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Lewis X Biosynthesis in *Helicobacter pylori*

MOLECULAR CLONING OF AN α(1,3)-FUCOSYLTRANSFERASE GENE*

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Stephen L. Martin‡§, Mark R. Edbrooke‡, T. Charles Hodgman¶, Dirk H. van den Eijnden‖, and Michael I. Bird‡

From the ‡Glycobiology Unit and the ¶Advanced Technologies and Informatics Unit, GlaxoWellcome Medicines Research Centre, Stevenage, Herts SG1 2NY, United Kingdom and the ‖Department of Medical Chemistry, Vrije Universiteit, Amsterdam, The Netherlands

The lipopolysaccharide of certain strains of *Helicobacter pylori* was recently shown to contain the Lewis X (Le^x) trisaccharide (Galβ-1,4-(Fucα(1,3))-GlcNAc). Le^x 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^x in mucosal adhesion, immune evasion, and autoantibody induction have been proposed (Moran, A. P., Prendergast, M. M., and Appelmelk, B. J. (1996) *FEMS Immunol. Med. Microbiol.* 16, 105–115). In mammals, the final step of Le^x biosynthesis is the α(1,3)-fucosylation of GlcNAc in a terminal Galβ(1→4)-GlcNAc unit, and a corresponding GDP-fucose:*N*-acetylglucosaminyl 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 *fuc*T, encoding a protein with localized homology to the human α(1,3)-Fuc-Ts. We have demonstrated that *fuc*T 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)[1] 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^x) and Lewis Y (Le^y) 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^x 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^x and/or Le^y epitopes and provided evidence that anti-$Le^{x/y}$ antibodies may be involved in *H. pylori*-associated gastritis. Interestingly, molecular mimicry of Le^x is also thought to be responsible for autoantibody production by *Schistosoma mansoni* (24, 25). In addition, surface carbohydrate antigens containing Le^x 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^x and/or Le^y 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^x 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 Gal-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-

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AF006039.

§ To whom correspondence should be addressed: Glycobiology Unit, GlaxoWellcome Medicines Research Centre, Gunnels Wood Rd., Stevenage, Herts SG1 2NY, UK. Tel.: 44-1438-764085; Fax: 44-1438-764810; E-mail: slm17885@ggr.co.uk.

[1] The abbreviations used are: Le^b, Lewis b; Le^x, Lewis X; Le^y, Lewis Y; α(1,3)-Fuc-T, α(1,3)-fucosyltransferase, GDP-fucose:β-D-*N*-acetylglucosaminide 3-α-fucosyltransferase; Gal-T, galactosyltransferase; bp, base pair(s); kb, kilobase(s); PCR, polymerase chain reaction; LacNAc, *N*-acetyllactosamine, Galβ1→4GlcNAc; LNT, lacto-*N*-tetraose, Galβ1→4GlcNAcβ1→3Galβ1→4Glc; lacto-*N*-biose, Galβ1→3GlcNAc; HPLC, high performance liquid chromatography; NEM, *N*-ethylmaleimide; BSA, bovine serum albumin.

ginning to suggest, cell-surface $Le^{x/y}$ epitopes play an important role in *H. pylori* persistence and pathogenesis (23, 39), the α(1,3)-Fuc-T may offer a nonbactericidal therapeutic target for eradication of *H. pylori* without otherwise disturbing the balance of gut fauna.

Five members of the human α(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 α(1,3)-Fuc-Ts and the recently cloned chicken α(1,3)-fucosyltransferase (CFT1) (45) suggests that other nonmammalian α(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 α(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}H]fucose (2.22 TBq/mmol) was obtained from Amersham. Oligosaccharides were obtained from Dextra and Oxford Glycosystems. *Xanthomonas manihotis* α(1,2)- and α(1,3/4)-fucosidases and *Streptomyces plicatus N*-acetyl-β-hexosaminidase were from New England Biolabs. Bovine testis β-galactosidase was from Böehringer Mannheim. *Bacteroides fragilis* endo-β-galactosidase was supplied by Oxford Glycosystems. Neutropak NH_2 HPLC columns (5 μm, 150 × 4.6 mm) were from Capital Analytical. *H. pylori* NCTC 11637 was obtained from the National Collection of Type Cultures (NCTC). Buffer and media components were obtained from Sigma, Difco, and Life Technologies, Inc. All chemicals were of the highest available purity. Chemiluminescent detection film was from Kodak.

