THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 278, No. 24, Issue of June 13, pp. 21893–21900, 2003
Printed in U.S.A.
© 2003 by The American Society for Biochemistry and Molecular Biology, Inc.

C-terminal Amino Acids of *Helicobacter pylori* α1,3/4 Fucosyltransferases Determine Type I and Type II Transfer*[S]

Received for publication, February 19, 2003, and in revised form, March 27, 2003
Published, JBC Papers in Press, April 3, 2003, DOI 10.1074/jbc.M301704200

Bing Ma‡, Ge Wang‡§, Monica M. Palcic¶, Bart Hazes‡‖, and Diane E. Taylor‡**

From the ‡Department of Medical Microbiology and Immunology, University of Alberta, Edmonton, Alberta T6G 2H7, Canada and the ¶Department of Chemistry, University of Alberta, Edmonton, Alberta T6G 2G2, Canada

The α1,3/4 fucosyltransferase (FucT) enzyme from *Helicobacter pylori* catalyzes fucose transfer from donor GDP-β-L-fucose to the GlcNAc group of two series of acceptor substrates in *H. pylori* lipopolysaccharide: βGal1,3βGlcNAc (Type I) or βGal1,4βGlcNAc (Type II). Fucose is added either in α1,3 linkage of Type II acceptor to produce Lewis X or in α1,4 linkage of Type I acceptor to produce Lewis A, respectively. *H. pylori* FucTs from different strains have distinct Type I or Type II substrate specificities. FucT in *H. pylori* strain NCTC11639 has an exclusive α1,3 activity because it recognizes only Type II substrates, whereas FucT in *H. pylori* strain UA948 can utilize both Type II and Type I acceptors; thus it has both α1,3 and α1,4 activity, respectively. To identify elements conferring substrate specificity, 12 chimeric FucTs were constructed by domain swapping between 11639FucT and UA948FucT and characterized for their ability to transfer fucose to Type I and Type II acceptors. Our results indicate that the C-terminal region of *H. pylori* FucTs controls Type I and Type II acceptor specificity. In particular, the highly divergent C-terminal portion, seven amino acids DNPFIFC at positions 347–353 in 11639FucT, and the corresponding 10 amino acids CNDAHYSALH at positions 345–354 in UA948FucT, controls the Type I and Type II acceptor recognition. This is the opposite of mammalian FucTs where acceptor preference is determined primarily by the N-terminal residues in the hypervariable stem domain.

The lipopolysaccharide of *Helicobacter pylori* contains fucosylated oligosaccharides, predominantly the Type II blood group antigens Lewis X and Lewis Y (1). Some *H. pylori* strains also express the Type I blood group antigens Lewis A and Lewis B (2). *H. pylori* fucosyltransferases (FucTs)[1] are the enzymes responsible for the last steps in the synthesis of Lewis antigens. Difucosylated Lewis antigens (Lewis Y and Lewis B) can be synthesized via two pathways: terminal fucosylation (in α1,2 linkage) followed by subterminal fucosylation (in α1,3 or α1,4 linkage) or subterminal fucosylation followed by terminal fucosylation. Unlike mammalian cells, the latter pathway is predominately used in *H. pylori* (3).

In *H. pylori* genomes (4, 5), there exist two homologous α1,3/4 FucT genes, *futA* and *futB*, and one gene *futC* for α1,2 FucT (6). These three *fut* genes do not always encode functional proteins. For instance, the *futA* gene, but not the *futB* gene, encodes an active α1,3/4 FucT in *H. pylori* strains NCTC11639 and UA948 (7, 8). The *futC* gene in strain NCTC11639 is functional (6), but this gene is not functional in strain UA948 (8). The on/off status of *fut* genes and the various levels of FucT activities exhibited in different *H. pylori* strains determine the Lewis antigen expression patterns of *H. pylori* lipopolysaccharide.

FucTs are inverting enzymes and belong to glycosyltransferase family 10 (9). They are widely expressed in vertebrates, invertebrates, and bacteria. Mammalian α1,3/4 FucTs are Golgi-anchored proteins with short cytoplasmic N-terminal tails, transmembrane segments, hypervariable stem domains, and C-terminal catalytic domains. The C-terminal catalytic domain of mammalian FucT displays a low level of amino acid sequence identity (40%) with the internal catalytic domain of *H. pylori* FucTs (8). This domain contains two highly conserved regions, referred to as the α1,3/4 FucT motifs (10, 11). In comparison with mammalian FucTs, *H. pylori* FucTs do not have N-terminal tails or transmembrane domains; instead, they contain heptad repeats at their C terminus, which are absent in human FucTs (8, 11).

α1,3/4 FucTs exhibit distinct acceptor preferences. For instance, despite the fact that human FucT III, V, and VI and bovine FucT share at least 70% protein sequence identity (12, 13), they displayed strikingly distinctive acceptor specificity patterns. FucT VI and bovine FucT display exclusive α1,3 activity; in contrast, FucT III and V show both α1,3 and α1,4 activity, although they exhibit different preferences toward Type I and Type II substrates. FucT III favors Type I acceptors and therefore is predominantly an α1,4 FucT, whereas FucT V prefers Type II acceptors and hence is primarily an α1,3 FucT (Refs. 12–14 and references therein). The domain swapping experiments performed between FucT III and VI (15) or between FucT III and V (16) demonstrated that the N-terminal hypervariable stem region in human FucTs is responsible for

* This work was supported by funding from the Canadian Bacterial Diseases Network Center of Excellent Program (to D. E. T.) and the National Sciences and Engineering Research Council (to M. M. P.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[S] The on-line version of this article (available at http://www.jbc.org) contains supplemental data.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AAF35291.

§ Present address: Dept. of Microbiology, University of Georgia, Athens, GA 30602.

‖ Medical Scholar with the Alberta Heritage Foundation for Medical Research.

** Medical Scientist with the Alberta Heritage Foundation for Medical Research. To whom correspondence should be addressed: Dept. of Medical Microbiology and Immunology, 1-28 Medical Sciences Building, University of Alberta, Edmonton, AB T6G 2H7, Canada. Tel.: 780-492-4777; Fax: 780-492-7521; E-mail: diane.taylor@ualberta.ca.

[1] The abbreviations used are: FucT, fucosyltransferase; Type I, Galβ1,3GlcNAc; Type II, Galβ1,4GlcNAc; Type I-R, Galβ1,3GlcNAc-O$(CH_2)_8CO_2CH_3$; Type II-R, Galβ1,4GlcNAc-O$(CH_2)_8CO_2CH_3$; WT, wild type.

conferring acceptor specificity. In particular, site-directed mutagenesis data showed that the substitution of as few as two amino acids (His[87]-Ile[88] in FucT III corresponding to Asn[86]-Thr[87] in FucT V) (17) or replacement of a single amino acid (Trp[111] in FucT V corresponding to Arg[115] in bovine FucT) (18) is able to change the Type I or Type II substrate specificity. The adjacent residue Asp[112] in FucT V, corresponding to Glu[116] in bovine FucT, was able to further modulate the relative Type I and Type II acceptor specificity (18). Because there is no sequence identity at the N terminus between human FucTs and *H. pylori* FucTs (8), no domain or amino acid residues in *H. pylori* FucTs correspond to the region or residues conferring Type I and Type II substrate specificity in human FucTs could be identified.

