The α(1-3)-fucosyltransferases come of age

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Introduction

Since the publication of the first molecular cloning of a mammalian α(1-3)-fucosyltransferase (α(1-3)FucT; GDP-fucose–β-D-N-acetylglucosamine 3-α-L-fucosyltransferase) enzyme in 1990 [1], there has been a transformation in our understanding of the importance of these enzymes. The α(1-3)FucTs are critical for the synthesis of the carbohydrate ligands involved in intercellular binding between leucocytes and endothelial cells, via the selectin adhesion molecules. This implies their involvement in the correct trafficking of leucocytes to areas of inflammation and tissue damage, and for lymphocyte homing to the skin and lymph nodes. The minimal carbohydrate structure of these selectin ligands includes the Lewis antigen, sLeX \{Neu5Aca(2-3)Galp(1-4)[Fuca(1-3)]GlcNAc\}. The order of synthesis of this moiety involves the action of an α-(3-2)-sialyltransferase on the lactosamine structure Galp(1-4)GlcNAc or Galp(1-3)GlcNAc, followed by fucosylation of the GlcNAc residue. Importantly, fucosylation is the terminal step, as sialylation cannot occur after the addition of fucose. A family of five enzymes have now been cloned from man, along with examples from other species including mouse and chicken. Detailed characterization of the
catalytic properties of the enzymes has been performed, progress has been made on the identification of amino acid residues crucial for enzyme activity, and the tissue-specific and regulatable expression of the enzymes has been investigated. However, conclusive evidence of the importance of these enzymes in leucocyte recruitment has come from the recent studies by John Lowe's group using transgenic ‘knockout’ mice. In this work, the activity of the recently identified FucT-VII gene was ablated, leading to profound changes in the phenotype of these animals [2]. In this review, we will use examples from our own and published work to illustrate the progress that has been made on the understanding of the α(1-3)FucT enzymes.

The α(1-3)FucT gene family

Five human coding sequences, designated FucT-III to FucT-VII, have been cloned (III [1]; IV [3–5]; V [6]; VI [7,8]; VII [9,10]), along with homologues from other species including mouse (IV [11–13]; VII [13]) and chicken (IV [14]). All encode an enzyme capable of transferring the fucose moiety from GDP-β-L-fucose in an α(1-3) linkage to the GlcNAc of type-II lactosamine-containing glycoprotein or glycolipid acceptors. The FucT-III enzyme is also capable of catalysing a second class of linkage by generating an α(1-4) linkage to the GlcNAc of type-I lactosamine-containing glycoprotein or glycolipid acceptors. The FucT-III enzyme is also capable of catalysing a second class of linkage by generating an α(1-4) linkage to type-I lactosamine acceptors. Where it is known, each α(1-3)FucT is present as a single-copy gene, although silent and enzyme-inactivating mutations have been identified from different individuals within the FucT-III, FucT-V and FucT-VI genes [15,16]. The first cDNAs to be cloned revealed that the coding sequence of each gene was contained within a single exon. However, subsequent cloning of the FucT-VII sequence from human and mouse has demonstrated that the open reading frame for this enzyme is interrupted by an intron between the 4th and 5th amino acid residues [13,17]. The enzymes encoded by these genes are between 342 and 405 amino acids in length, representing proteins of between 44 and 47 kDa in size, although each enzyme may be post-translationally modified by N- or O-glycosylation. The enzymes have a putative topology common to all mammalian glycosyltransferases, with a short N-terminal intracytoplasmic tail, a hydrophobic membrane-spanning region of between 19 and 22 amino acids (34 residues in FucT-IV) and an intra-Golgi domain encompassing the C-terminal catalytic region. Residence of the enzymes in the Golgi apparatus has recently been confirmed using antisera raised to FucT-V, where the enzyme was shown to be colocalized with β(1-4)galactosyltransferase in transfected COS-7 cells [18].

Chromosomal localization has shown that the FucT-III, -V and -VI genes are syntenically linked on chromosome 19p13.3 (in the order V-III-VI) [7,19,20], FucT-IV is on 11q21 [20] and FucT-VII is telomeric on the long arm of chromosome 9 at q34.3 ([9]; Chee Gee See, personal communication). The coding regions of the genes reflect a high degree of nucleotide and amino acid homology, particularly within the C-terminal catalytic region. Alignment of the amino acid sequences of the α(1-3)FucTs reveals three distinct families within higher organisms (Figure 1).