Hybridization to the H. pylori Plasmid Library—A 363-bp sequence fragment from the *H. pylori* genomic data base containing the Fuc-T homology region was amplified from *H. pylori* NCTC 11637 genomic DNA by PCR using the primers HPFT1 (5′-CTT TGA AAA GAG GGT TTG CCA) and HPFT2 (5′-CAA GTA TCT CAC GTA ATC AAT). Amplified product was purified using the QiaQuick PCR purification system (Qiagen) following the manufacturer's instructions. Approximately 0.5 μg of the purified fragment was used to prepare DIG-labeled probe using the DIG Hi-prime labeling kit (Boehringer Mannheim). Probe was used without further purification.

Nylon membranes carrying plasmid DNA from an *H. pylori* plasmid library in high density gridded format were kindly supplied by Dr C. L. Clayton (Genomics Unit, GlaxoWellcome). Membranes were prehybridized for 4 h at 42 °C in 20 ml of 50% formamide (v/v), 1% SDS (w/v), 7.5% (w/v) dextran sulfate, 1 M sodium chloride, 1.5 × Denhardt's solution (46), 1.7 mM sodium pyrophosphate, 37.5 mM Tris·HCl, pH 7.5, containing 0.1 mg/ml denatured salmon sperm DNA. Probe was denatured by boiling for 5 min and added to the prehybridization buffer; hybridizations were carried out overnight at 42 °C. Membranes were washed twice for 30 min in 2 × SSC, 0.1% (w/v) SDS, room temperature; 1 × SSC, 0.1% (w/v) SDS, 45 °C; 0.1 × SSC, 0.1% (w/v) SDS, 68 °C. Detection was performed using the Boehringer Mannheim chemiluminescent DIG detection system with CSPD luminescent substrate. Membranes were exposed to film for 5–120 min at room temperature.

Library Clone Retrieval and Sequence Analysis—Clones which hybridized strongly to the probe were retrieved from 384-well library storage microtiter plates (stored at −80 °C) and grown overnight in 2 ml of L broth containing 100 μg/ml ampicillin. DNA was prepared by the rapid alkaline lysis method (46). Larger quantities of plasmid DNA were obtained from 1–200-ml cultures using the Qiagen plasmid (maxi) system. DNA sequencing reactions performed using AmpliTaq FS with dye terminators (Perkin-Elmer) and run on an Applied Biosystems ABI 373 automated sequencer. Sequence analysis was performed using GCG 8.1 (47) and BLAST (48) software. Sequence alignments were created with Pileup (part of the GCG package) with a gap penalty of 5.0 and gap extension penalty of 0.5.

Subcloning of fucT into an E. coli Expression Vector—A 1.4-kb DNA fragment containing the *fuc*T gene was amplified from *H. pylori* NCTC11637 genomic DNA by PCR using the primers HPFT3 (5′-GAG TGT CTA ATG GGA TCC TTA TTT TTT AAC CCA CCT) and HPFT5 (5′-TAG CCC TAA TCA AGC CTT TG). PCR product was purified using the QiaQuick PCR purification system (Qiagen), ligated to the A/T cloning vector pCR™II (Invitrogen) and introduced into *Escherichia coli* XL-1 Blue (Stratagene) by electroporation. Recombinant (white) clones were mapped with *Bss*HII to identify plasmids containing the cloned fragment in the desired orientation (direction of transcription of *fuc*T in the same direction as *lac*Z). A 1.4-kb fragment containing the *fuc*T gene was excised from a suitable clone with *Bam*HI and ligated to pET-11a vector DNA (Novagen) which had been linearized with *Bam*HI and dephosphorylated using shrimp alkaline phosphatase (Amersham, Little Chalfont, UK). Ligated DNA was introduced into *E. coli* BL21(DE3), and transformants were selected on L agar containing ampicillin (100 μg/ml). Recombinant clones were identified by restriction mapping with *Bss*HII.

Preparation of Cell Extracts—Recombinant clones were grown overnight at 37 °C from single colony inocula in 5 ml of L broth containing 100 μg/ml ampicillin. 20 ml of fresh L broth was inoculated with 200 μl of the overnight culture and incubated at 37 °C. *E. coli* XL-1 Blue containing library plasmids were grown for 5–8 h prior to harvesting. *E. coli* BL21(DE3) containing the expression plasmid pHPFT was incubated until an A_{600} of 0.4–0.6 was attained. Isopropyl-1-thio-β-D-galactopyranoside was added to a final concentration of 0.5 mM, and incubation continued for an additional 3 h. Bacteria were harvested by centrifugation (4000 × *g*, 15 min, 4 °C) and washed in phosphate-buffered saline. Pelleted bacteria were resuspended in 0.5 ml of chilled solubilization buffer (0.1% (w/v) Triton X-100, 0.1 M NaCl, 25% (w/v) glycerol, 0.1 M NaCl, 2 mM dithiothreitol, 50 mM Tris-HCl, pH 7.0) and sonicated on ice for 4 × 15 s bursts (MSE Soniprep 150), with a 60-s cooling period on ice between bursts. Sonicate was cleared by centrifugation (20,000 × *g*, 30 min, 4 °C) and stored at −80 °C.