The main goal of this study was to identify the determinants of *H. pylori* FucTs responsible for discriminating acceptor substrate preference. Characterization of structure-function relationships of *H. pylori* FucTs would enable us to manipulate the Lewis antigen expression pattern by mutation of FucT genes. This could lead to a clearer understanding of the role of Lewis antigen in persistent *H. pylori* infection. In addition, *H. pylori* FucTs are promising candidates for chemoenzymatic glycoconjugate synthesis (19–21). Finally, better understanding of the bacterial FucTs could help us understand the divergent evolution of various lineages in the FucT kingdom.

EXPERIMENTAL PROCEDURES

Materials—The primers were synthesized by Invitrogen. pGEM-T vector was obtained from Promega Co. (Madison, WI). Type II-R (βGal1–4βGlcNAc-O-$(CH_2)_8CO_2CH_3$) and Type I-R (βGal1–3βGlcNAc-O-$(CH_2)_8CO_2CH_3$) were kindly provided by Dr. O. Hindsgaul. Anti-penta-histidine monoclonal antibody was purchased from Qiagen. GDP-fucose and horseradish peroxidase-conjugated goat anti-mouse IgG were from Sigma. [^{3}H]GDP-fucose (0.1 mCi ml^{-1}, 17.3 Ci mmol^{-1}) was obtained from American Radiolabeled Chemicals Inc. (St. Louis, MO). The BCA protein assay kit was purchased from Pierce. Nitrocellulose membrane was obtained from Micron Separation Inc. (Westboro, MA). The ECL kit was supplied by Amersham Biosciences. BioMax MR film was obtained from Eastman Kodak Co. (Rochester, NY).

Cloning of Chimeric FucTs—H. pylori strains NCTC11639 and UA948 were cultured by standard methods, as described by Taylor *et al.* (22). Chromosomal DNA was isolated following standard protocol (23). Two primers BM11 and BM12 were designed to amply the *futA* gene. The domain swapping experiments were accomplished by primer overlap extension with two steps of sequential PCR (23). The sequences of primers are available in the supplemental data. Two pairs of proximal primers were used in the second step of PCR: BM91 and BM77, complementary to *futA* in strain 11639; and BM101 and BM87, complementary to *futA* in strain UA948. A Shine-Dalgarno sequence was included at the sense primers BM91 and BM101. The codons for six histidine residues were included in the antisense primers BM77 and BM87. The PCR products were cloned into A/T cloning vector pGEM-T under the control of a T7 promoter. Cloned chimeric *futA* genes were sequenced to assure that no mutation had occurred during their construction.

Induction and Expression of H. pylori FucT Genes—Expression of FucT genes were induced as described previously (7, 8) with minor modifications. *Escherichia coli* HMS174DE3 cells were used for FucT expression, and DE3 carries the gene encoding T7 RNA polymerase under the control of an isopropyl-β-D-thiogalactopyranoside-induced lactose promoter. The cells were grown at 30 °C with vigorous shaking (200 rpm min^{-1}) in SOC medium (20% tryptone, 5% yeast extract, 0.05% (w/v) NaCl, 10 mM $MgCl_2$, 2.5 mM KCl, 20 mM glucose) (23) including ampicillin (100 μg ml^{-1}) and rifampicin (200 μg ml^{-1}) for 2 h until the A_{600} reached ~0.3, at which point 1 mM of isopropyl-β-D-thiogalactopyranoside was added. After 4 h, the cultures were centrifuged and resuspended in HEPES buffer (20 mM, pH 7.0, containing 0.5 mM phenylmethanesulfonyl fluoride), and the cells were lysed using three freeze-thaw cycles (24). The cells were treated with lysozyme (200 μg ml^{-1}) and DNase (125 units ml^{-1}) for 20 min on ice after the first freeze-thaw process. The total protein concentrations of the crude cell extracts were determined using the BCA protein assay kit, with bovine serum albumin as a standard according to the supplier's instructions. The crude cell lysates (isolated membrane fractions) were used for the enzyme assays, enzyme kinetics determination, and immunoblot analysis.

Fucosyltransferase Assay—FucT assays were performed as described previously (7, 8, 25). A protein preparation from HMS174DE3 *E. coli* cells containing the pGEM vector was used as a negative control. The α1,3 and α1,4 FucT activities of wild type (WT) and chimeric FucTs were assayed with Type II-R at 1.8 mM or Type I-R at 7.5 mM and GDP-fucose at 200 μM. [^{3}H]GDP-fucose (~60,000 dpm) was included in each reaction. One milliunit represents the amount of enzyme that converts 1 nmol of acceptor substrates to the product per min. The specific activity (milliunit mg^{-1}) was obtained by dividing the enzyme activity (milliunit) by total protein concentration. Specific enzyme activity below 0.01 milliunit mg^{-1} was considered undetectable.

Immunoblot Analysis of Native and Chimeric FucT Expression—Crude cell extracts were boiled for 5 min in 4% (w/v) SDS and 0.002% (w/v) bromphenol blue. The cell extracts containing equal amounts of total protein (7.35 μg) were separated by SDS-PAGE, and the proteins were electrically transferred to nitrocellulose membrane (pore size, 0.22 μm). The nitrocellulose blots were probed with primary antibody (mouse anti-penta-histidine monoclonal antibody) at 1:1000 dilution and secondary antibody (horseradish peroxidase-conjugated goat anti-mouse IgG) at 1:2000 dilution. The blots were developed using an ECL kit, and the images were visualized on BioMax MR films. The FucT band densities were determined and quantified using Alpha Ease FC software (Alpha Innotech Corporation, San Leandro, CA). A standard curve with six points quantifying FucT band densities was performed using WT UA948FucT sample containing total protein between 1.84 and 18.4 μg. For WT and chimeric FucT samples, 7.35 μg of total protein was loaded for immunoblot analysis, which was in the middle of the linear range (data not shown). The expression level of UA948FucT was set to 1, and the expression levels of 11639FucT and chimeric FucTs were obtained relative to that of UA948FucT. The enzyme activity of mutants was normalized by dividing the specific activity (milliunit mg^{-1}) by the expression level of the chimeric FucT.

Determination of Kinetic Parameters—Acceptor kinetics were determined using 0.03–2 mM Type II-R or 0.4–25 mM Type I-R with GDP-fucose at 200 μM including [^{3}H]GDP-fucose. 2 mM of Type II-R is the highest concentration that can be reached in the current study because of its low solubility. Because of the limited supply of the acceptors, donor kinetics were determined using 3–200 μM GDP-fucose with Type II-R at 2 mM or Type I-R at 15 mM including [^{3}H]GDP-fucose. The kinetic parameters were obtained by fitting the initial rate data to the Michaelis-Menten equation using nonlinear regression analysis with Prism 2.0 software (GraphPad Software, Inc. San Diego, CA).

RESULTS

Sequence Alignment of H. pylori FucTs—Seven FucT sequences from five different *H. pylori* strains, 11639, UA948, 11637, 26695, and J99 (Fig. 1), were aligned using ClustalW (26) with LaserGene99 software (DNAStar Inc., Madison, WI). UA948FucT displays both α1,3 and α1,4 activities and like human FucT V, it favors Type II over Type I acceptors (8). 11639FucT as well as 11637FucTb have exclusive α1,3 activity (7, 27). There is no experimental data available for enzyme activity and specificity of 26695FucTa, 26695FucTb, J99FucTa, and J99FucTb. Because *H. pylori* strain 26695 and J99 lipopolysaccharide express Lewis X and Lewis Y (28) but not Lewis A and Lewis B structures, α1,3/4 FucTs in 26695 and J99 are expected to have α1,3 activity only. Whether or not the active FucT protein is encoded by the *futA* gene, the *futB* gene, or both remains to be determined.

