The lipopolysaccharide of certain strains of *Helicobacter pylori* was recently shown to contain the Lewis X (Le^a^) trisaccharide (Galβ1,4(Fuco)(1,3)-GlcNAc). Le^a^ is an oncofetal antigen which appears on human gastric epithelium, and its mimicry by carbohydrate structures on the surface of *H. pylori* may play an important part in the interaction of this pathogen with its host. Potential roles for bacterial Le^a^ in mucosal adhesion, immune evasion, and autoantibody induction have been proposed (Moran, A. P., Prendergast, M. M., and Appelmelk, B. J. (1996) *FEBS Immunol. Med. Microbial.* 16, 105–115). In mammals, the final step of Le^a^ biosynthesis is the α(1,3)-fucosylation of GlcNAc in a terminal Galβ(1→4)-GlcNAc unit, and a corresponding GDP-fucose:α(1,3)-fucosyltransferase (α(1,3)-Fuc-T) activity was recently discovered in *H. pylori* extracts. We used part of a human α(1,3)-Fuc-T amino acid sequence to search an *H. pylori* genomic data base for related sequences. Using a probe based upon weakly matching data base sequences, we retrieved clones from a plasmid library of *H. pylori* DNA. DNA sequence analysis of the library clones revealed a gene which we have named *fucT*, encoding a protein with localized homology to the human α(1,3)-Fuc-Ts. We have demonstrated that *fucT* encodes an active Fuc-T enzyme by expressing the gene in *Escherichia coli*. The recombinant enzyme shows a strong preference for type 2 (e.g. LacNAc) over type 1 (e.g. lacto-N-biose) acceptors in vitro. Certain residues in a short segment of the *H. pylori* protein are completely conserved throughout the α(1,3)-Fuc-T family, defining an α(1,3)-Fuc-T motif which may be of use in identifying new fucosyltransferase genes.

The Gram-negative bacterium *Helicobacter pylori* is a major cause of chronic gastritis and peptic and duodenal ulcers (1–5). It has also been implicated in gastric adenocarcinoma (6–9) and gastric lymphoma (10), leading to its classification as a type I human carcinogen (11). *H. pylori* is a chronic pathogen, and the means by which this organism is able to persist in the stomach and resist or evade destruction by the immune system is central to its involvement in disease. Some aspects of the host-pathogen interaction have been resolved, including the involvement of the Lewis b (Le^b^) epitope on epithelial cells in attachment of *H. pylori* (12), and characterization of a bacterial cytotoxin responsible for gastric epithelial damage (for a review see Ref. 13), but clearly much remains to be discovered.

Recent structural analysis of *H. pylori* lipopolysaccharides revealed that the O antigen contains fucosylated carbohydrate structures identical to the mammalian Lewis X (Le^a^) and Lewis Y (Le^b^) epitopes (14–17). It was further established that the bacterium contains endogenous galactosyltransferase (Gal-T) and fucosyltransferase (Fuc-T) activities necessary for biosynthesis of these structures (18) suggesting that they are synthesized *de novo* by *H. pylori* rather than scavenged from the surface of mammalian cells. Le^a^ is an oncofetal antigen (19, 20) also expressed on adult human gastric mucosa (21), and its presence on *H. pylori* lipopolysaccharides may play a role in survival and pathogenesis. *H. pylori* infection is known to induce antibodies that cross-react with human gastric mucosa (22). In a recent report, Appelmelk et al. (23) demonstrated that the targets of this autoimmune response include Le^a^ and/or Le^b^ epitopes and provided evidence that anti-Le^a/b^ antibodies may be involved in *H. pylori*-associated gastritis. Interestingly, molecular mimicry of Le^a^ is also thought to be responsible for autoantibody production by *Schistosoma mansoni* (24, 25). In addition, surface carbohydrate antigens containing Le^a^ structures may play a part in the immunopathology of *H. pylori* infection by promoting Th-1 to Th-2 switching as has been reported in schistosomal infections (26). Two recent reports (27, 28) that over 85% of *H. pylori* isolates from geographically widespread locations express Le^a^ and/or Le^b^ antigens would also seem to imply selective pressure for maintenance of these structures, given the considerable structural variability often shown by lipopolysaccharides from Gram-negative bacteria.