Fucosyltransferase Assays—Fucosyltransferase activity was measured by a modification (49) of the method of Prieels *et al.* (50). Briefly, 12.5 μl of cell extract was incubated with 20 μM GDP-fucose, 100,000 cpm of GDP-[^{3}H]fucose, 5 mM acceptor, 5 mM $MnCl_2$, 1 mM ATP, buffered to pH 7.2 with 50 mM HEPES-NaOH in a total volume of 50 μl for 1 h at 37 °C. Sensitivity of the *H. pylori* enzyme to the inhibitor *N*-ethylmaleimide (NEM) was assessed by including NEM at final concentrations of up to 15 mM in Fuc-T assay reactions. Reactions were stopped by addition of 1 ml of mixed bed resin slurry (AG 1-X8 (Cl^- form) 1:4 (w/v) in water), vortexed briefly, and centrifuged for 5 min at 20,000 × *g* at room temperature. Radioactivity in 600 μl of supernatant was measured by scintillation counting. Allowance was made for nonspecific breakdown of GDP-fucose and fucose transfer to endogenous acceptors by performing control reactions in the absence of acceptor. Assay reactions were performed in duplicate. K_m for the acceptor *N*-acetyllactosamine (LacNAc) was determined by measuring reaction rates with 0–100 mM LacNAc and 200 μM GDP-fucose, while the donor K_m was obtained using 0–100 μM GDP-fucose and 5 mM LacNAc.

Purification of Fucosylated Oligosaccharides Generated using H. pylori Fuc-T—0.5 ml of *H. pylori* Fuc-T (approximately 0.2 milliunit) was incubated with 5 mM acceptor (LacNAc or LNT), 3 mM GDP-fucose, 800,000 cpm of GDP-[^{3}H] fucose, 0.1% (w/v) BSA, 2 μl (2 units) of shrimp alkaline phosphatase, 5 mM $MnCl_2$, buffered to pH 7.2 with 50 mM HEPES-NaOH in 1 ml total volume for 40 h at 37 °C. Incubation mixtures were passed through a 2.5-ml Dowex AG 1-X8 ion exchange column, washed through with 7.5 ml of water. Column eluant and washings were pooled, evaporated to dryness, and redissolved in 0.5 ml of water. Samples from the Dowex column were applied to a Bio-Gel P2 gel filtration column (20 × 1 cm) eluted with water at 20 ml/h. 1-ml fractions were collected, and 5-μl aliquots were removed for liquid scintillation counting. Radioactive fractions were pooled and lyophilized, keeping discrete eluant peaks separate. To remove residual Triton X-100, products were dissolved in 500 μl of water and applied to disposable 100-mg Amprep C18 reverse phase columns (Amersham), washed through with 3 ml of water. Pooled eluant and washings were lyophilized and redissolved in 100 μl of water. Finally, fucosylated products were purified by HPLC on a Neutropak NH_2 column in water/acetonitrile (25:75 by volume for products derived from LacNAc, 30:70 by volume for those produced from LNT). 0.5-ml fractions were collected, and radioactive fractions were pooled, lyophilized, and redissolved in 100 μl of water.

Glycosidase Treatment of Fucosylated Oligosaccharides—20 μl of the purified LacNAc-derived product was incubated overnight at 37 °C with 3 units (units as defined by the supplier) of α(1,3/4)- or α(1,2)-fucosidase from *X. manihotis* in 30 μl of 50 mM sodium citrate, pH 6.0, containing 100 μg/ml BSA. 20 μl of the LNT-derived product was incubated overnight at 37 °C with 6.25 milliunits of *B. fragilis* endo-β-galactosidase in 50 μl of 50 mM sodium acetate, pH 5.7, containing 250 μg/ml BSA; 10 milliunits of bovine testis β-galactosidase in 50 μl of 50 mM sodium citrate, pH 4.5; or 10 milliunits of bovine testis β-galactosidase and 10 milliunits of *S. plicatus N*-acetyl-β-hexosaminidase

in 50 μl of 50 mM sodium citrate, pH 4.5.

Glycosidase reaction products were analyzed by HPLC on the Neutropak NH_2 column in water/acetonitrile (25:75 by volume for products derived from LacNAc, 30:70 by volume for those generated from LNT). Elution profiles were generated by collecting 0.5-ml fractions for scintillation counting. Retention times for unlabeled oligosaccharide standards (LacNAc and for products derived from LacNAc using *H. pylori* Fuc-T, LNT, LacNAc, and Le^x for those generated using LNT as acceptor) under corresponding chromatographic conditions were determined by monitoring absorbance at 205 nm.