Three regions of low sequence identity are evident in the alignment of seven *H. pylori* FucTs (displayed in *boxed areas* in Fig. 1). The first divergent region is located at the N terminus between residues 13 and 41 (11639FucT numbering). The second region resides immediately upstream of the heptad repeats, with most of the divergence caused by the unique sequences in UA948FucT and 26695FucTa. The third divergent region contains the C-terminal heptad repeats. There are between two and 10 heptad repeats with a conserved D(D/N)LR(V/I)NY sequence in all of the listed FucTs except for UA948FucT (Fig. 1). UA948FucT contains five internal repeats with this consensus sequence, whereas the first and

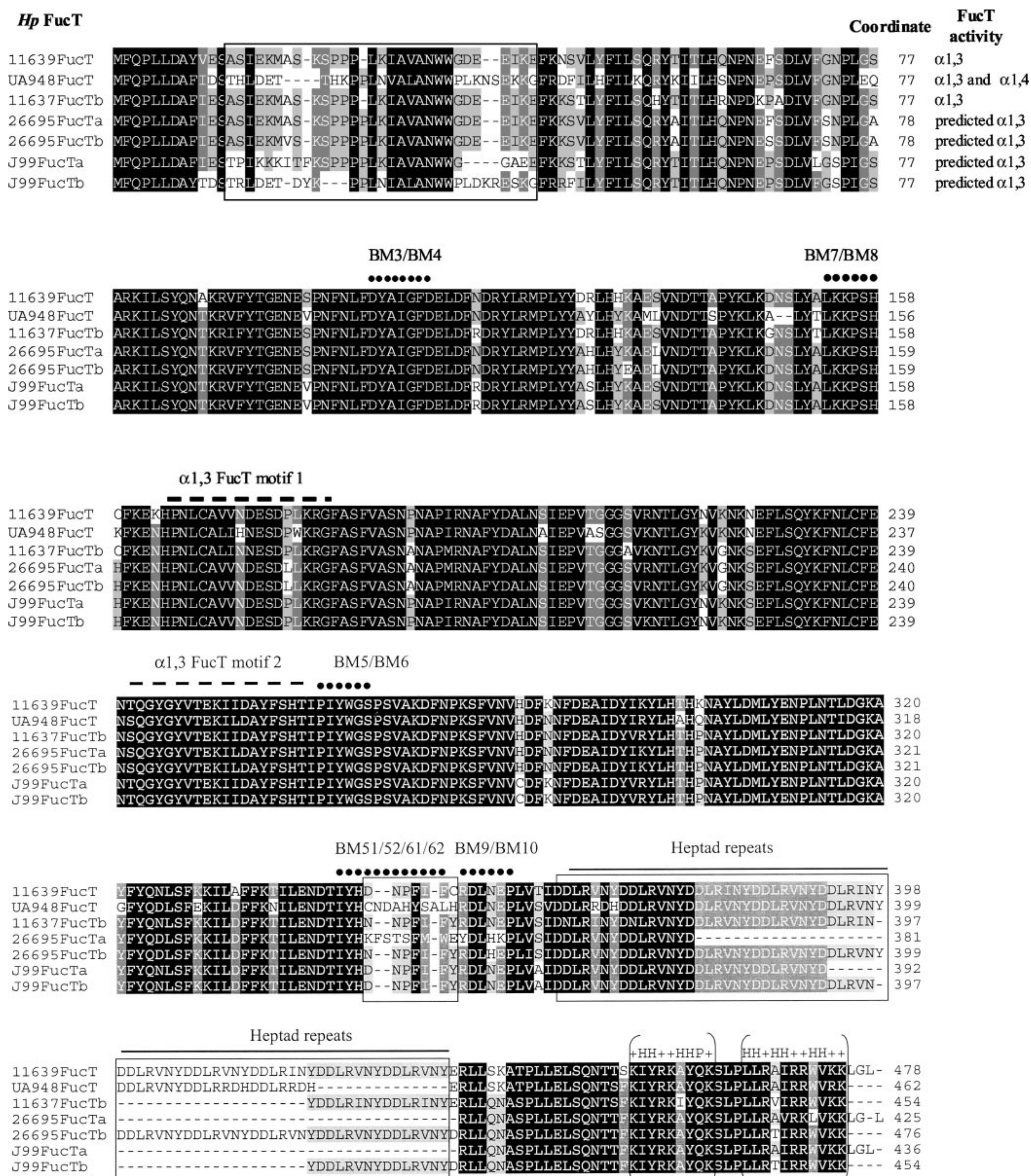

FIG. 1. **Alignment of *H. pylori* FucTs.** Sequence alignment was performed using ClustalW with LaserGene99 software (DNASTAR). Highly conserved residues have a *black background*, whereas partially conserved residues are shown with a *shaded background*. Numbering at the end of each line refers to the position in the alignment. The positions of primers are marked with *filled circles*. Two highly conserved α1,3/4 motifs are marked with *dashed lines*. The heptad repeats are marked with *straight lines*. Three major divergent sequences among *H. pylori* FucTs are *boxed*. The sequences forming two putative amphipathic α-helices are shown in *brackets*, and the positively charged, hydrophobic, and polar residues are marked with +, *H*, and *P*, respectively.

the last two heptad repeats contain the amino acid sequence DDLRRDH.

Expression of Native and Chimeric FucTs—The expected molecular masses of 11639FucT and UA948FucT are 56.0 and 54.6, kDa, respectively, and both proteins migrate to the expected locations on the SDS-PAGE gel (Fig. 2). The chimeric FucTs were expressed at similar levels as WT FucTs in HMS174DE3 cells (Fig. 2), indicating that the domain swapping did not cause significant changes in FucT enzyme expression levels.

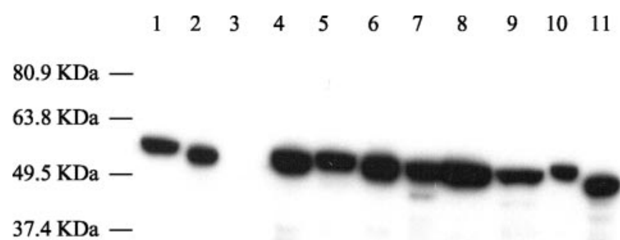

FIG. 2. **Immunoblot of wild type and chimeric FucTs.** Wild type and chimeric FucTs with His_6 tag were expressed in *E. coli* HMS174DE3 cells with induction using 1 mM of isopropyl-β-D-thiogalactopyranoside and growth at 30 °C for 4 h. The FucT proteins were detected with mouse anti-penta-histidine monoclonal antibody. *Lane 1*, 11639FucT; *lane 2*, UA948FucT; *lane 3*, pGEM vector alone; *lane 4*, $11639^{1–110}UA948^{111–462}$; *lane 5*, $UA948^{1–110}11639^{111–478}$; *lane 6*, $11639^{1–158}UA948^{157–462}$; *lane 7*, $UA948^{1–156}11639^{159–478}$; *lane 8*, $11639^{1–296}UA948^{295–462}$; *lane 9*, $UA948^{1–294}11639^{297–478}$; *lane 10*, $11639^{347CNDAHYSALH}$; *lane 11*, $UA948^{345DNPFIFC}$.

