repeats were replaced by heterologous
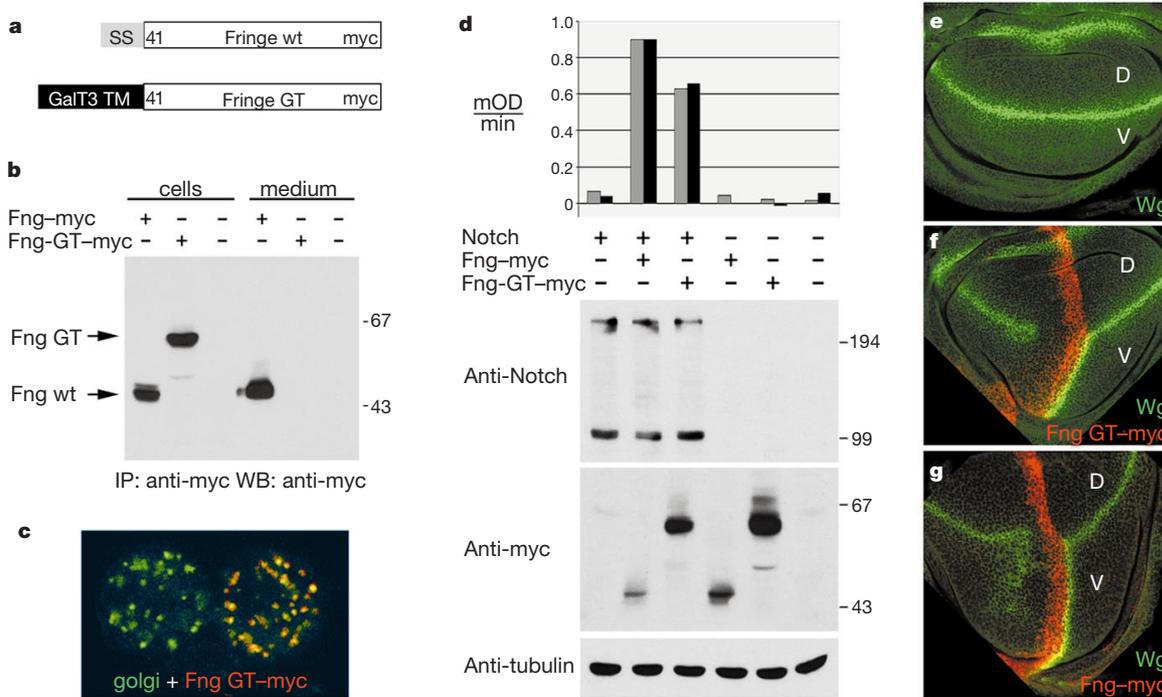

Figure 2 Golgi-tethered Fringe increases binding of Delta to Notch. **a**, Representation of wild-type and Golgi-tethered Fringe (Fng GT). The N-terminal signal sequence (ss) of wild-type fringe (residues 1–40) was replaced by the transmembrane and juxtamembrane region (1–121) of the Golgi-resident GalNAc-T3 protein. Both proteins carry a C-terminal myc tag. **b**, Western blot of myc-tagged proteins immunoprecipitated from whole cell lysates (cells) and from conditioned medium. Cells were transfected to express wild-type Fringe–myc or Fringe–GT–myc. Control cells were transfected with empty vector. **c**, Immunofluorescent labelling of SL2 cells transfected to express Fringe–GT–myc and labelled with antibody to *Drosophila* Golgi (green) and anti-myc (red). The cell on the right

expressed Fringe–GT–myc. **d**, Delta–AP binding to Notch-expressing cells was increased by co-expression of Fringe–myc or Fringe–GT–myc. Replicate experiments are shown. Immunoblots of cell lysates probed with anti-Notch, anti-myc and anti-Tubulin are shown below. **e–g**, Wing imaginal discs labelled to visualize Wingless protein (green) and the myc epitope tag (red). **e**, *patched[GAL4]*/+ control wing disc. D, dorsal compartment; V, ventral compartment. **f**, *patched[GAL4]*/UAS–Fringe–GT–myc. **g**, *patched[GAL4]*/UAS–Fringe–myc. Fringe–GT–myc and wild-type Fringe–myc expression in the *patched[GAL4]* stripe are shown in red.