RESULTS

Identification of a Fucosyltransferase Gene in H. pylori NCTC 11637—Human α(1,3)-fucosyltransferases (Fuc-TIII–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[2] search of a GlaxoWellcome *H. pylori* genomic data 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 Le^x 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. 1*A*). The probe sequence occurs within the only complete open reading frame in the sequence (designated *fuc*T), spanning 1002 bp and coding for a predicted 333-amino acid polypeptide with localized sequence homology to the human α(1,3)-Fuc-Ts. A partial open reading frame occurs approximately 500 bp upstream of the *fuc*T gene, running in the same direction. The predicted translation of this part of the *H. pylori* sequence shows homology to phosphoserine phosphatase (*ser*B) 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 fuc*T 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. 1*B*). 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 *fuc*T 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 Le^x 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 fuc*T 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 tested 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 *fuc*T. To exclude the possibility that coding sequences outside the identified *fuc*T gene were responsible for the observed Fuc-T activity, and in an effort to increase levels of recombinant fucosyltransferase activity, we subcloned *H. pylori fuc*T into the *E. coli* expression vector pET-11a. The resulting plasmid, pHPFT, contains *fuc*T as the sole *H. pylori*-derived coding sequence under control of the T7*lac* promoter. *E. coli* BL21(DE3) containing pHPFT produced Fuc-T activity when induced with isopropyl-1-thio-β-D-galactopyranoside, 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 (**1**) suggesting that *H. pylori* FucT may prefer to fucosylate β-configured GlcNAc. Similar preferences have been reported for human Fuc-TIV 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).

[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.

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GATCACTTTG AAAGCTATGA ATGGCGAGAC AGATTTTCAT AAAAGTCTTA TTTTAAGGGT TTCCAAACTC AAAAACATGC    80
CCTTAAAACT AGCCAAAGAA GTTTGTGAAA GTCTGCCTTT ATTTGAGGGG GCGTTTGAAC TCATTAGCAC CTTAAAAGAG   160
AAAAATTACA AGGTGGTTTG CTTCAGCGGA GGCTTTGATC TAGCGACCAA TCATTACAGG GATTTATTGC ATTTAGATGC   240
GGCTTTCAGT AACACGCTGG TAGTGGAAAA TAACGCCTTA AACGGCTTGG TTACGGGGCA TATGATGTTT TCACACTCTA   320
AAGGCGAAAT GCTACTCGCT TTACAACGCT TATTAAATAT CAGTAAAACG AACACTTTAG TCGTGGGCGA TGGGGCGAAT   400
GACTTGAGCA TGTTCAAACA TGCCCATATT AAAATCGCTT TCAACGCTAA AGAGGTTTTA AAACAGCACG CTACGCATTG   480
CATCAATGAG CCTGATTTAG CCCTAATCAA GCCTTTGATT TAAAAAAATT TTTTTGTAAA ATACTCCTTT TAAAGGATAA   560
CCATGTTCCA ACCCCTATTA GACGCCTTTA TAGAAAGCGC TTCCATTGAA AAAATGGCCT CTAAATCTCC CCCCCCCTAA   640
AAATCGCTGT GGCGAATTGG TGGGGAGATG AAGAAATTAA AGAATTTAAA AAGAGCACTC TGTATTTCAT TTTAAGTCAG   720
CATTACACAA TCACTTTACA CCGAAACCCT GATAAACCTG CGGACATCGT TTTTGGTAAC CCCCTTGGAT CAGCCAGAAA   800
AATCTTATCC TATCAAAACA CTAAACGAAT ATTTTACACC GGTGAAAACG AATCGCCTAA TTTCAACCTC TTTGATTACG   880
CCATAGGCTT TGATGAATTA GACTTTAGAG ATCGTTATTT GAGA                                          924

ATG CCT TTA TAT TAT GAT AGG CTA CAC CAT AAA GCC GAG AGC GTG AAT GAC ACC ACC GCA CCC TAC    990
M   P   L   Y   Y   D   R   L   H   H   K   A   E   S   V   N   D   T   T   A   P   Y     22

AAG ATT AAA GGC AAC AGC CTT TAT ACT TTA AAA AAA CCC TCC CAT TGT TTT AAA GAA AAC CAC CCT   1056
K   I   K   G   N   S   L   Y   T   L   K   K   P   S   H   C   F   K   E   N   H   P     44

AAT TTG TGC GCG CTC ATC AAT AAT GAG AGC GAT CCT TTG AAA AGA GGG TTT GCC AGT TTT GTA GCG   1122
N   L   C   A   L   I   N   N   E   S   D   P   L   K   R   G   F   A   S   F   V   A     66