In mammals, the defining step of Le^a^ biosynthesis is fucosylation of a type 2 core structure (Galβ1→4GlcNAc). This reaction is catalyzed in humans by one or more members of a family of α(1,3)-fucosyltransferases which employ GDP-fucose as an activated sugar donor (29–38). Fuc-T and Ga-L-T activities have been detected in *H. pylori* extracts (18), but although the order of sugar transfer appears to follow the same course as in mammalian systems, with galactosylation preceding fucosylation, little is known about the bacterial Fuc-T and how it is related to the mammalian transferases. If, as evidence is be-
Helicobacter pylori Fucosyltransferase

Gaining to suggest, cell-surface Le\(^\text{a}\) epitopes play an important role in \(H. pylori\) persistence and pathogenesis (23, 39), the \(\alpha(1,3)-\)Fuc-T may offer a nonbacterial therapeutic target for eradication of \(H. pylori\) without otherwise disturbing the balance of gut fauna.

Five members of the human \(\alpha(1,3)-\)Fuc-T gene family have been cloned (Fuc-TIII–VII) (29–38). Homologs of some of these genes have also been cloned from mouse (40–42), rat (43), and cow (44) cDNA. The remarkable degree of sequence conservation between mammalian \(\alpha(1,3)-\)Fuc-Ts and the recently cloned chicken \(\alpha(1,3)-\)fucosyltransferase (CPT1) (45) suggests that other nonmammalian \(\alpha(1,3)-\)Fuc-Ts may also show significant homology to the known members of this enzyme family. We describe here the identification and cloning of a gene from \(H. pylori\), \(fuc\)T, which encodes an active Fuc-T with localized sequence similarity to the \(\alpha(1,3)-\)Fuc-Ts.

**EXPERIMENTAL PROCEDURES**

**Materials—** GDP-fucose and N-acetyllactosamine were from Sigma. AG 1-X8 mixed bed resin was from Bio-Rad. GDP-[\(^3\text{H}\)]fucose (2.22 TBq/nmol) was obtained from Amersham. Oligosaccharides were obtained from Dextra and Oxford Glycosystems. RPMI 1640 medium (Gibco) was from Welgenalin. Xanthomonas manihotis nucleohistine (1,3)-fucosyltransferase (CFT1) (45) suggests that other nonmammalian \(\alpha(1,3)-\)Fuc-Ts may also show significant homology to the known members of this enzyme family. We describe here the identification and cloning of a gene from \(H. pylori\), \(fuc\)T, which encodes an active Fuc-T with localized sequence similarity to the \(\alpha(1,3)-\)Fuc-Ts.

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Identification of a Fucosyltransferase Gene in H. pylori

**RESULTS**

Identification of a Fucosyltransferase Gene in H. pylori NCTC 11637—Human α(1,3)-fucosyltransferases (Fuc-TII-VII) show a high degree of sequence similarity at the amino acid level. To identify *H. pylori* sequences with homology to the human fucosyltransferase enzymes, we performed a TBLASTN search of a GlaxoWellcome *H. pylori* genomic database set with part of the catalytic domain (residues 152–303) of human Fuc-TVI, a strongly conserved region among the human α(1,3)-Fuc-T family. A number of *H. pylori* sequence fragments showed weak similarity to the query (maximum BLAST score 0.0025), with matches localized to a short region in each case (17 identities in 30 amino acids). Codon usage plots indicated that this reading frame was likely to be protein coding (data not shown). Closer examination of the sequence alignments revealed that several of the matching residues from the *H. pylori* sequence are conserved across all five human α(1,3)-Fuc-Ts, suggesting that the data base sequence fragments may be part of a related *H. pylori* gene. Since both α(1,3)-Fuc-T and β(1,4)-Gal-T Leα forming activities have been reported in *H. pylori* (18), we carried out a similar search with part of the catalytic domain of human β(1,4-Gal-T), but found no matching sequences.