sequences from the transmembrane protein CD2 (Fig. 4a). Cells expressing Notch–CD2 and Golgi-tethered Fringe–GT bound over 50-fold more Delta–AP than cells expressing Notch–CD2 alone or control cells (Fig. 4b). This observation indicates that the EGF repeats of Notch are sufficient to mediate binding to Delta when taken out of their normal context. Under normal circumstances, expression of Notch on the cell surface requires proteolytic cleavage in the extracellular domain by a furin-like protease[24,25]. The cleaved extracellular domain remains attached to the transmembrane and intracellular domain to form an active receptor complex. In the case of Notch–CD2, proteolytic processing does not appear to be required for cell-surface expression and Delta–AP interaction, as the protein lacks the cleavage site located between Notch–Lin repeats and the transmembrane domain that is used in mouse Notch1.

As the EGF modules of Notch appear to be sufficient to mediate ligand interaction, we reasoned that the corresponding domain of Notch expressed as a soluble AP fusion protein might retain ligand-binding activity (Fig. 4a). We produced Notch–AP in presence or absence of co-expressed Fringe–GT. AP activity of the supernatants was first normalized and binding of the secreted AP fusion proteins was carried out on SL2 cells expressing the full-length form of Delta.
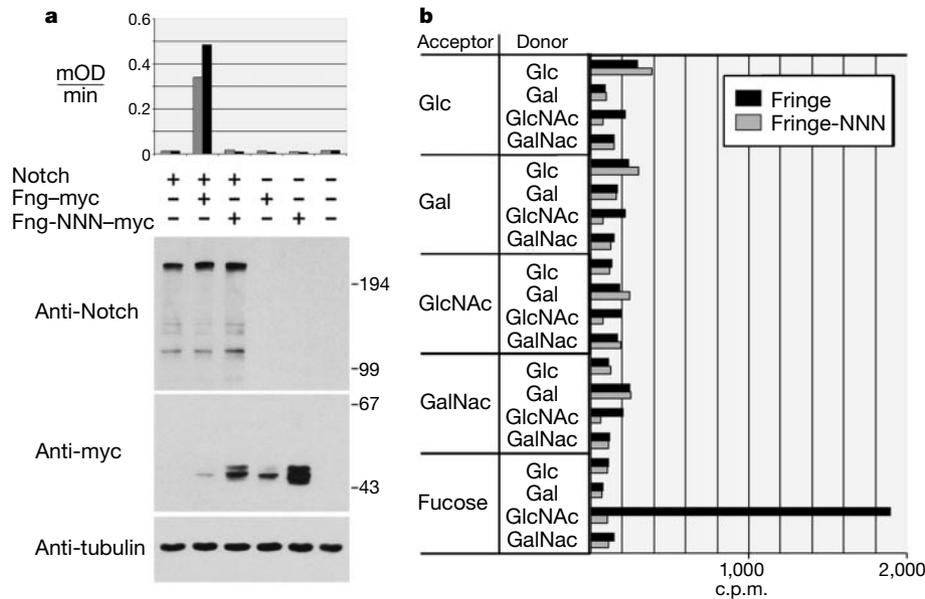