AGC AAC GCT AAC GCT CCT ATG AGG AAC GCT TTC TAT GAC GCT TTA AAT TCT ATT GAG CCA GTT ACT   1189
S   N   A   N   A   P   M   R   N   A   F   Y   D   A   L   N   S   I   E   P   V   T     88

GGG GGA GGA GCC GTG AAA AAC ACT TTA GGC TAT AAG GTT GGA AAC AAA AGC GAG TTT TTA AGC CAA   1254
G   G   G   A   V   K   N   T   L   G   Y   K   V   G   N   K   S   E   F   L   S   Q     110

TAC AAA TTC AAC CTG TGT TTT GAA AAC TCA CAA GGC TAT GGC TAT GTA ACC GAA AAA ATC ATT GAC   1320
Y   K   F   N   L   C   F   E   N   S   Q   G   Y   G   Y   V   T   E   K   I   I   D     132

GCT TAC TTT AGC CAT ACT ATT CCC ATT TAT TGG GGG AGT CCC AGC GTG GCG AAA GAT TTT AAC CCT   1386
A   Y   F   S   H   T   I   P   I   Y   W   G   S   P   S   V   A   K   D   F   N   P     154

AAG AGT TTT GTG AAT GTC CAT GAT TTC AAC AAC TTT GAT GAA GCG ATT GAT TAC GTG AGA TAC TTG   1452
K   S   F   V   N   V   H   D   F   N   N   F   D   E   A   I   D   Y   V   R   Y   L     176

CAC ACG CAC CCA AAC GCT TAT TTA GAC ATG CTC TAT GAA AAC CCT TTA AAC ACC CTT GAT GGG AAA   1518
H   T   H   P   N   A   Y   L   D   M   L   Y   E   N   P   L   N   T   L   D   G   K     198

GCT TAC TTT TAC CAA AAT TTG AGT TTT AAA AAA ATC CTA GAT TTT TTT AAA ACG ATT TTA GAA AAC   1584
A   Y   F   Y   Q   N   L   S   F   K   K   I   L   D   F   F   K   T   I   L   E   N     220

GAC ACG ATT TAT CAT AAT AAC CCT TTC ATT TTC TAT CGT GAT TTG AAT GAG CCG TTA GTA TCC ATT   1650
D   T   I   Y   H   N   N   P   F   I   F   Y   R   D   L   N   E   P   L   V   S   I     242

GAT AAT TTG AGA ATC AAT TAT GAT AAT TTG AGG GTT AAT TAT GAT GAT TTG AGG GTT AAT TAT GAT   1716
D   N   L   R   I   N   Y   D   N   L   R   V   N   Y   D   D   L   R   V   N   Y   D     264

GAT TTG AGG GTT AAT TAT GAT GAT TTG AGA ATC AAT TAT GAT GAT TTG AGA ATC AAT TAT GAT GAT   1782
D   L   R   V   N   Y   D   D   L   R   I   N   Y   D   D   L   R   I   N   Y   D   D     286

TTG AGA ATT AAT TAT GAG CGC CTT TTG CAA AAC GCT TCA CCT TTA TTG GAA TTG TCC CAA AAC ACC   1848
L   R   I   N   Y   E   R   L   L   Q   N   A   S   P   L   L   E   L   S   Q   N   T     308

TCT TTT AAA ATC TAT CGC AAA ATT TAT CAA AAA TCC TTA CCC TTA TTG CGT GTA ATA AGG AGG TGG   1914
S   F   K   I   Y   R   K   I   Y   Q   K   S   L   P   L   L   R   V   I   R   R   W     330

GTT AAA AAA TAA                                                                          1926
V   K   K   *                                                                            334

GGCGTTTTTT AAGACTGATT AAGAAACTGA ACGCTATTTT AAAATGCGCT AACGCTTCTT TTTTTGAGCG TGGGGTTTTT   2006
GAGCATGTCC TCTAAAGCAT GGGTGCTTAA AAAATGTTTT GTTTTTAAAG ACACGATGCG CCCAAAGGAC TCTTCTTTAA   2086
AAAGGTTTAA AAGGCGTTTG GGCAAAATCT CGCCAAATAC CACAATGACT TTTGAAGCGC TGTTGTCTAA TTGCCAGGTT   2166
AAATGAAATA GGCATGCGTT GATTTCTTCT TCTAAATTAA GGCTGTTAGA GTCGCATTTA AGGAGCGATA AAATACTGCA   2246
ATCTTTTAAG GGGTAGTTAA AAACCTTTTG GATAATGCTC TCTAACATGG CTGCTTTTAA ATTGTTTAAG AAATTTAATT   2326
GGCTATCCAG CATAGGGGTG AGCGTGATGA AAGCGAGCTT GGAAGTGGGG TTAAAAAGCC CAATCACCGG TTTTGAATGT   2406
TGATGGCGTT TGCACAGATT GCAATTTTCT ATGCCCTCAT GAACTTGTTC TGTTAAGGGG CTTATTTTGA GGCTTGGTAA   2486
GGCTAGTATT GGTGTAAGTT TCGCCCAAAA GACGCTCCAT ATAAAGAGAG CGTAAAGTTT GAAGGCGTGA GTGTTGCAAA   2566
GCGTTCAGTC TTTATGCTTT TTGAAAGCGT GAGGGCTTAG CATGTTTTCT GGCTTGAAAA TATCGTCCAG TTGCTCTTTA   2646
GTTAAGATTT TCTTTTCTAA AGCGATGTCA TAGATAGAAC GATCGCTTT                                     2696
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B