Using primers derived from one of the matching data base sequences, we amplified a short (approximately 400 bp) DNA fragment from *H. pylori* NCTC 11637 genomic DNA which was subsequently labeled with digoxigenin and used to identify hybridizing clones in a plasmid library of DNA from the same organism. Seven strongly hybridizing clones were retrieved from the library for DNA sequence analysis, which revealed considerable overlap between the cloned sequences in all seven plasmids. DNA sequencing of all seven clones in both strands yielded a total of approximately 2.7 kb of contiguous sequence (Fig. 1A). The probe sequence occurs within the only complete open reading frame in the sequence (designated fucT), spanning 1002 bp and coding for a predicted 333-amino acid polypeptide with localized sequence homology to the human α(1,3)-Fuc-Ts. The open reading frame occurs approximately 500 bp upstream of the fucT gene, running in the same direction. The predicted translation of this part of the *H. pylori* sequence shows homology to phosphoserine phosphatase (serB) genes from Gram-negative bacteria, with greatest similarity to the *Haemophilus influenzae* sequence (37% identity, 65 matching residues over 173 amino acids).

Primary Structure of *H. pylori* fucT—The nucleotide sequence and predicted translation of *H. pylori* fucT are shown in Fig. 1. The GC content of the gene (36%) is typical for *H. pylori* coding sequences (3). The predicted amino acid sequence contains no recognizable signal peptide or transmembrane domain, a Kyte-Doolittle plot revealing that hydrophobic regions of the sequence are small and infrequent (Fig. 1B). A repetitive element occupies 49 amino acids of the C-terminal part of the protein. The repeat unit is imperfect, but leucine appears consistently at 7-amino acid intervals in a pattern reminiscent of the eukaryotic leucine zipper motif.

The similarity between the *fucT* gene product and other α(1,3)-Fuc-T is weak outside the short region originally identified by the data base search, spanning residues 101 to 129 of the *H. pylori* protein. Within this part of the sequence, however, 10 residues are completely conserved in all five human α(1,3)-Fuc-Ts and also appear unchanged in bovine, murine, and avian α(1,3)-Fuc-T enzymes (Fig. 2). In addition, there are a number of partially conserved positions (occupied by one of two amino acids). Outside this region, similarity to other members of the α(1,3)-Fuc-T family diminishes very quickly, although a number of isolated conserved residues can be identified. No significant similarity to any enzyme class other than the α(1,3)-Fuc-T family could be found for the *H. pylori* sequence.

Given the reported occurrence of Leα structures on the *H. pylori* O antigen and detection by others (18) and ourselves (3) of corresponding α(1,3)-Fuc-T activity in cell extracts from this bacterium, we took the exclusive, albeit localized, similarity between the deduced amino acid sequence of *H. pylori* fucT and α(1,3)-Fuc-T enzymes as an indication that it may encode an *H. pylori* fucosyltransferase enzyme.

Fucosyltransferase Activity—We assayed cell lysates from the clones retrieved from the *H. pylori* plasmid library for α(1,3)-Fuc-T activity using N-acetyllactosamine (Galβ1→4GlcNAc) as an acceptor. All seven of the library clones showed measurable Fuc-T activity, while neither control clones containing pUC18 nor untransformed *E. coli* possessed any activity (Table I), demonstrating that cloned *H. pylori* sequences contained in the library plasmids encode an active Fuc-T.

Cloning *H. pylori* fucT into an *E. coli* Expression Vector—The *H. pylori* library plasmids contain stretches of flanking sequence on either side of fucT. To exclude the possibility that coding sequences outside the identified fucT gene were responsible for the observed Fuc-T activity, and in an effort to increase levels of recombinant fucosyltransferase activity, we subcloned *H. pylori* fucT into the *E. coli* expression vector pET-11a. The resulting plasmid, pHPFT, contains fucT as the sole *H. pylori*-derived coding sequence under control of the T7lac promoter. *E. coli* BL21(DE3) containing pHPFT produced Fuc-T activity when induced with isopropyl-1-thiogalactopyranoside, extracts typically showing a specific activity of 100–200 pmol/min/mg with N-acetyllactosamine as acceptor. Some activity could also be detected in uninduced samples (10–20% of induced levels), presumably as a result of "leaky" promoter repression. Maximal activity levels produced from pHPFT were not, as we had hoped, substantially higher than those in the library clones, nor was a highly expressed protein of the expected molecular mass (approximately 40 kDa) apparent from SDS-polyacrylamide gel electrophoresis analysis of uncleared cell extracts (data not shown). The limited Fuc-T activity produced by pHPFT thus appears to result from limited expression rather than accumulation of highly expressed but insoluble and inactive recombinant protein.