Figure 3 Fringe has glycosyltransferase activity. **a**, Delta–AP binding to control and Notch-transfected SL2 cells. Cells were transfected to express Notch, wild-type Fringe–myc, or mutant Fringe-NNN–myc as indicated. Fringe-NNN–myc has no activity in the binding assay, despite being expressed at higher levels than wild-type Fringe–myc. Lower panels, western blots of cell lysates probed with anti-Notch, with anti-myc and with anti-Tubulin. MOD, absorbance units $\times 10^{-3}$. **b**, Glycosyltransferase activity was measured in microsomal fractions from cells expressing wild-type Fringe–myc or Fringe-NNN–myc. Enzyme activity was measured by transfer of ^{14}C-labelled sugars from UDP donor sugars onto acceptor sugars. Average results from two experiments are shown. Donors tested were UDP-glucose (Glc), UDP-galactose (Gal), UDP-N-acetyl-glucosamine (GlcNAc) and N-acetyl-galactosamine (GalNAc). Acceptors tested were D-glucose, D-galactose, D-GlcNAc, D-GalNAc and L-fucose.
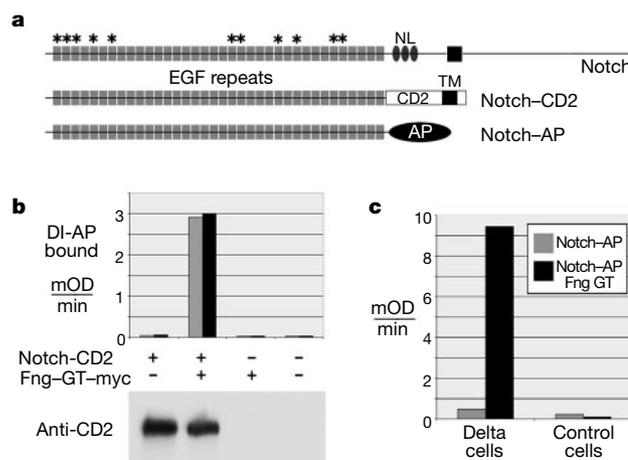

Figure 4 Secreted Notch produced by Fringe–GT-expressing cells binds Delta-expressing cells. **a**, Representation of Notch, Notch–CD2 and the secreted Notch–AP fusion proteins. Notch–CD2 consists of the EGF modules fused to the transmembrane protein CD2. Notch–AP consists of the same EGF modules expressed as a secreted AP fusion protein. Asterisks indicate EGF modules containing a perfect consensus sequence for O-linked fucose modification. NL, Notch–Lin repeats; TM, membrane-spanning domain. **b**, Quantitation of Delta–AP binding in replicate experiments. Cells were transfected to express Notch–CD2 or Fringe–GT–myc as indicated. Lower panel, immunoblot probed with anti-CD2. Notch–CD2 expression was comparable in the presence or absence of Fringe–GT–myc. Notch–CD2 lacks the site for furin-mediated cleavage located near the Notch–Lin repeats[25] and migrates at a relative molecular mass of ~200,000. **c**, Quantitation of Notch–AP binding to cells expressing full-length Delta or control cells. Grey bars, Notch–AP produced by SL2 cells; black bars, Notch–AP produced by SL2 cells co-expressing Golgi-tethered Fringe–GT–myc. The level of Notch–AP activity in the conditioned media was normalized.
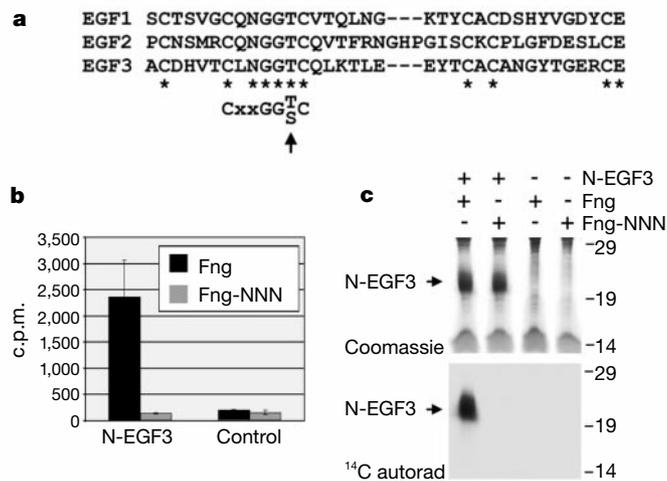
Figure 5 Glycosylation of Notch by Fringe *in vitro*. **a**, Aligned amino-acid sequence of the first three EGF repeats of Notch. Conserved residues and the consensus sequence for *O*-fucosylation are indicated (arrow). **b**, Glycosyltransferase activity of microsomal fractions from cells expressing wild-type Fringe–myc or Fringe-NNN–myc. Enzyme activity was measured by transfer of ^{14}C-labelled UDP-GlcNAc onto Notch–EGF3–His (N-EGF3). Average results from two experiments are shown. **c**, SDS–PAGE of samples from **b** run on a 15% acrylamide gel. Upper panel, Coomassie blue stained gel. The Notch–EGF3 protein is indicated. Lower panel, ^{14}C-UDP-GlcNAc-labelled Notch–EGF3 protein visualized by autoradiography. Background proteins from the microsome fractions were not ^{14}C-labelled.