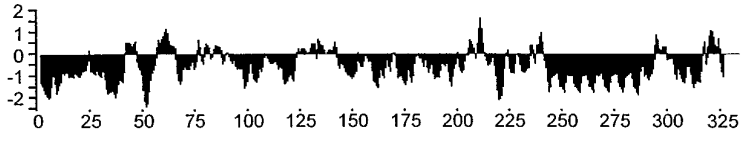

FIG. 1. *A*, nucleotide sequence and predicted translation of *H. pylori fuc*T. An upstream partial open reading frame with homology to *H. influenzae* and *E. coli ser*B genes is *underlined*. *B*, Kyte-Doolittle hydrophobicity plot of *H. pylori fuc*T. Hydrophobic domains are plotted above the axis, hydrophilic domains below.

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 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* Fuc-T showed no activity toward 2′-fucosyllactose (**5**), implying that in the synthesis of Le^y by *H. pylori* α2-fucosylation of Gal may occur *after* α3-fucosylation of GlcNAc. Taken together, these results suggest that the *Helicobacter* enzyme has little α(1,2)- or α(1,4)-Fuc-T activity, but efficiently α(1,3)-fucosylates neutral and α(2,3)-sialylated type 2 acceptors. To further define the catalytic properties of *H. pylori* Fuc-T, K_m values were determined for LacNAc (0.5 mM) and GDP-fucose (9 μM). Sensitivity to the