Kinetic Parameters of WT FucTs with or without His_6 Tag—Kinetic parameters for both WT and His_6-tagged WT FucTs are shown in Table I. The K_m for Type II acceptor of 11639FucT is 0.37 mM, 4-fold lower than that of UA948FucT. UA948FucT had a 7-fold lower K_m for Type II than Type I. 11639FucT and UA948FucT have similar donor K_m values with Type II acceptor. UA948FucT had a slightly lower donor K_m when Type I was used as an acceptor. Acceptor and donor K_m values were not affected by the addition of a His_6 tag, except that UA948FucT with a His_6 tag had a slightly higher K_m for Type I acceptor.

Enzyme Activity and Substrate Specificity of Chimeric FucTs—Twelve chimeric FucTs (Fig 3*A*) containing peptide fragments from two parental FucTs were constructed. The exchange of the three highly divergent segments (*boxed areas* in Fig. 1) between two parental FucTs was the major focus of domain swapping. In addition, the exchange of comparatively conserved internal regions containing two α1,3 motifs was also included. The normalized α1,3 and α1,4 activities (milliunit mg^{-1}) according to the FucT protein expression level of WT and chimeric FucTs are given in Fig. 3*B*. In addition, the α1,4/α1,3 activity ratio (%) was calculated (Fig. 3*B*).

The α1,3 activity of 11639FucT was 16.3 milliunit mg^{-1}, which is lower than that of UA948FucT (23.8 milliunit mg^{-1}). The α1,4 activity of UA948FucT was one-third of its α1,3 activity. Constructs $11639^{1–110}UA948^{111–462}$, $11639^{1–158}UA948^{157–462}$, and $11639^{1–296}UA948^{295–462}$ containing the 11639FucT N terminus and the UA948FucT C terminus had both α1,3 and α1,4 FucT activities. In contrast, their counterparts, constructs $UA948^{1–110}11639^{111–478}$, $UA948^{1–156}11639^{159–478}$, and $UA948^{1–294}11639^{297–478}$, containing the UA948FucT N terminus and the 11639FucT C terminus, displayed α1,3 activity with little or no α1,4 activity. Therefore, it is the C terminus that controls Type I or Type II acceptor specificity in *H. pylori* FucTs. This is different from human FucTs, where the N-terminal hypervariable stem domain determines acceptor specificity. $UA948^{1–156}11639^{159–478}$ and $UA948^{1–294}11639^{297–478}$ exhibited very low α1,3 FucT activity, although they were stably expressed (Fig. 2). Apparently the two segments of the chimeras are not fully compatible in these cases, which results in the low enzyme activity.

Mutant $11639^{1–359}UA948^{361–462}$ displayed α1,3 activity at WT level without gaining α1,4 activity, indicating that the C-terminal heptad repeats of UA948FucT cannot confer Type I acceptor specificity. This is further supported by the substitution of heptad repeats of UA948FucT by those of 11639FucT (construct $UA948^{1–360}11639^{360–478}$), which failed to abolish the α1,4 activity. However, although chimera $UA948^{1–360}11639^{360–478}$ exhibited a nearly WT UA948FucT α1,4/α1,3 activity ratio (24% *versus* 31%), both its α1,3 and α1,4 activities were low. Apparently, the heptad repeats of 11639FucT have a negative impact on catalysis by the UA948 catalytic domain, affecting α1,3 and α1,4 activities to a similar extent. In contrast, the 11639FucT catalytic domain can tolerate the exchange of heptad repeats by those of UA948 FucT.

Mosaic construct $11639^{1–110}UA948^{111–294}11639^{297–478}$ had normal α1,3 activity but no α1,4 activity, demonstrating that the internal region (110–294) of UA948FucT was unable to confer α1,4 activity either. In contrast, construct $11639^{1–158}UA948^{157–360}11639^{360–478}$ exhibited both α1,3 and α1,4 activity, although both at low levels. Comparing all 10 chimeras it becomes obvious that those containing residues 295–361 of UA948FucT have α1,4 activity, whereas all without it lack α1,4 activity. Therefore, it is likely that residues in this segment confer α1,4 activity. The segment (295–361) includes the second divergent region. Remarkably, chimera $11639^{347CNDAHYSALH}$ gained 11% of WT UA948FucT α1,4 activity. In contrast, its counterpart $UA948^{345DNPFIFC}$ lost almost all of its α1,4 activity without losing its α1,3 activity. This suggests that the second divergent region determines the absence or presence of α1,4 activity. Our data indicate that residues $^{345}CNDAHYSALH^{354}$ of UA948FucT are essential for conferring Type I substrate specificity.

It should be noted that $11639^{347CNDAHYNALH}$ gained relatively low α1,4 activity and that its α1,4/α1,3 ratio is also lower than that of WT UA948FucT (Fig. 3*B*), suggesting that some other residues may further contribute to acceptor substrate specificity. $UA948^{1–360}11639^{360–478}$, but not $11639^{1–296}UA948^{295–462}$, has a near WT α1,4/α1,3 ratio, suggesting that additional acceptor specificity determinants are located at the N terminus, prior to residue 296. Chimera $11639^{1–110}UA948^{111–462}$ displays a 2-fold drop in α1,4/α1,3 ratio that does not deteriorate further in the $11639^{1–158}UA948^{157–462}$ construct. This suggests that the additional acceptor specificity determinants reside in the N-terminal 110 residues. However, at this moment we do not yet want to rule out a role for residues 158–296 because chimera $11639^{1–158}UA948^{157–462}$ has a noticeably higher α1,4/α1,3 ratio than chimera $11639^{1–296}UA948^{295–462}$. Our data indicate that the second divergent region is crucial for α1,4 activity, but more detailed studies will be needed to delineate the additional acceptor determinants more precisely.

Kinetics Parameters of Mutants—The kinetic parameters of two WT FucTs and constructs $11639^{1–359}UA948^{361–462}$, $UA948^{1–360}11639^{360–478}$, $11639^{347CNDAHYNALH}$, and $UA948^{345DNPFIFC}$ are shown in Table II. The α1,4 activity of $11639^{1–359}UA948^{361–462}$ was too low to obtain reliable kinetic parameters.

The V_{max} of 11639FucT using Type II acceptor was about one third of that of UA948FucT, but the V_{max}/K_m of 11639FucT was higher than that of UA948FucT because of its lower K_m. Therefore, 11639FucT has a higher fucose transfer efficiency in α1,3 linkage than UA948FucT. Type II is a better acceptor than Type I for UA948FucT because of its higher fucose incorporation (V_{max}) and lower K_m. Similar acceptor K_m values were obtained for $11639^{1–359}UA948^{361–462}$ and $UA948^{1–360}11639^{360–478}$ compared with their corresponding WT enzymes, which confirmed that the C-terminal heptad repeats are not responsible for acceptor recognition.

In comparison with WT 11639FucT, $11639^{347CNDAHYSALH}$ possessed a higher K_m for Type II acceptor (2.2 mM), which was close to the K_m value of UA948FucT. Similarly, the K_m value of $UA948^{345DNPFIFC}$ for Type II was reduced from 2.2 to 0.17 mM,

TABLE I

The acceptor and donor kinetic parameters of wild type 11639FucT and UA948FucT with or without the C-terminal His_6 *tag*

Acceptor kinetics were determined using 0.03–2 mM Type II-R or 0.4-25 mM Type I-R with GDP-fucose at 200 μM including [^{3}H]GDP-fucose at 0.2 μM. Donor kinetics were determined using 3–200 μM GDP-fucose with Type II-R at 2 mM or Type I-R at 15 mM.