Notch–AP produced in cells co-expressing Fringe–GT–myc bound to Delta-expressing cells 20 times more effectively than Notch–AP produced in the absence of Fringe (Fig. 4b). This suggests that the observed binding relies solely on the EGF modules of Notch being present as a secreted soluble protein.

To determine whether Fringe acts directly to modify one or more EGF modules of Notch, we carried out an *in vitro* glycosylation assay using a short protein consisting of the first three EGF modules of Notch as the substrate (EGF3). The first three EGF modules of Notch contain perfect consensus sites for addition of *O*-linked fucose (Fig. 5a). The results presented above suggest that Fringe might act by elongating *O*-linked fucose through addition of GlcNAc. The Notch–EGF3 protein was expressed in SL2 cells with a carboxy-terminal histidine tag to permit purification of the secreted protein. Equal amounts of Notch–EGF3 were incubated with ^{14}C-labelled UDP-GlcNAc and microsomal lysates from cells expressing wild-type Fringe or Fringe-NNN. Over 10-fold more labelled GlcNAc was incorporated into Notch–EGF3 by wild-type Fringe than by the mutant form Fringe-NNN (Fig. 5b, c). We conclude that Fringe can act directly to modify the EGF repeats of Notch.

Our results provide evidence that Fringe is a glycosyltransferase enzyme that acts in the Golgi to modify Notch. Fringe-dependent glycosylation of Notch increased its ability to bind Delta. Unexpectedly, we were unable to detect measurable binding of Notch–AP to cells expressing Serrate, or of Serrate–AP to cells expressing Notch. Fringe had no measurable effect on Serrate–AP binding to Notch in our assays when expressed in either the Serrate–AP producing cells or in Notch-expressing cells. This suggests that another factor may be required to promote Notch–Serrate binding. *Drosophila* Brainiac shows limited sequence similarity to Fringe[16] and has been implicated as a modulator of the activities of Notch and EGF signalling pathways[26]. Mammalian Brainiac-related proteins have been characterized as β3Gal or β3GlcNac glycosyltransferases with acceptor substrate specificities distinct from Fringe[17]. It remains to be determined whether Brainiac modifies Notch–ligand or other receptor–ligand interactions.

We propose that Fringe activity determines the type of *O*-linked fucose extension on the EGF repeats of Notch, and possibly on other EGF-repeat-containing proteins. *O*-linked fucose may be extended by the addition of β1-3-glucose or β1-3GlcNAc, and the latter may be followed by addition of galactose and sialic acid residues[22,23]. Our results suggest that Fringe directs elongation of *O*-linked fucose in the EGF modules of Notch by addition of GlcNAc. Fringe-mediated modification changes the properties of Notch–Delta binding and has an important role in conferring signalling specificity *in vivo*. The identification of enzymes that selectively modify oligosaccharide side chains suggests a new range of possibilities for the regulation of ligand–receptor interactions in a cell-type-specific and protein-specific manner. □

Methods

Constructs

Notch–AP was constructed by cloning sequences encoding amino acids 1–1,467 of Notch in frame with human placental alkaline phosphatase from pcDNA3-AP (ref. 27). The fusion junction is located at the *Bsp*EI site between the last EGF repeat and the first Notch–Lin repeat. The same Notch fragment was used to make Notch–CD2 and was linked in frame to rat CD2 at residue 2. For Notch–EGF3, residues GHHHHHH and a stop codon were introduced after residue 177 of Notch. For Delta–AP, a *Bgl*II site was introduced by PCR after residue N592 and sequences encoding residues 1–592 of Delta were fused in frame with AP. For Fringe–myc, residues EFEQKLISEEDL were introduced at the C terminus of Fringe by PCR. Fringe–myc was cloned into pRmHa3 for expression in SL2 cells and into pUAST for GAL4-regulated expression in *Drosophila*. For Fringe-NNN–myc, residues D236–D238 of Fringe–myc were converted to N236–N238 by PCR. For Fringe–GT–myc, fragments encoding the first 121 amino acids of GalNAc-T3 (GenBank accession number X92689) and 40–424 of Fringe–myc were amplified by PCR using oligonucleotides that produce a 15-bp overlapping sequence at the fusion junction. The first two PCR products were used as template to amplify the full-length fusion.