**Acceptor Specificity of H. pylori Fuc-T—**We measured the activity of recombinant *H. pylori* FucT with a panel of oligosaccharide acceptors, as shown in Table II. The enzyme strongly preferred type 2 (Galβ1→4GlcNAc) structures over type 1 (Galβ1→3GlcNAc) acceptors. The type 2 tetrasaccharide (9) was a better acceptor than LacNAc (4) suggesting that *H. pylori* FucT may prefer to fucosylate β-configured GlcNAc. Similar preferences have been reported for human Fuc-IV and to a lesser extent for Fuc-TV with these two acceptors (51). With sialylated LacNAc acceptors the *H. pylori* FucT most closely resembled human Fuc-Ts V and VII in that 3′-sialyl-LacNAc (6) was a substrate, while 6′-sialyl-LacNAc (7) was not (51, 52).

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2 TBLASTN compares a peptide query sequence with the translation in all six frames of a nucleotide data base.

3 V. A. Kelly, C. J. Britten, and S. L. Martin, unpublished observation.
No activity was observed with the type 1 disaccharide lacto-N-biose (2), although lacto-N-tetraose (LNT) (10) was an efficient acceptor. Fucosylation of the terminal galactose of LNT in the 2-position (11) or GlcNAc in the 4-position (12) significantly reduced incorporation rates, while fucosylation on both GlcNAc and glucose (15) abolished fucose incorporation altogether. This suggests that *H. pylori* FucT may be capable of fucosylating predominantly the glucose residue of LNT-based acceptors, as has been demonstrated for Fuc-TV (51).

6'-Sialylation of GlcNAc also blocked fucosylation of type 2 structures with the recombinant *H. pylori* enzyme (18) whereas 3'-sialylation of the terminal galactose residue (17) caused only a minor reduction in relative activity. Unlike Fuc-TV (but like Fuc-TVI and Fuc-TVII), however, the *H. pylori* FucT showed no activity toward 2'-fucosyllactose (5), implying that in the synthesis of Ley by *H. pylori* a 2-fucosylation of Gal may occur after a 3-fucosylation of GlcNAc. Taken together, these results suggest that the *Helicobacter* enzyme has little a(1,2)- or a(1,4)-Fuc-T activity, but efficiently a(1,3)-fucosylates neutral and a(2,3)-sialylated type 2 acceptors. To further define the catalytic properties of *H. pylori* Fuc-T, Km values were determined for LacNAc (0.5 mM) and GDP-fucose (9 μM). Sensitivity to the
inhibitor NEM was measured by performing assays in the presence of NEM at concentrations up to 15 mM. The enzyme showed very limited NEM sensitivity, with Fuc-T activity reduced by only 34% in the presence of 15 mM NEM. For comparison, FucT-III (expressed in COS cells) is inhibited approximately 85% at the same NEM concentration, while extracts from *Schistosoma mansoni* and COS cells expressing Fuc-TIV retain about 50% of their Fuc-T activity (53).

Analysis of Fucosylated Products Generated by H. pylori Fuc-T—Some of the inferences drawn from the acceptor preferences of *H. pylori* Fuc-T were investigated further by examining the sensitivity of fucosylated products to glycosidase treatment. Radiolabeled oligosaccharide products were generated by incubating the acceptors LacNAc and LNT with *H. pylori* Fuc-T in the presence of GDP-[3H]fucose. Following removal of excess sugar nucleotide, free fucose and residual Triton X-100 from the reaction mixture by successive ion exchange, size exclusion, and reverse phase chromatographic steps, oligosaccharide products were purified by HPLC on a Neutropak NH2 column.