FucT constructs	K_m		K_m of GDP-fucose	
	Type II-R[a]	Type I-R[b]	With Type II-R[a]	With Type I-R[b]
	mM		μM	
11639	0.31 ± 0.06	NA[c]	48.0 ± 8.6	NA[c]
11639-C-His_6	0.37 ± 0.03	NA[c]	44.7 ± 5.5	NA[c]
UA948	1.2 ± 0.2	8.4 ± 1.1	68.3 ± 8.3	35.7 ± 7.9
UA948-C-His_6	1.5 ± 0.2	13.4 ± 1.4	56.0 ± 10.1	33.7 ± 7.8

[a] Galβ1,4GlcNAc-O$(CH_2)_8CO_2CH_3$.
[b] Galβ1,3GlcNAc-O$(CH_2)_8CO_2CH_3$.
[c] Not applicable.

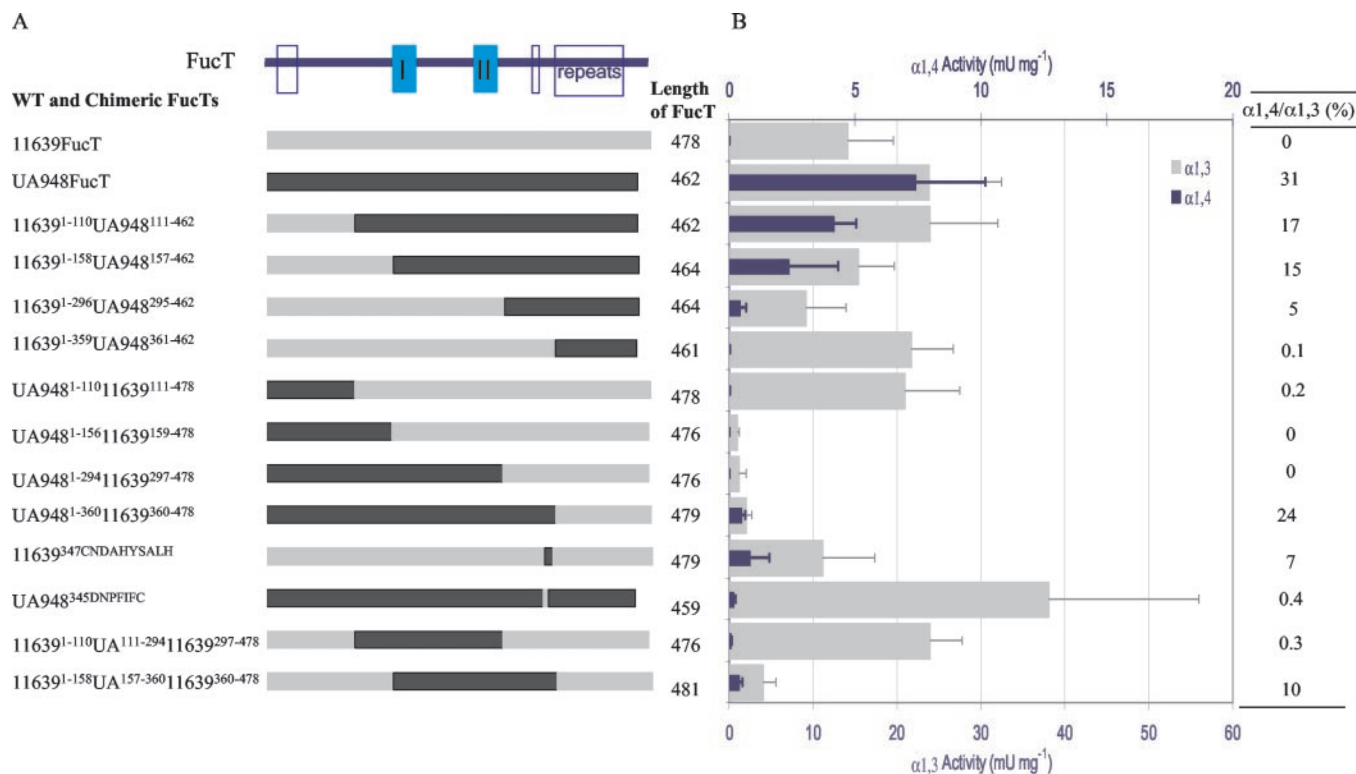

FIG. 3. **Schematic representations of chimeric FucTs (*A*) and their α1,3 and α1,4 activities (*B*).** *A*, *top*, the *bar* represents the FucT protein. The two catalytic domains are shown as *vertical light blue blocks*. The three highly divergent regions are *boxed*. *Bottom*, schematic *bars* represent the structure of chimeric FucTs. The length (number of total amino acids) of each FucT construct is given at the *right side* of each *bar*. *B*, enzyme activities (milliunit mg^{-1}) for α1,3-FucT activity (*gray bars*, *bottom y axis*) and α1,4-FucT activity (*dark blue bars*, *top y axis*) of each FucT construct are standardized by FucT expression level. Each value represents the average of three or four determinations with standard deviations indicated. The ratio of α1,4/α1,3 activity (%) for each FucT construct is shown on the *right*.

which is close to that of 11639FucT (0.37 mM). This strongly implies that the second divergent region plays an essential role in Type II acceptor recognition. In addition, chimera11639^{347CNDAHYSALH} gained α1,4 activity with the K_m value for Type I substrate near the WT UA948FucT level. This verifies the vital role of those 10 amino acids in conferring α1,4 activity. On the other hand, as expected, the catalysis of Type I substrate was severely diminished in the UA948^{345DNPFIFC} chimera, and despite the fact that acceptor K_m of UA948^{345DNPFIFC} for acceptor Type I was similar to the level of WT UA948FucT, the V_{max} of this chimera was dramatically decreased. This suggests that those 10 amino acids present in UA948FucT play an important role in efficient fucose transfer in α1,4 linkage.

DISCUSSION

Mammalian α1,3/4 FucTs, especially human FucTs, have been extensively characterized with regard to the key polar group of the acceptors (25, 29), the domain or amino acid residues responsible for donor binding (30–33) and acceptor recognition (15–18, 29–32), and kinetic mechanisms (33–35). The study of bacterial α1,3/4 FucTs, in contrast, is not as far advanced. To date, bacterial α1,3/4 FucTs have only been characterized in *H. pylori* (7, 8, 27) and *Vibrio cholerae* (36), despite identification of putative FucT homologs in *Rickettsia conorii* (37), *Salmonella enterica serovar Typhi* (38, 39), *Yersinia pestis* (40), and *Mesorhizobium loti* (41).

The aim of this study was to identify the determinants of *H. pylori* α1,3/4 FucTs that confer recognition of Type I and Type II substrates. Our data demonstrate that the C-terminal region of *H. pylori* FucTs controls Type I and Type II substrate recognition. Particularly, residues ^{345}CNDAHYSALH^{354} of UA948FucT are essential for α1,4 activity. It is worth mentioning that the donor binding affinity was modified in constructs 11639$^{1-359}$UA948$^{361-462}$, UA948$^{1-360}$11639$^{360-478}$, and 11639^{347CNDAHYSALH} with Type II-R acceptor and

TABLE II
Acceptor and donor kinetic parameters of wild type and chimeric FucTs

Acceptor kinetics were determined using 0.03-2 mM Type II-R or 0.4-25 mM Type I-R with GDP-fucose at 200 μM including [^{3}H]GDP-fucose at 0.2 μM. Donor kinetics were determined using 3-200 μM GDP-fucose with Type II-R at 2 mM or Type I-R at 15 mM.