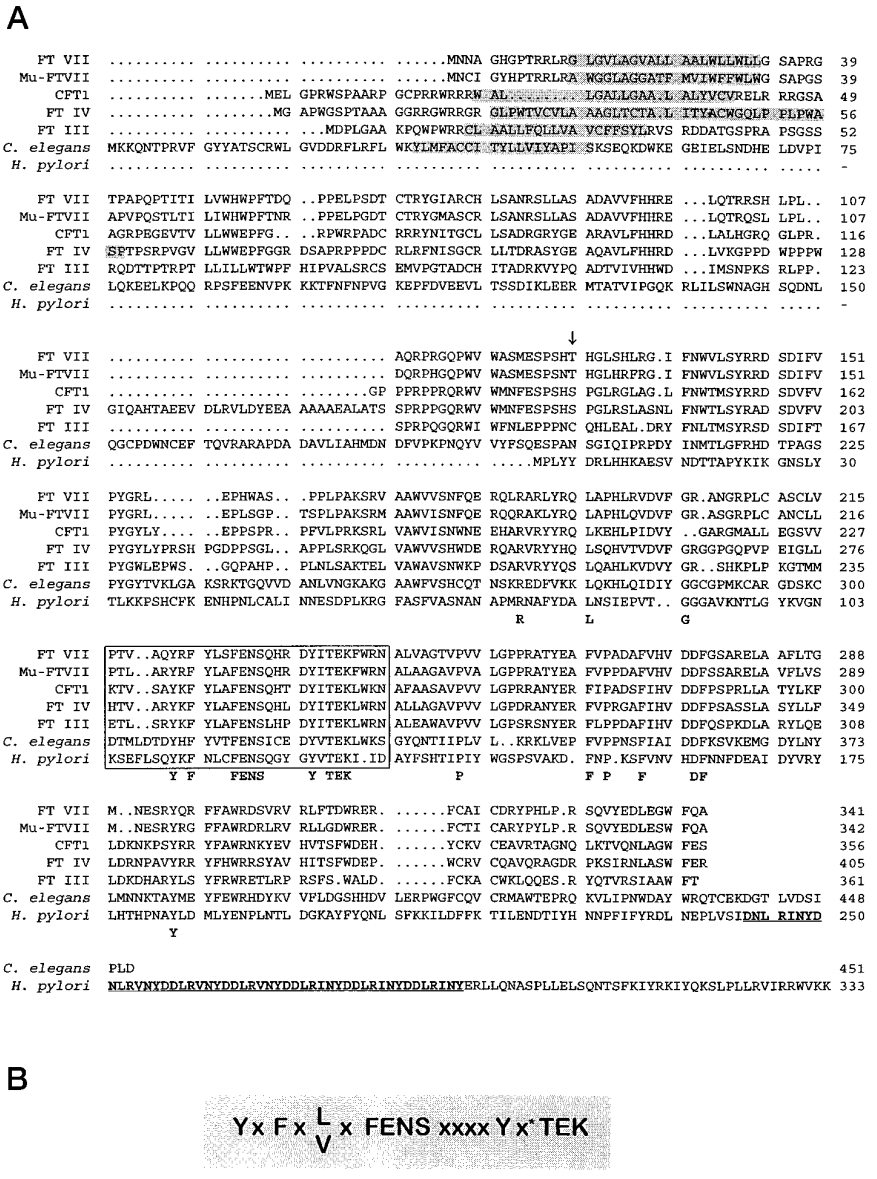

FIG. 2. *A*, sequence alignment of *H. pylori* Fuc-T with other Fuc-Ts. Aligned sequences are: human Fuc-TIII, -IV, and -VII (*FT-III*, *-IV*, *-VII*, respectively), murine Fuc-TVII (*Mu-FTVII*), chick Fuc-T (*CFT1*), a cosmid-derived *C. elegans* sequence (*C. elegans*),[4] and a translation of the *H. pylori fuc*T gene *(H. pylori*). Sequences are ordered according to similarity. Transmembrane domains are *shaded.* The position of Cys^{143} in Fuc-TIII, 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*. *B*, α(1,3)-Fuc-T motif. All known α(1,3)-Fuc-Ts contain this short peptide motif. Fully conserved residues are indicated in *one-letter code*, *two letters arranged vertically* indicate that either of the corresponding amino acids may occupy this position. 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 α(1,3)-Fuc-Ts except in the recently cloned rat gene, where it is occupied by Asn.

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-[^{3}H]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 NH_2 column.

Incubation of LacNAc with *H. pylori* Fuc-T yielded a single radiolabeled product with a retention time on HPLC corresponding to that of Le^x (Fig. 3*A*). The product was unaffected by treatment with a selective α(1,2)-fucosidase, while treatment with α(1,3/4)-fucosidase resulted in complete conversion to a new product which eluted from the Neutropak NH_2 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 Le^x trisaccharide. Accordingly, the product is resistant to α(1,2)-fucosidase, but sensitive to α(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. 3*B*. As expected, endo-β-galactosidase had no effect on the

TABLE I
Fucosyltransferase activity of H. pylori fucT-containing plasmid library clones

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
16I14	96
16E24	58
5F21	2.5
15M19	78
16G9	99
4F3	77
1C10	48
pUC18	0
No plasmid	0

TABLE II
Acceptor specificity of recombinant H. pylori FucT expressed in E. coli

Relative Fuc-T activity with a panel of oligosaccharide acceptors (5 mM) expressed as a percentage of activity with 5 mM LacNAc as substrate (211 pmol/min/mg). A dash indicates a relative activity of < 2.5%.

Acceptor	Relative activity
	%
Galβ1→4GlcNAc (**1**)	100
Galβ1→3GlcNAc (**2**)	—
Galβ1→4(Fucα1–3)GlcNAc (**3**)	—
Fucα1→3Galβ1–4Glc (**4**)	—
Fucα1→2Galβ1→4Glc (**5**)	—
NeuAcα2→3Galβ1→4GlcNAc (**6**)	44
NeuAcα2→6Galβ1→4GlcNAc (**7**)	—
Manβ1→4GlcNAc (**8**)	20
Galβ1→4GlcNAcβ1→3Galβ1→4Glc (**9**)	171
Galβ1→3GlcNAcβ1→3Galβ1→4Glc (**10**)	57
Fucα1→2Galβ1→3GlcNAcβ1→3Galβ1→4Glc (**11**)	20
Galβ1→3(Fucα1→4)GlcNAcβ1→3Galβ1→4Glc (**12**)	18
Galβ1→4(Fucα1→3)GlcNAcβ1→3Galβ1→4Glc (**13**)	63
Fucα1→2Galβ1→3(Fucα1→4)GlcNAcβ1→3Galβ1→4Glc (**14**)	17
Galβ1→3(Fucα1→4)GlcNAcβ1→3Galβ1→4(Fucα1→3)Glc (**15**)	—
Galβ1→4GlcNAcβ1→3Galβ1→4(Fucα1→3)GlcNAc (**16**)	65
NeuAcα2→3Galβ1→3GlcNAcβ1→3Galβ1→4Glc (**17**)	39
Galβ1→3(NeuAcα2→6)GlcNAcβ1→3Galβ1→4Glc (**18**)	—
NeuAcα2→6Galβ1→4GlcNAcβ1→3Galβ1→4Glc (**19**)	25