Incubation of LacNAc with *H. pylori* Fuc-T yielded a single radiolabeled product with a retention time on HPLC corresponding to that of Lex (Fig. 3A). The product was unaffected by treatment with a selective *a*(1,2)-fucosidase, while treatment with a *a*(1,3/4)-fucosidase resulted in complete conversion to a new product which eluted from the Neutropak NH2 column more rapidly than LacNAc. These observations support the conclusion that the major fucosylated product generated by *H. pylori* Fuc-T with LacNAc as acceptor is the Lex trisaccharide. Accordingly, the product is resistant to *a*(1,2)-fucosidase, but sensitive to *a*(1,3/4)-fucosidase, which liberates fucose as the sole radiolabeled product.

To investigate the fucosylation of the type 1 acceptor LNT (10) by *H. pylori* Fuc-T, labeled product was treated with endo-*β*-galactosidase. The action of this glycosidase is inhibited by fucosylation of residues flanking the *β*-galactoside linkage (54, 55) and thus if, as substrate preferences seem to suggest, *H. pylori* Fuc-T fucosylates the glucose residue of LNT, the product should be resistant to endo-*β*-galactosidase cleavage. Since endo-*β*-galactosidase activity may also be hampered by fucosylation at more distant sites (for example, on GlcNAc or the distal Gal residue of LNT) (55), the product was also treated with bovine testis *β*-galactosidase, alone or in combination with *β*-N-acetylhexosaminidase, to examine this possibility. Analysis of the labeled oligosaccharides produced by glycosidase digestion of *H. pylori* Fuc-T-generated fucosyl-LNT is shown in Fig. 3B. As expected, endo-*β*-galactosidase had no effect on the

**Fig. 2. A**, sequence alignment of *H. pylori* Fuc-T with other Fuc-Ts. Aligned sequences are: human Fuc-TIII, -IV, and -VII (FT-III, -IV, -VII, respectively), murine Fuc-TVII (Mu-FT-VII), chick Fuc-T (CFTI), a cosmid-derived C. elegans sequence (C. elegans), and a translation of the *H. pylori* fuc gene (*H. pylori*). Sequences are ordered according to similarity. Transmembrane domains are shaded. The position of Cys412 in *H. pylori* Fuc-T, implicated in inhibition by NEM, is arrowed. A repetitive element in the *H. pylori* sequence is underlined and in bold. Completely conserved residues are indicated below the alignment. A region of strong, localized homology is boxed. As shown here, the motif represents a minimal consensus and those positions which may be occupied by more than two different amino acids, or by two unrelated residues, are indicated as unconserved positions, designated X. The asterisked position is occupied by Ile or Val in all *a*(1,3)-Fuc-Ts except in the recently cloned rat gene, where it is occupied by Asn.
fucosylated oligosaccharide, while LNT itself was readily cleaved under similar conditions (data not shown). Digestion with bovine testis exo-β-galactosidase resulted in substantial (>50%) conversion to a product with a retention time close to that of LNT. This indicates that the distal Gal residue of the major product is not fucosylated, as this would otherwise block exo-β-galactosidase action. Combined treatment with β-galactosidase and N-acetyl-β-hexosaminidase yielded a more rapidly eluted product, consistent with removal of distal Gal and GlcNAc residues to leave a fucose-containing trisaccharide. Further β-galactosidase action is presumably blocked by fucose branching at the Glc residue of the remaining galactoside. These findings suggest that *H. pylori* Fuc-T fucosylates LNT predominantly at the glucose residue. Resistance of part of the product material to bovine testis β-galactosidase may reflect some degree of (1,2)- or (1,4)-fucosylation, although the substrate preferences of this Fuc-T indicate that incomplete galactosidase digestion is perhaps a more likely explanation.