WT/mutants[a]	Type II-R[b]			Type I-R[c]			K_m for GDP-fucose	
	K_m	V_{max}[d]	V_{max}/K_m[e]	K_m	V_{max}[d]	V_{max}/K_m[e]	Type II-R[b]	Type I-R[e]
	mM			*mM*			*μM*	
11639	0.37 ± 0.03	11.8 ± 0.3	31.9	NA[f]	NA[f]	NA[f]	44.7 ± 5.5	NA[f]
UA948	1.5 ± 0.2	29.0 ± 1.8	19.3	13.4 ± 1.4	11.7 ± 0.6	0.87	56.0 ± 10.1	33.7 ± 7.8
$11639^{1\text{-}359}$ $UA948^{361\text{-}462}$	0.7 ± 0.1	41.6 ± 0.01	48.5	ND[g]	ND[g]	ND[g]	153 ± 8	ND[g]
$UA948^{1\text{-}360}$ $11639^{360\text{-}478}$	1.3 ± 0.2	4.1 ± 0.3	3.1	9.7 ± 1.7	1.1 ± 0.08	0.11	236 ± 47	188 ± 20
$11639^{347CNDAHYSALH}$	2.2 ± 0.6	8.8 ± 1.5	4.0	20.8 ± 5.9	0.7 ± 0.1	0.03	244 ± 34	54.6 ± 6.6
$UA948^{345DNPFIFC}$	0.17 ± 0.01	28.9 ± 0.6	170.0	22.7 ± 7.4	0.7 ± 0.1	0.03	48.1 ± 7.0	213 ± 52

[a] All FucTs possessed a His_6 tag at the C-terminal.
[b] Galβ1, 4GlcNAc-$O(CH_2)_8CO_2CH_3$.
[c] Galβ1,3GlcNAc-$O(CH_2)_8CO_2CH_3$.
[d] V_{max} (milliunits mg^{-1}) was standardized based on FucT expression detected by Western blot.
[e] Milliunits mg^{-1} mM^{-1}.
[f] Not applicable.
[g] Not determined; the activity was too low to determine the kinetic parameters with confidence.

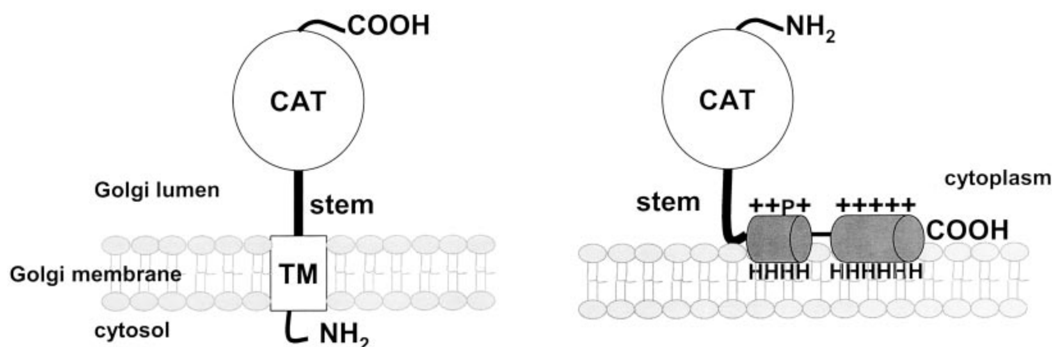

FIG. 4. **Schematic structures of mammalian FucTs (*left*) and *H. pylori* FucTs (*right*) anchored at Golgi and *H. pylori* cell membrane, respectively.** The two *cylinders* of *H. pylori* FucTs show two putative amphipathic α-helices. +, positively charged residue; *H*, hydrophobic residue; *P*, a polar residue; *NH_2*, N terminus; *COOH*, C terminus; *TM*, transmembrane domain; *CAT*, catalytic domain.

$UA948^{345DNPFIFC}$ with Type I-R acceptor. It is most likely that the binding of *H. pylori* FucT enzyme with acceptor and donor is a coordinated process. The recently published crystal structures of catalytic domains from other families of glycosyltransferases demonstrate that for both inverting enzymes, bovine β1,4-galactosyltransferase (42) and bovine *N*-acetylglucosaminyltransferase I (43), as well as retaining enzymes, bovine α1,3-galactosyltransferase (44), α1,4-galactosyltransferase (45), human galactosyltransferase, and human *N*-acetylgalactosaminyltransferase (46), the donor binding caused a conformational change that was essential to create the acceptor binding site. The data suggest that these enzymes followed a sequential ordered bi-bi catalytic mechanism. The same mechanism was reported for *E. coli* MurG (47) and human FucT V (33) based on product inhibition studies. The donor K_m modification of chimeras observed in the current study implies that *H. pylori* FucTs might follow the same mechanism. However, it is also possible that the domain swapping experiments cause significant folding changes that could affect donor and acceptor binding. More detailed kinetic studies and/or crystallography structure analysis would clarify this issue.

To date, FucT enzymes that display α1,4 activity have also been found in the chimpanzee (48), Rhesus macaque (Old World Monkey) (49), and plants (50, 51). According to sequence alignment, all of these FucTs contain a tryptophan residue within the N-terminal hypervariable stem region, which was found to be essential for conferring α1,4 activity in mammalian FucTs (18). This tryptophan residue is conserved in human FucT III and FucT V, which have both α1,3 and α1,4 activities, but is not present in other human FucTs, which possess only α1,3 activity. In *H. pylori*,[2] UA1111FucTa and UA1111FucTb also display α1,4 activity, although their activities are much lower than that of UA948FucT (52). Neither of these two FucTs contains those 10 crucial amino acids ^{345}CNDAHYSALH^{354} that are present in UA948FucT. Instead, UA1111FucTa has ^{346}DNPFIFY^{352}, which resembles the majority of *H. pylori* FucTs (Fig. 1), whereas UA1111FucTb has ^{347}KSSTSFMWE^{355} (53), which is similar to 26695FucTa (Fig. 1). Apparently, acceptor specificity determinants in *H. pylori* FucTs are not as conserved as in eukaryotic FucTs. The residues conferring α1,4 activity in UA1111FucTa and UA1111FutTb remain to be determined.

The results in the current study demonstrate that the C-terminal region of *H. pylori* FucTs controls Type I and Type II substrate recognition. In particular, residues ^{345}CNDAHYSALH^{354} of UA948FucT are essential for α1,4 activity, whereas the 110 N-terminal residues may further contribute to α1,4 activity. In complete contrast, acceptor specificity in human FucTs is determined by the residues at the N-terminal hypervariable stem domain (15–18), with possibly a further contribution by C-terminal residues (30). However, despite the different location of the acceptor specificity determinants in mammalian and *H. pylori* FucTs, sequence analysis suggests that they may be more similar on a structural level in terms of domain organization.

[2] 11637 variant 3a was recently found to be Lewis A positive as detected by enzyme-linked immunosorbent assay (57), leading these authors to suggest that FucT in this strain may have α1,4 activity. This hypothesis remains to be confirmed by the enzyme assay.

Analysis of the C-terminal sequence of *H. pylori* FucTs showed two remarkable features: the heptad repeats and two conserved regions rich in positive and hydrophobic residues (in *brackets* in Fig. 1). The C-terminal heptad repeats are predicted to fold into an α-helix (PepTool), and it has been suggested that the heptad repeats form a leucine zipper motif that may serve as a dimerization domain (7). Deletion of the C-terminal 99 amino acids of 11639FucT has been reported to abolish enzyme activity (7), suggesting that the heptad repeats do play a role in the enzyme function.