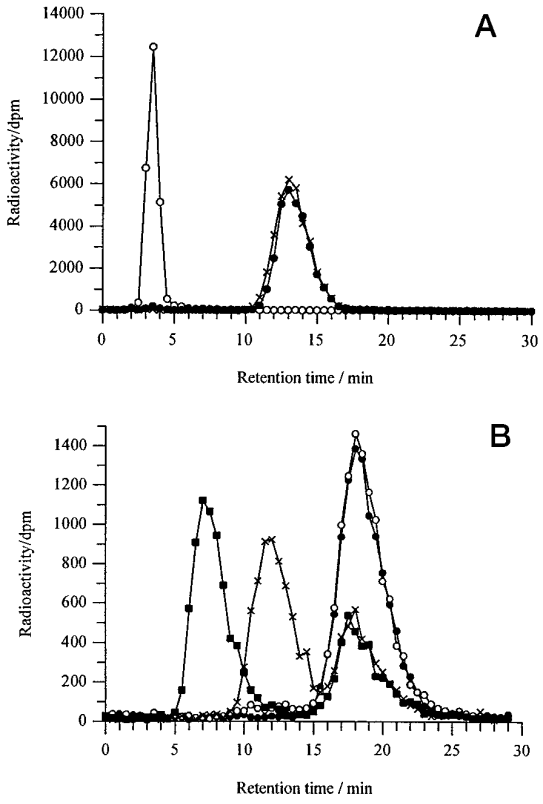

FIG. 3. *A*, glycosidase/HPLC analysis of fucosylated LacNAc generated by *H. pylori* Fuc-T. Purified labeled product was analyzed by chromatography on a Neutropak NH_2 column under isocratic conditions using water/acetonitrile (25:75 by volume) following no treatment (●), digestion with *Xanthomonas manihotis* α(1,3)-fucosidase (○), or digestion with *X. manihotis* α(1,2)-fucosidase (×). LacNAc and Le^x standards eluted at 7.8 and 13.2 min, respectively, under these conditions. *B*, glycosidase analysis of *H. pylori* Fuc-T-generated product with LNT as acceptor. Purified labeled product was analyzed by chromatography on the Neutropak NH_2 column using water/acetonitrile (30:70 by volume) following no treatment (●), digestion with *B. fragilis* endo-β-galactosidase (○), digestion with bovine testis β-galactosidase (×), or digestion with bovine testis β-galactosidase and *S. plicatus* β-*N*-acetylhexosaminidase (■). In this system, LacNAc, Le^x, and LNT standards eluted at 4.3, 6.1, and 13.4 min, respectively. Endo-β-galactosidase cleavage of LNT generated a product with a retention time of 9.3 min (data not shown).

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 eluting 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 α(1,2)- or α(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 (*fuc*T) with highly localized similarity to known α(1,3)-Fuc-T enzymes. Cell extracts from library clones containing the *H. pylori* gene possessed Fuc-T activity, and by subcloning *fuc*T into an *E. coli* expression vector we were able to confirm that it encodes an active α(1,3)-Fuc-T. *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* FucT 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-T genes, 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-

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 Le^x 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 α(1,2)- or α(1,4)-Fuc-T activity, as has been reported for human Fuc-TV (51). Biosynthesis of the Le^y epitope found on the surface of many *H. pylori* isolates is therefore likely to involve a separate α(1,2)-Fuc-T activity. Overall, the acceptor specificity of *H. pylori* Fuc-T does not match that reported for any of the human enzymes or indeed that of *S. mansoni* α(1,3)-Fuc-T (53). Like the schistosome 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-Le^x (sLe^x) structure which was recently detected in a small number of *H. pylori* isolates by Wirth *et al.* (27). The absence of sLe^x from the majority of *H. pylori* isolates may therefore reflect a lack of sialyltransferase 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. 2*B*) 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[4] (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 argue 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-TVII uses 2,3-sialylated acceptors almost exclusively, Fuc-TIV strongly prefers neutral type 2 substrates (41) in *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 Mn^{2+}. The motif lies some considerable distance from a cysteine residue implicated in GDP-fucose protectable 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. 2*A*), 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).[5] Interestingly, the conserved motif contains a lysine residue (Fig. 2*A*), 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 Le^x by *H. pylori in vivo* via disruption of *fuc*T and may make it possible to test the role of Le^x directly in models of *H. pylori* pathogenesis.

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