**DISCUSSION**

By searching an *H. pylori* genomic data set with part of the catalytic domain sequence of a human α(1,3)-Fuc-T and sequencing corresponding clones from a plasmid library of *H. pylori* DNA we were able to identify a gene (*fucT*) with highly localized similarity to known α(1,3)-Fuc-T enzymes. Cell extracts from *E. coli* XL-1 Blue containing library plasmids were assayed for fucosyltransferase activity with LacNAc (5 mM) as a substrate.

| Clone   | Specific activity (pmol/mg/min) | Relative activity |
|---------|--------------------------------|------------------|
| 16H14   | 96                             | 100              |
| 16E24   | 58                             | 100              |
| 5F21    | 2.5                            | 100              |
| 15M19   | 78                             | —                |
| 16G9    | 99                             | —                |
| 4F3     | 77                             | —                |
| 1C10    | 48                             | —                |
| pUC18   | 0                              | —                |
| No plasmid | 0                      | —                |

These findings suggest that *H. pylori* Fuc-T is the first Fuc-T gene to be cloned from an invertebrate, although enzyme activity has been detected in the freshwater snail *Lymnea stagnalis* (56) and in the parasite *S. mansoni* (53).

Sequence similarity between the mammalian Fuc-Ts and chick fucosyltransferase (CFT1) provided evidence for evolutionary conservation of α(1,3)-Fuc-T sequences (45). Conservation between *H. pylori* Fuc-T and the mammalian enzymes, although limited and highly localized, suggests that aspects of the α(1,3)-Fuc-T sequence have survived unchanged through evolution from bacteria to higher mammals and man. The lack of overall sequence similarity to human α(1,3)-Fuc-Ts would seem to preclude the idea that *H. pylori* acquired the Fuc-T gene from a mammalian source. The base composition of the gene (35% GC) is also much closer to the average for *H. pylori* (36%) than to mammalian and avian α(1,3)-Fuc-Ts, which are typically GC-rich (e.g., CFT-1, 69% GC).

Unlike eukaryotic Fuc-Ts which have a hydrophobic transmembrane domain near their N terminus and share a common type II membrane protein topology, the *H. pylori* enzyme con-
H. pylori Fucosyltransferase

C. Britten and M. I. Bird, manuscript in preparation.

tains no recognizable membrane insertion elements. Aligned on the basis of the short, highly conserved region of homology (Fig. 2), the bacterial enzyme appears to lack a region corresponding to the transmembrane and stem domains of other Fuc-Ts. Most of the "hypervariable region" previously implicated in acceptor binding specificity in human Fuc-TIII and -V (residues 34 to 161 in Fuc-TIII) (57) is also absent, suggesting that the architecture of the H. pylori protein is substantially different from the rest of the enzyme family. The alignment also reveals that the C terminus of the bacterial sequence extends for approximately 100 amino acids beyond that of the other Fuc-Ts, half of this C-terminal extension being taken up by a periodic 7-amino acid leucine zipper-like motif. The function of this region, which has no counterpart in mammalian or avian Fuc-T sequences, is unknown. One possibility is that it mediates homo- or heteromultimer formation through coiled-coil type interactions, but at present the subunit structure of the H. pylori protein is unknown and further work will be necessary to establish the role of the zipper-like region.

Recombinant H. pylori Fuc-T has a strong preference for type 2 acceptors, and analysis of oligosaccharides generated by fucosidase digestion of the product generated by this Fuc-T with LacNAc indicates that H. pylori Fuc-T is indeed capable of synthesizing the Leα epitope. Some type 1 structures are also fucosylated, but our studies suggest that with these acceptors fucose may be transferred predominantly to glucose rather than GlcNAc, implying that the enzyme has little similarity to mammalian Fuc-TIV. This is not the case with the bacterial enzymes or indeed that of H. pylori Fuc-T (51). Biosynthesis of the Leα epitope was reported for the enzyme and human Fuc-T (51). Biosynthesis of the Leα epitope found on the surface of many H. pylori isolates is therefore likely to involve a separate α(1,2)-Fuc-T activity, as has been reported for human Fuc-TIV (51). Biosynthesis of the Leα epitope identified by enzyme and human Fuc-Ts IV and VII, however, H. pylori Fuc-T shows only slight sensitivity to NEM inhibition. Interestingly, 3′-sialyl-LacNAc is an efficient acceptor (although 6′-sialyl-LacNAc is not), implying that H. pylori Fuc-T may be capable of synthesizing the sialyl-Leα (sLeα) structure which was recently detected in a small number of H. pylori isolates by Wirth et al. (27). The absence of sLeα from the majority of H. pylori isolates may therefore reflect a lack of sialylationtransferase activity in these strains.