Alternatively spliced transcripts have been identified for FucT-III and -VI from normal

Figure 1

Phylogram of the known α(1-3)FucT amino acid sequences revealing three distinct families within higher organisms (FucT-III/V/VI, -VII and -IV), and the more distantly related Caenorhabditis elegans (celegans) and H. pylori (hp) enzymes
tissues including kidney, colon (III/VI) and liver (IV), and alternative polyadenylation sites have been identified in the 3'-untranslated region of FucT-V [21]. This implies that there may be multiple levels of control of expression of the enzymes, be it tissue-specific transcription, post-transcriptional alternative splicing or the potential for post-translational modification, particularly as all the enzymes possess either two or four potential N-glycosylation sites.

Most surprising is the recent identification of α(1-3)FucT activities in prokaryotes, including Schistosoma mansoni [22] and Helicobacter pylori [23]. This finding suggests that these enzymes are not the exclusive domain of higher organisms. These organisms have been shown to bear Le^a (Galβ(1-4)[Fucα(1-3)]GlcNAc; S. mansoni and H. pylori) and Le^b (Fucα(1-2)Galβ(1-4)[Fucα(1-3)]GlcNAc; H. pylori only-type structures on their surface, similar to those found on higher organisms. With H. pylori, recent evidence suggests that these structures could be involved in molecular mimicry between the pathogen and the host, leading to an autoimmune mechanism for H. pylori-associated gastritis [24]. We have cloned an α(1-3)FucT sequence from H. pylori, which is a Gram-negative bacterium. A H. pylori genomic database, derived from the strain NCTC11637, was searched using a conserved FucT sequence from the catalytic domain of human FucT-VI, and a homologous H. pylori sequence was identified. The H. pylori enzyme can use simple di- and tri-saccharide acceptors in vitro with a strong preference for type-II structures, although its presumed normal acceptor is the complex O-specific polysaccharide chains of lipopolysaccharide. Even more intriguing is the observation that the enzyme has very limited overall homology with the other α(1-3)FucTs and does not bear the same general topology of the other enzymes (i.e. it has no transmembrane region and an extended C-terminal region), but a common set of amino acid residues has been found to be present in all α(1-3)FucTs cloned to date. This 'α(1-3)FucT motif' may be useful in the isolation of novel α(1-3)FucT enzymes from other species, including mice and humans (S. L. Martin, M. R. Edbrooke, T. C. Hodgman, D. H. van den Eijnden and M. I. Bird, unpublished work).

**Characterization of enzyme activity**

The mammalian α(1-3)FucT enzymes have been characterized principally by their ability to utilize simple acceptor saccharides (for examples, see [25]), to use both glycoprotein and glycolipid acceptors [26,27], as well as their ability to generate specific antigens on the surface of transfected cells [1,3-10]. These results are summarized in Table 1. Of particular note is the ability of the enzymes encoded by genes found on chromosome 19, i.e. III, V and VI, to use both neutral and sialylated acceptor substrates, whereas FucT-IV and VII can only utilize neutral or sialylated acceptors respectively. It is not yet clear if the enzymes have a preference for N- or O-linked glycoconjugates.

Domain-swapping experiments have shown that as few as 11 non-identical amino acids, found within the hypervariable region at the N-terminus of the C-terminal catalytic region of FucT-III, -V and -VI, may dictate whether the enzyme can utilize type-I acceptors to make the

<table>
<thead>
<tr>
<th>FucT classification</th>
<th>General</th>
<th>Acceptor specificity</th>
<th>Cell-surface antigens</th>
<th>N-Ethylmaleimide sensitivity</th>
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<tbody>
<tr>
<td>III</td>
<td>Lewis</td>
<td>/ / / / /</td>
<td>Le^a * Le^x / Le^s * / / / /</td>
<td>+</td>
</tr>
<tr>
<td>IV</td>
<td>Myeloid</td>
<td>/ / x /</td>
<td>Le^a  / Le^x / Le^s / / / / /</td>
<td>-</td>
</tr>
<tr>
<td>V</td>
<td></td>
<td>/ / / / /</td>
<td>Le^a * Le^s / Le^x / / / / / /</td>
<td>+</td>
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<td>VI</td>
<td>Plasma</td>
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<td>VII</td>
<td>Leucocyte</td>
<td>x / / / /</td>
<td>Le^x / Le^s / Le^a / / / / / / / /</td>
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**Table 1: Properties of the cloned human α(1-3)FucT enzymes**

The properties include acceptor specificity for di- and tri-saccharides and production of cell-surface antigens after transfection into COS or CHO cells. NAL, Galβ(1-4)GlcNAc; SLN, Neu5Acα(2-3)Galβ(1-4)GlcNAc; 2'FL, Fucα(1-2)Galβ(1-4)Glc; LNB, Galβ(1-3)GlcNAc; VIM-2, Neu5Acα(2-3)Galβ(1-4)GlcNAcβ(1-3)Galβ(1-4)[Fucα(1-3)]GlcNAc.
Lewis a antigen \((\text{Le}^a = \text{Gal}\beta(1-3)[\text{Fucx}(1-4)]\text{GlcNAc})\) [28,29]. Holmes and colleagues [30] showed that Ser-178 was important in donor substrate binding for the FucT-IV enzyme, and their evidence strongly suggested that analogous cysteine residues are the GDP-fucose-protectable, N-ethylmaleimide-sensitive sites present in FucT-III and -V (see Table 1). Our own analyses have revealed that, for FucT-VI, an N-ethylmaleimide-reactive cysteine is in or adjacent to the substrate-binding site of the enzyme, and that FucT-VI possesses histidine residues that are also essential for enzyme activity [31].