The patterns of positive and hydrophobic residues in the C-terminal tail region, KIYRK(A/I)YQK and LLR(A/V/T)(I/V)RR(W/L)V(K/R)K (in *brackets* in Fig. 1) perfectly match the requirements to form extremely amphipathic helices with positive and hydrophobic residues occupying the opposite faces of the helix (Fig. 4). Such helices can act as membrane anchors, with their hydrophobic face embedded in the membrane and the positive charges interacting with phospholipids head groups (54, 55). Notably, the C-terminal 50 amino acids of *Neisseria meningitidis* α1,4 galactosyltransferase are also rich in positively charged and hydrophobic residues and have been found to be responsible for membrane association (45). Finally, the existence of a membrane anchor also fits the experimental data that *H. pylori* FucTs are membrane-associated (7).

The sequence properties described above suggest that two amphipathic helices at the C terminus of *H. pylori* FucTs could correspond to the N-terminal membrane anchor of mammalian FucTs. Similarly, the heptad repeat region of *H. pylori* FucTs acts as a spacer linking the catalytic domain to the membrane anchor, corresponding in function to the stem region of mammalian FucTs. This suggests that the domains of mammalian FucTs and *H. pylori* FucTs are organized in a similar manner: a membrane anchor is linked by a hypervariable stem region to the catalytic domain (Fig. 4). As a result, the substrate recognition sites for both FucT families are located at their hypervariable stem region. Similarly, the stem region of β-galactoside-α2,6-sialyltransferase, another family of glycosyltransferases, was also found to govern the preference for glycoprotein acceptors (56).

In conclusion we have characterized the substrate specificity determinants of *H. pylori* FucTs, which represent a unique family of FucT enzymes. Although there is only a low level of sequence identity between mammalian FucTs and *H. pylori* FucTs, we discovered a putative similarity in their structural organization. As a result, acceptor specificity may in both cases be determined by divergent sequences in the stem region. However, the molecular basis for acceptor specificity remains to be determined by further structure and function studies, ideally in combination with a crystal structure.

Acknowledgments—We thank Dr. O. Hindsgaul for providing us with the Type I-R and Type II-R acceptors for this study. We thank Drs. S. Connell and J. Simala-Grant for helpful discussion during the course of this work and James Gunton for critical reading of the manuscript.

REFERENCES

1. Wirth, H. P., Yang, M., Karita, M., and Blaser, M. J. (1996) *Infect. Immun.* **64,** 4598–4605
2. Monteiro, M. A., Chan, K. H., Rasko, D. A., Taylor, D. E., Zheng, P. Y., Appelmelk, B. J., Wirth, H. P., Yang, M., Blaser, M. J., Hynes, S. O., Moran, A. P., and Perry, M. B. (1998) *J. Biol. Chem.* **273,** 11533–11543
3. Wang, G., Ge, Z., Rasko, D. A., and Taylor, D. E. (2000) *Mol. Microbiol.* **36,** 1187–1196
4. Tomb, J. F., White, O., Kerlavage, A. R., Clayton, R. A., Sutton, G. G., Fleischmann, R. D., Ketchum, K. A., Klenk, H. P., Gill, S., Dougherty, B. A., Nelson, K., Quackenbush, J., Zhou, L., Kirkness, E. F., Peterson, S., Loftus, B., Richardson, D., Dodson, R., Khalak, H. G., Glodek, A., McKenney, K., Fitzegerald, L. M., Lee, N., Adams, M. D., Venter, J. C., *et al.* (1997) *Nature* **388,** 539–547
5. Alm, R. A., Ling, L. S., Moir, D. T., King, B. L., Brown, E. D., Doig, P. C., Smith, D. R., Noonan, B., Guild, B. C., deJonge, B. L., Carmel, G., Tummino, P. J., Caruso, A., Uria-Nickelsen, M., Mills, D. M., Ives, C., Gibson, R., Merberg, D., Mills, S. D., Jiang, Q., Taylor, D. E., Vovis, G. F., and Trust, T. J. (1999) *Nature* **397,** 176–180
6. Wang, G., Rasko, D. A., Sherburne, R., and Taylor, D. E. (1999) *Mol. Microbiol.* **31,** 1265–1274
7. Ge, Z., Chan, N. W., Palcic, M. M., and Taylor, D. E. (1997) *J. Biol. Chem.* **272,** 21357–21363
8. Rasko, D. A., Wang, G., Palcic, M. M., and Taylor, D. E. (2000) *J. Biol. Chem.* **275,** 4988–4994
9. Campbell, J. A., Davies, G. J., Bulone, V., and Henrissat, B. (1997) *Biochem. J.* **326,** 929–939
10. Breton, C., Oriol, R., and Imberty, A. (1996) *Glycobiology* **6,** vii–xii
11. Breton, C., Oriol, R., and Imberty, A. (1998) *Glycobiology* **8,** 87–94
12. Weston, B. W., Smith, P. L., Kelly, R. J., and Lowe, J. B. (1992) *J. Biol. Chem.* **267,** 24575–24584
13. Oulmouden, A., Wierinckx, A., Petit, J. M., Costache, M., Palcic, M. M., Mollicone, R., Oriol, R., and Julien, R. (1997) *J. Biol. Chem.* **272,** 8764–8773
14. de Vries, T., Knegtel, R. M., Holmes, E. H., and Macher, B. A. (2001) *Glycobiology* **11,** 119R–128R
15. Legault, D. J., Kelly, R. J., Natsuka, Y., and Lowe, J. B. (1995) *J. Biol. Chem.* **270,** 20987–20996
16. Xu, Z., Vo, L., and Macher, B. A. (1996) *J. Biol. Chem.* **271,** 8818–8823
17. Nguyen, A. T., Holmes, E. H., Whitaker, J. M., Ho, S., Shetterly, S., and Macher, B. A. (1998) *J. Biol. Chem.* **273,** 25244–25249
18. Dupuy, F., Petit, J. M., Mollicone, R., Oriol, R., Julien, R., and Maftah, A. (1999) *J. Biol. Chem.* **274,** 12257–12262
19. Meldal, M., and St. Hilaire, P. M. (1997) *Curr. Opin. Chem. Biol.* **1,** 552–563
20. Ernst, B., and Oehrlein, R. (1999) *Glycoconj. J.* **16,** 161–170
21. Johnson, K. F. (1999) *Glycoconj. J.* **16,** 141–146
22. Taylor, D. E., Hargreaves, J. A., Ng, L. K., Sherbaniuk, R. W., and Jewell, L. D. (1987) *Am. J. Clin. Pathol.* **87,** 49–54
23. Sambrook, J., and Russell, D. W. (2000) *Molecular Cloning: A Laboratory Manual*, 3rd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
24. Wang, G., Rahman, M. S., and Humayun, M. Z. (1997) *Biochemistry* **36,** 9486–9492
25. Gosselin, S., and Palcic, M. M. (1996) *Bioorg. Med. Chem.* **4,** 2023–2028
26. Thompson, J. D., Higgins, D. G., and Gibson, T. J. (1994) *Nucleic Acids Res.* **22,** 4673–4680
27. Martin, S. L., Edbrooke, M. R., Hodgman, T. C., van den Eijnden, D. H., and Bird, M. I. (1997) *J. Biol. Chem.* **272,** 21349–21356
28. Appelmelk, B. J., Martin, S. L., Monteiro, M. A., Clayton, C. A., McColm, A. A., Zheng, P., Verboom, T., Maaskant, J. J., van den Eijnden, D. H., Hokke, C. H., Perry, M. B., Vandenbroucke-Grauls, C. M., and Kusters, J. G. (1999) *Infect. Immun.* **67,** 5361–5366
29. de Vries, T., Srnka, C. A., Palcic, M. M., Swiedler, S. J., van den Eijnden, D. H., and Macher, B. A. (1995) *J. Biol. Chem.* **270,** 8712–8722
30. Vo, L., Lee, S., Marcinko, M. C., Holmes, E. H., and Macher, B. A. (1998) *J. Biol. Chem.* **273,** 25250–25255
31. Pykari, M., Toivonen, S., Natunen, J., Niemela, R., Salminen, H., Aitio, O., Ekstrom, M., Parmanne, P., Valimaki, M., Alais, J., Auge, C., Lowe, J. B., Renkonen, O., and Renkonen, R. (2000) *J. Biol. Chem.* **275,** 40057–40063
32. Sherwood, A. L., Upchurch, D. A., Stroud, M. R., Davis, W. C., and Holmes, E. H. (2002) *Glycobiology* **12,** 599–606
33. Qiao, L. M. B., Shimazaki, M., Schultz, J., and Wong, C. H. (1996) *J. Am. Chem. Soc.* **118,** 7653–7662
34. Murray, B. W., Takayama, S., Schultz, J., and Wong, C. H. (1996) *Biochemistry* **35,** 11183–11195
35. Murray, B. W., Wittmann, V., Burkart, M. D., Hung, S. C., and Wong, C. H. (1997) *Biochemistry* **36,** 823–831
36. Stroeher, U. H., Parasivam, G., Dredge, B. K., and Manning, P. A. (1997) *J. Bacteriol.* **179,** 2740–2747
37. Ogata, H., Audic, S., Barbe, V., Artiguenave, F., Fournier, P. E., Raoult, D., and Claverie, J. M. (2000) *Science* **290,** 347–350
38. McClelland, M., Sanderson, K. E., Spieth, J., Clifton, S. W., Latreille, P., Courtney, L., Porwollik, S., Ali, J., Dante, M., Du, F., Hou, S., Layman, D., Leonard, S., Nguyen, C., Scott, K., Holmes, A., Grewal, N., Mulvaney, E., Ryan, E., Sun, H., Florea, L., Miller, W., Stoneking, T., Nhan, M., Waterston, R., and Wilson, R. K. (2001) *Nature* **413,** 852–856
39. Parkhill, J., Dougan, G., James, K. D., Thomson, N. R., Pickard, D., Wain, J., Churcher, C., Mungall, K. L., Bentley, S. D., Holden, M. T., Sebaihia, M., Baker, S., Basham, D., Brooks, K., Chillingworth, T., Connerton, P., Cronin, A., Davis, P., Davies, R. M., Dowd, L., White, N., Farrar, J., Feltwell, T., Hamlin, N., Haque, A., Hien, T. T., Holroyd, S., Jagels, K., Krogh, A., Larsen, T. S., Leather, S., Moule, S., O'Gaora, P., Parry, C., Quail, M., Rutherford, K., Simmonds, M., Skelton, J., Stevens, K., Whitehead, S., and Barrell, B. G. (2001) *Nature* **413,** 848–852
40. Parkhill, J., Wren, B. W., Thomson, N. R., Titball, R. W., Holden, M. T., Prentice, M. B., Sebaihia, M., James, K. D., Churcher, C., Mungall, K. L., Baker, S., Basham, D., Bentley, S. D., Brooks, K., Cerdeno-Tarraga, A. M., Chillingworth, T., Cronin, A., Davies, R. M., Davis, P., Dougan, G., Feltwell, T., Hamlin, N., Holroyd, S., Jagels, K., Karlyshev, A. V., Leather, S., Moule, S., Oyston, P. C., Quail, M., Rutherford, K., Simmonds, M., Skelton, J., Stevens, K., Whitehead, S., and Barrell, B. G. (2001) *Nature* **413,** 523–527
41. Sullivan, J. T., Trzebiatowski, J. R., Cruickshank, R. W., Gouzy, J., Brown, S. D., Elliot, R. M., Fleetwood, D. J., McCallum, N. G., Rossbach, U., Stuart, G. S., Weaver, J. E., Webby, R. J., De Bruijn, F. J., and Ronson, C. W. (2002) *J. Bacteriol.* **184,** 3086–3095
42. Ramakrishnan, B., Balaji, P. V., and Qasba, P. K. (2002) *J. Mol. Biol.* **318,** 491–502
43. Unligil, U. M., Zhou, S., Yuwaraj, S., Sarkar, M., Schachter, H., and Rini, J. M. (2000) *EMBO J.* **19,** 5269–5280
44. Boix, E., Zhang, Y., Swaminathan, G. J., Brew, K., and Acharya, K. R. (2002)