Cell culture and binding assays

Complementary DNAs for expression in *Drosophila* Schneider SL2 cells were cloned into pRmHa3. Cells were transiently transfected by the CaPO$_4$ method using 4–8 μg of DNA per well in 6-well plates. Expression was induced by addition of 0.7 mM CuSO$_4$ for 2 days. Conditioned medium was collected from Notch–AP and Delta–AP transfected cells 2–4 days after induction. The activity level of Notch–AP expressed with and without Fringe was normalized by addition of SL2 conditioned medium. AP-containing supernatants were supplemented with 0.1% NaN$_3$ and incubated with adherent cells for 90 min at room temperature. Cells were washed 5 times with HBSS containing 0.05% BSA and 0.1% azide, and lysed in 10 mM Tris pH 8, 1% Triton-X100. Endogenous AP was inactivated by heat treatment for 10 min at 65 °C and the lysates clarified by centrifugation. AP activity was measured in 1 M diethanolamine, 5 mM MgCl$_2$, 6.25 mM *p*-nitrophenyl phosphate. Bound AP activity was quantified in 96-well plates using a microplate reader and Micromanager software (BioRad). An additional replicate of each transfection was prepared for immunoblot analysis. Lysates were prepared separately for immunoblot analysis to allow inclusion of protease inhibitors (which were not used in the binding assay).

Glycosyltransferase assays

Fringe–Myc and Fringe-NNN–myc were cloned into baculovirus vector pVL1393

(Pharmingen) and expressed in High Five cells. Microsomal fractions were prepared by hypotonic lysis followed by ultracentrifugation. Membrane pellets were solubilized 1:2 (vol/vol) in 20 mM cacodylate pH 6.5, 1% Triton-CF54 and 5 mM $MnCl_2$ containing leupeptin and aprotinin. This suspension (5 µl) was added to a total of 50 µl reaction mixture containing 25 mM cacodylate pH 6.5, 0.25% Triton-CF54, 5 mM $MnCl_2$, 500 mM free sugar and 100 µM UDP-[^{14}C]sugar (1,280–2,000 c.p.m. nmol^{-1}). Reactions were incubated at 37 °C for 45–60 min, followed by Dowex-1 anion exchange chromatography and scintillation counting of the flow through[28]. For in vitro glycosylation of Notch–EGF3, we transfected SL2 cells and purified secreted His-tagged Notch–EGF3 from conditioned medium by Ni-NTA affinity chromatography. We carried out in vitro glycosylation as described for acceptor sugars using 0.25 µCi [^{14}C]GlcNAc per reaction. After incubation, the Ni-NTA beads were washed 4 times, and labelled Notch–EGF3 protein was eluted with 250 mM imidazole in SDS–PAGE sample buffer.

Immunoprecipitation and western blots

Cells were lysed in 50 mM Tris pH 7.5, 1% TritonX100, 120 mM NaCl and 30 mM NaF, containing protease inhibitors (see ref. 29). Antibodies for immunoprecipitation and western blots included mouse monoclonal anti-Myc (9E10), rabbit anti-Myc (Santa Cruz Biotechnology), mouse anti-CD2 (Serotec) and mouse anti-Notch 9C6. Mouse anti-Golgi (ref. 30). Protein bands were visualized with peroxidase conjugated secondary antibodies and enhanced chemiluminescense (Amersham).