Mammalian α(1,3)-Fuc-Ts are a closely-related family of enzymes, making it difficult to identify residues of potential structural and/or catalytic importance from sequence alignments. The recently cloned avian α(1,3)-Fuc-T, CFT-1 (45), also shows a high level of sequence similarity to the corresponding mammalian proteins, with 46.3% sequence identity to human Fuc-TIV. This is not the case with the H. pylori enzyme, which shows significant homology to the other α(1,3)-Fuc-Ts only in one short region. A consensus motif derived from this local area of homology (Fig. 2B) is unique to members of the α(1,3)-Fuc-T family, including the H. pylori enzyme and an open reading frame from a Caenorhabditis elegans cosmid (58). This highly conserved α(1,3)-Fuc-T motif may be useful in identifying novel α(1,3)-Fuc-T genes in genomic and expressed sequence tag sequence data, since its appearance seems to be a reliable predictor of membership of this enzyme family. It may also provide a tool for cloning α(1,3)-Fuc-Ts in a manner similar to the demonstrated utility of the L- and S-sialyl motifs in cloning novel sialyltransferases (59).

The functional significance of the α(1,3)-Fuc-T motif is at present unclear. Marked differences in acceptor preferences among members of the α(1,3)-Fuc-T family would seem to arise against a role in acceptor binding. Human Fuc-TIV and VII for example both contain the α(1,3)-Fuc-T motif, but while Fuc-TIVII uses 2,3-sialylated acceptors almost exclusively, Fuc-TVstrongly prefers neutral type 2 substrates (41) in vitro assays. The behavior of Fuc-TIV in vivo is apparently more complex (60). The α(1,3)-Fuc-T motif lies outside sequence regions implicated by efforts to define acceptor-discriminating residues in α(1,3)-Fuc-Ts (51, 57, 61). Given that the enzymes transfer fucose from a common sugar nucleotide donor, it seems more likely that the α(1,3)-Fuc-T motif is involved in binding GDP-fucose or Mn2+. The motif lies some considerable distance from a cysteine residue implicated in GDP-fucose protectible inhibition by NEM (62), although it may be much closer in space within the folded protein than the primary sequence suggests. The corresponding position in the H. pylori Fuc-T is occupied by tyrosine (Fig. 2A), in keeping with observations that enzymes with Cys at this location are inhibited by NEM while those with other amino acids (Fuc-TIV has Ser, Fuc-TVII has Thr) are resistant to NEM inhibition (62). Interestingly, the conserved motif contains a lysine residue (Fig. 2A), possibly a candidate for the so far unidentified GDP-fucose-protected lysine residue identified by pyridoxal phosphate labeling of a human fucosyltransferase (63). Further work is clearly needed to test these speculations, but in this respect the lack of overall similarity between the H. pylori and mammalian transferase sequences may be advantageous. The relatively small number of conserved residues inside and outside the α(1,3)-Fuc-T motif may provide a useful focus for mutagenesis experiments to probe structural and mechanistic aspects of the α(1,3)-fucosyltransferases. The dissimilarity of H. pylori and human Fuc-T enzymes would also seem to auger well for the design of selective inhibitors of the bacterial enzyme.

The H. pylori enzyme, which lacks a transmembrane domain and is, presumably, nonglycosylated, is devoid of some of the features which make eukaryotic Fuc-Ts difficult to work with. The possibility of bacterial expression also makes this enzyme a promising candidate for chemoenzymatic glycoconjugate synthesis. The same features may simplify the task of structural determination. Given the conserved motif, it seems reasonable to assume that this enzyme shares at least some structural features in common with its mammalian counterparts which have so far resisted structural elucidation.

Mounting evidence appears to point to a role for Lewis antigen mimicry in H. pylori pathogenesis. Identification and cloning of a Fuc-T gene from this organism will allow us to probe the biosynthesis of Leα by H. pylori in vivo via disruption of fucT and may make it possible to test the role of Leα directly in models of H. pylori pathogenesis.

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