J. Biol. Chem. **277,** 28310–28318
45. Persson, K., Ly, H. D., Dieckelmann, M., Wakarchuk, W. W., Withers, S. G., and Strynadka, N. C. (2001) *Nat. Struct. Biol.* **8,** 166–175
46. Patenaude, S. I., Seto, N. O., Borisova, S. N., Szpacenko, A., Marcus, S. L., Palcic, M. M., and Evans, S. V. (2002) *Nat. Struct. Biol.* **9,** 685–690
47. Chen, L., Men, H., Ha, S., Ye, X. Y., Brunner, L., Hu, Y., and Walker, S. (2002) *Biochemistry* **41,** 6824–6833
48. Costache, M., Apoil, P. A., Cailleau, A., Elmgren, A., Larson, G., Henry, S., Blancher, A., Iordachescu, D., Oriol, R., and Mollicone, R. (1997) *J. Biol. Chem.* **272,** 29721–29728
49. Dupuy, F., Germot, A., Marenda, M., Oriol, R., Blancher, A., Julien, R., and Maftah, A. (2002) *Mol. Biol. Evol.* **19,** 815–824
50. Bakker, H., Schijlen, E., de Vries, T., Schiphorst, W. E., Jordi, W., Lommen, A., Bosch, D., and van Die, I. (2001) *FEBS Lett.* **507,** 307–312
51. Leonard, R., Costa, G., Darrambide, E., Lhernould, S., Fleurat-Lessard, P., Carlue, M., Gomord, V., Faye, L., and Maftah, A. (2002) *Glycobiology* **12,** 299–306
52. Rasko, D. A., Wang, G., Monteiro, M. A., Palcic, M. M., and Taylor, D. E. (2000) *Eur. J. Biochem.* **267,** 6059–6066
53. Rasko, D. A. (2000) *Expression and Synthesis of Lewis Antigens in Helicobacter pylori*. Ph.D. thesis, pp. 220, University of Alberta, Edmonton, Canada
54. Chen, C., Seow, K. T., Guo, K., Yaw, L. P., and Lin, S. C. (1999) *J. Biol. Chem.* **274,** 19799–19806
55. Wakarchuk, W. W., Cunningham, A., Watson, D. C., and Young, N. M. (1998) *Protein Eng.* **11,** 295–302
56. Legaigneur, P., Breton, C., El Battari, A., Guillemot, J. C., Auge, C., Malissard, M., Berger, E. G., and Ronin, C. (2001) *J. Biol. Chem.* **276,** 21608–21617
57. Appelmelk, B. J., Martino, M. C., Veenhof, E., Monteiro, M. A., Maaskant, J. J., Negrini, R., Lindh, F., Perry, M., Del Giudice, G., and Vandenbroucke-Grauls, C. M. (2000) *Infect. Immun.* **68,** 5928–5932