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Mice overexpressing human uncoupling protein-3 in skeletal muscle are hyperphagic and lean

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Uncoupling protein-3 (UCP-3) is a recently identified member of the mitochondrial transporter superfamily[1,2] that is expressed predominantly in skeletal muscle[1,2]. However, its close relative UCP-1 is expressed exclusively in brown adipose tissue, a tissue whose main function is fat combustion and thermogenesis. Studies on the expression of UCP-3 in animals and humans in different physiological situations support a role for UCP-3 in energy balance and lipid metabolism[3,4]. However, direct evidence for these roles is lacking. Here we describe the creation of transgenic mice that overexpress human UCP-3 in skeletal muscle. These mice are hyperphagic but weigh less than their wild-type littermates. Magnetic resonance imaging shows a striking reduction in adipose tissue mass. The mice also exhibit lower fasting plasma glucose and insulin levels and an increased glucose clearance rate. This provides evidence that skeletal muscle UCP-3 has the potential to influence metabolic rate and glucose homeostasis in the whole animal.

The human α-skeletal actin promoter was used to drive tissue-

from the biochemical literature. As of December 1999, this database provides descriptions for 6 archaea, 32 bacteria and 5 eukaryotes. The downloaded data were manually rechecked, removing synonyms and substrates without defined chemical identity.

Construction of metabolic network matrices

Biochemical reactions described within a WIT database are composed of substrates and enzymes connected by directed links. For each reaction, educts and products were considered as nodes connected to the temporary educt–educt complexes and associated enzymes. Bidirectional reactions were considered separately. For a given organism with N substrates, E enzymes and R intermediate complexes the full stoichiometric interactions were compiled into an $(N+E+R) \times (N+E+R)$ matrix, generated separately for each of the 43 organisms.

Connectivity distribution $P(k)$

Substrates generated by a biochemical reaction are products, and are characterized by incoming links pointing to them. For each substrate we have determined k_{in}, and prepared a histogram for each organism, showing how many substrates have exactly $k_{in} = 0,1,\ldots$. Dividing each point of the histogram with the total number of substrates in the organism provided $P(k_{in})$, or the probability that a substrate has k_{in} incoming links. Substrates that participate as educts in a reaction have outgoing links. We have performed the analysis described above for k_{in}, determining the number of outgoing links (k_{out}) for each substrate. To reduce noise logarithmic binning was applied.

Biochemical pathway lengths [$\Pi(l)$]

For all pairs of substrates, the shortest biochemical pathway, $\Pi(l)$ (that is, the smallest number of reactions by which one can reach substrate B from substrate A) was determined using a burning algorithm. From $\Pi(l)$ we determined the diameter, $D = \Sigma_l l \cdot \Pi(l)/\Sigma_l \Pi(l)$, which represents the average path length between any two substrates.

Substrate ranking $\langle r \rangle_o$, $\sigma(r)$

Substrates present in all 43 organisms (a total of 51 substrates) were ranked on the basis of the number of links each had in each organisms, having considered incoming and outgoing links separately ($r = 1$ was assigned for the substrate with the largest number of connections, $r = 2$ for the second most connected one, and so on). This gave a well defined r value in each organism for each substrate. The average rank $\langle r \rangle_o$ for each substrate was determined by averaging r over the 43 organisms. We also determined the standard deviation, $\sigma(r) = \langle r^2 \rangle_o - \langle r \rangle_o^2$ for all 51 substrates present in all organisms.

Analysis of the effect of database errors

Of the 43 organisms whose metabolic network we have analysed, the genomes of 25 have been completely sequenced (5 archaea, 18 bacteria and 2 eukaryotes), whereas the remaining 18 are only partially sequenced. Therefore two main sources of possible errors in the database could affect our analysis: the erroneous annotation of enzymes and, consequently, biochemical reactions (the likely source of error for the organisms with completely sequenced genomes); and reactions and pathways missing from the database (for organisms with incompletely sequenced genomes, both sources of error are possible). We investigated the effect of database errors on the validity of our findings. The data, presented in Supplementary Information, indicate that our results are robust to these errors.

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errata

Determining multiple length scales in rocks

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On page 179 of this paper, the six occurrences of $\pi 2$ on lines 10 and 23 of the text should have been $\pi/2$. ☐

Glycosyltransferase activity of Fringe modulates Notch–Delta interactions

Katja Brücker, Lidia Perez, Henrik Clausen & Stephen Cohen

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In Fig. 1b, the fifth column of the Fng–myc row should have shown a plus sign instead of a minus sign. ☐

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