A limited number of \(\alpha(1-3)\)FucT enzyme inhibitors have been synthesized [32]. The most potent to date is guanosine 5'-diphospho-2-deoxy-2-fluoro-\(\alpha\)-l-fucose (GDP-2F-Fuc), which has a reported inhibition constant of 4.2 \(\mu\)M against the FucT-V enzyme [33].

Soluble forms of the \(\alpha(1-3)\)FucT enzymes have been identified in a number of body fluids including serum [34] and milk [35,36]. Thus recombinant expression could be used to generate soluble truncated forms of the enzymes in order to allow detailed structural and enzymic studies. FucT-III has been expressed in such a way in COS-1 cells [1], and FucT-III, -IV, and -V in COS-7 cells [26]. In order to isolate large quantities of enzyme, we have successfully generated a soluble, truncated and purifiable form of the FucT-VII enzyme that bears all the properties of the full-length enzyme. The coding sequence was truncated immediately after the N-terminal hydrophobic region, and attached to an IgG-binding domain via a cleavable factor Xa site. This was expressed in baculovirus-infected Sf9 and/or Tni cells, and recombinant enzyme was purified by binding to IgG-Sepharose (N. Smithers, D. Brodie, V. A. M. Kelly and C. J. Britten, unpublished work; [37]). Expression was optimized to 2 mg/l, and purification generated an increase in specific activity of approx. 80-fold. The \(K_m\) values for the enzyme were within the expected ranges for both the substrate (8 \(\mu\)M for GDP-fucose) and the acceptor (1.6 mM for 3'-sialyl-lactosamine). This work will now be extended to the other family members.

Physiological functions of the enzymes

There has been a generalized classification of the FucT enzymes as they were isolated and characterized (see Table 1). However, it is essential to establish the exact role of the enzymes and their catalytic products in physiological processes. \(\text{Le}^a\) was first identified as a stage-specific embryonic antigen [38], and has been implicated in embryonic development. The presence of Lewis antigens and FucT enzymes has been widely documented in transformed and metastatic cells, most notably colon carcinoma cells that bear \(\text{aLe}^a\) structures [39]. The human FucT-III enzyme has been used in the generation of transgenic mice capable of expressing the Lewis b epitope \((\text{Le}^b = \text{Fucx}(1-2)\text{Gal}\beta(1-3)[\text{Fucx}(1-4)]\text{GlcNAc})\) in the pit cells of the stomach, with the resultant binding of the human pathogen, \(H.\) pylori [40], suggesting a role for fucosylated sugars in bacterial adhesion (Table 2).

Natural mutations have also been identified in certain members of the \(\alpha(1-3)\)FucT family. Some 9% of Indonesians carry an inactivating lesion in the FucT-VI plasma enzyme [15], and up to 10% of Caucasians and Japanese carry an inactivating mutation in the FucT-III gene. Neither of these mutations display any noticeable phenotype [16,41]. The most well-documented

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<tr>
<th>(\alpha(1-3))FucT enzyme</th>
<th>Target or phenotype</th>
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<tr>
<td>Human III</td>
<td>(\text{Le}^a) synthesis enables binding of (H.) pylori to murine gastric mucosa</td>
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<tr>
<td>Human IV/VII</td>
<td>ESL-1 activation</td>
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<tr>
<td>Human III/IV/VII</td>
<td>PSGL-1 activation</td>
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<tr>
<td>Human VII</td>
<td>E-selectin ligand synthesis on T- and erythroleukaemia cells</td>
</tr>
<tr>
<td>Murine VII</td>
<td>E-, P- and L-selectin ligand synthesis, involvement in leucocyte trafficking</td>
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example of an inability to metabolize fucose is in patients with leucocyte adhesion deficiency type II; two unrelated children suffer from Rambam–Hasharon syndrome and are severely handicapped with mental retardation, developmental problems and severe recurrent infections. This apparent defect in the ability to synthesize GDP-fucose leads to the Bombay blood phenotype [which is due to the absence of the α(1-2)-linked fucosylated red-cell H antigen], as well as an absence of the E-selectin ligand, sLeα [42].

The best-characterized physiological role for the α(1-3)FucTs is in the construction of ligands for E-, P- and L-selectin, already shown to play a crucial role in the trafficking of leucocytes and lymphocytes [43]. A 150 kDa glycoprotein ligand for E-selectin was recently identified on mouse neutrophils, and termed ESL-1 [44]. Zollner and Vestweber [45] have subsequently shown that this ligand is selectively activated in the presence of the FucT-IV and -VII enzymes, known to be expressed in myeloid cells. Further to this, it has been shown in human T-lymphoblasts that FucT-VII controls the synthesis of E-selectin ligands, and treatment of Jurkat cells with PMA induces FucT-VII mRNA and enzyme activity [46]. Also, transfection of the FucT-VII cDNA into K562 erythroleukaemia cells renders the cells E-selectin-binding positive [47].

Similarly, fucosylation appears to be important in the activation of the P-selectin glycoprotein ligand, PSGL-1, where up-regulation of α(1-3)FucT activity is linked to up-regulation of PSGL-1 function on activated T-cells [48]. Analysis of the post-translational modifications of PSGL-1 revealed that sulphation, fucosylation and sialylation are all important for binding [49], and that the α(1-3)FucT enzymes, including III, IV and VII, can be involved. This work also demonstrated the ability of these enzymes to be involved in the construction of O-linked glycans, as the modifications on PSGL-1 could only be achieved in the presence of the branching enzyme for O-linked glycans, core 2 β1-6-N-acetylgalcosaminyltransferase.

However, the most convincing evidence for the role of α(1-3)FucTs in cellular processes has come from the use of in vitro antisense studies and the generation of transgenic 'knock-out' mice. By transfection of an antisense cDNA construct into a cultured human adult T-cell leukaemia cell line, Hiraiai and colleagues [50] showed that selective inhibition of FucT-VII expression suppressed sLeα synthesis and blocked E-selectin-mediated cell adhesion. Transgenic-gene-ablation studies with the murine FucT-IV gene have resulted in limited phenotypic changes except for increased rolling speeds of peripheral blood leucocytes on endothelial cells isolated from cremaster muscle (John B. Lowe, personal communication). However, when mice were generated that were deficient in the FucT-VII gene, significant phenotypic changes were seen. The mice were healthy and bred normally (unlike the fucose-metabolism compromised patients with leucocyte adhesion deficiency syndrome type II), but were completely lacking in leucocyte E- and P-selectin ligands, and were deficient in high endothelial venule L-selectin ligands [2]. The mice were noted for their blood leucocytosis, impaired leucocyte extravasation during an inflammatory response, and faulty lymphocyte homing. This is the clearest evidence yet for the importance of α(1-3)FucTs in physiological processes, and raises the possibility that other specific pathophysiological processes can now be investigated. This work corroborates our own in vitro analyses of the FucT-VII enzyme, where it appears to preferentially fucosylate the terminal N-acetylgalcosamine residue of polylactosamine chains, with another enzyme, perhaps FucT-IV, acting on the internal sites (C. J. Britten, D. H. van den Eijnden, W. McDowell, V. A. M. Kelly, S. E. Hutchinson, M. R. Edbrooke, M. I. Bird, T. de Vries and N. Smithers, unpublished work). This suggests that the α(1-3)FucT enzymes are able to work in concert, as polyfucosylated structures (e.g. sialyl-disLeα [51] and sialyl-trisLeα [52]) have been shown to be potent ligands for the selectins.

The future for the α(1-3)FucT enzymes

More than ten molecular clones for the α(1-3)FucT enzymes have now been reported, including sequences from the domestic cow, Bos taurus [53], and from the nematode worm, C. elegans [54]. Are there other enzymes yet to be isolated? It has been suggested that the brain contains a distinct α(1-3)FucT activity that is similar to the myeloid enzyme, i.e. FucT-IV, yet is able to use Co2+ as an activator, unlike the other enzymes, which utilize Mn2+ [55]. Only the human homologues of FucT-IV, -VII and a -III-like pseudogene have been cloned from mice [11,12]. If fucosylated glycoconjugate structures, such as Leα, are important for implantation and development of these animals, it may be that
other murine sequences have yet to be found. Similarly, molecular clones have yet to be obtained for other organisms in which fucose metabolism has been identified, e.g. S. mansoni and Lymnea stagnalis [56]. Why is there a family of FucTs and is there a distinct role for each one? Inhibition of gene expression using antisense and transgenic-gene-ABLATION technology, coupled to enzyme-specific blocking antibody and/or low-molecular-mass inhibitor studies, should provide the complete story for these enzymes.

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