were washed once with buffer A [10 mM tris-HCl (pH 8.0), 10 μM phosphoserine, 10 μM phosphothreonine, 10 μM phosphotyrosine, 0.1 mM vanadate, and 0.1% CHAPS] containing 500 mM NaCl, 5 mM EGTA, 20 mM β-glycerolphosphate, and 1 μM microcystin. After three additional washes with the same buffer lacking microcystin, bound proteins were eluted from the column with buffer A containing 500 mM NaCl and 200 mM imidazole. The eluate was loaded on a Superdex 200 column equilibrated with buffer A containing 50 mM NaCl, 10 mM EGTA, and 10 mM EDTA. Column fractions containing Xcdc25-specific kinase activity were combined and incubated for 90 min at 4°C with a one-tenth volume of phosphocellulose P11 (Whatman, Hillsboro, OR) prepared as described [Y. Wang and P. J. Roach, in *Protein Phosphorylation: A Practical Approach*, D. G. Hardie, Ed. (IRL Press, Oxford, 1993), pp. 121–144]. The phosphocellulose was washed with 5 volumes of buffer A containing 50 mM NaCl. Bound proteins were eluted in buffer A containing 500 mM NaCl and 20 mM β-glycerolphosphate. The phosphocellulose eluate fraction was dialyzed against buffer A containing 25 mM NaCl and 20 mM β-glycerolphosphate and loaded onto a Mono Q PC 1.6/5 column (Pharmacia) equilibrated in the same buffer. The Mono Q column was eluted with a linear gradient up to 1 M NaCl in the same buffer.

13. The Mono Q fractions containing kinase activity were pooled and loaded onto a 5 to 20% sucrose gradient in buffer A containing 20 mM β-glycerolphosphate. The gradient was centrifuged at 40,000 rpm for 16 hours in a Beckman SW55 rotor at 4°C. The fractions were collected and assayed for Xcdc25-specific kinase activity (29).
14. Proteins were separated by electrophoresis and blotted onto Problot (Applied Biosystems). p67 was excised and digested with sequencing-grade trypsin (Boehringer Mannheim) in the presence of hydrogenated Triton X-100 as described [J. Fernandez, L. Andrews, S. M. Mische, *Anal. Biochem.* **218**, 112 (1994)]. The tryptic peptides were separated by reverse phase chromatography in an μRPC C2/C18 2.1/10 column with the use of the SMART system (Pharmacia). Peptide sequence analysis was performed with an ABI 476A sequencer (Applied Biosystems) in the Protein/Peptide Micro Analytical Facility at the California Institute of Technology.
15. Three degenerate primers corresponding to the peptide sequences KKXLX(T/G)TPNY\APEVL, (A/S)GANTTP, and XX(D/I)APSTIDQ (*30*) were designed: p67-1, AA(A/G)AA(A/G)AA(T/C)(T/C)TITG(T/C)(G/A)(G/C)-IACICC; p67-2, CCAIGGIGTIGT(A/G)TTIGCICC; and p67-3, GCICCI(A/T)(G/C)IA(C/T)IAT(T/C/A)GA(T/C)-CA. X denotes an unreadable amino acid residue. PCR was done with *AmpliTaq* DNA polymerase (Perkin-Elmer), the p67-1 and p67-2 primers, and *Xenopus* oocyte cDNA for 35 cycles at 94°C for 1 min, 45°C for 2 min, and 72°C for 3 min. The reaction yielded a single 850-bp product. A PCR reaction with primer p67-3 indicated that the third peptide sequence was also encoded in the 850-bp fragment.
16. A *Xenopus* oocyte cDNA library in the pAX-NMT vector (*2*) was screened by colony hybridization with the 850-bp PCR fragment that had been labeled in the presence of α-^{32}P-labeled deoxycytidine triphosphate by the random primer method. A Xho I–to–Xho I fragment from the cDNA containing the full coding sequence of Ptx1 was subcloned into pBluescript SK$^-$ (pBluescript-Ptx1). Plasmids containing nested deletions of the cDNA generated by the Erase-a-base system (Promega Biotech) were sequenced with an ABI 373A automated DNA sequencer (Applied Biosystems).
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22. The catalytically inactive N172A mutant of Ptx1 was created by PCR with the following oligonucleotides: p67-N172A-1, AACAGGGCCCCGAGCTTGAGGTC-TCTGTG; and p67-N172A-2, TCGGGGCCCTGTTC-CTTAATGATGAAATGGAGG.
23. For purification of various His6-Ptx1 proteins, Sf9 insect cells (2×10^7 cells) were treated for 3 hours with okadaic acid 46 hours after infection. The cells were harvested, washed once with phosphate-buffered saline, and lysed in 1 ml of buffer B [20 mM β-glycerolphosphate, 10 mM Hepes-KOH (pH 7.5), 0.1 mM vanadate, 5 mM EGTA, 5 mM β-mercaptoethanol, 10 μM phosphoserine, 10 μM phosphothreonine, and 10 μM phosphotyrosine] containing 150 mM NaCl, 1% CHAPS, 3 μM microcystin, 1 mM PMSF, pepstatin (10 μg/ml), chymostatin (10 μg/ml), and leupeptin (10 μg/ml). The clarified lysate was incubated with nickel agarose, which was subsequently washed with buffer B (containing 500 mM NaCl and 0.1% CHAPS) and eluted with buffer B (containing 25 mM NaCl and 200 mM imidazole). The eluate was supplemented with 2 mM dithiothreitol (DTT) and 1 mM EDTA and dialyzed against 10 mM Hepes-KOH (pH 7.5), 10 mM NaCl, 1 mM DTT, and 1 mM EDTA for 2 hours at 4°C. Portions were frozen in liquid N_2 and stored at −80°C.
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30. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
31. We thank members of the Dunphy lab for comments on the manuscript, P. R. Mueller for the *Xenopus* oocyte cDNA library, and D. S. Krapf and G. Hathaway for peptide sequencing. Supported in part by a grant from NIH. W.G.D. is an investigator of the Howard Hughes Medical Institute.

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A Model of Host-Microbial Interactions in an Open Mammalian Ecosystem

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The maintenance and significance of the complex populations of microbes present in the mammalian intestine are poorly understood. Comparison of conventionally housed and germ-free NMRI mice revealed that production of fucosylated glycoconjugates and an α1,2-fucosyltransferase messenger RNA in the small-intestinal epithelium requires the normal microflora. Colonization of germ-free mice with *Bacteroides thetaiotaomicron*, a component of this flora, restored the fucosylation program, whereas an isogenic strain carrying a transposon insertion that disrupts its ability to use L-fucose as a carbon source did not. Simplified models such as this should aid the study of open microbial ecosystems.

A complex and dynamic microbial ecosystem inhabits the human intestine. Establishment of a microflora begins at birth and progresses through a series of colonizations involving more than 400 bacterial species (*1*). These colonizations result in a metabolically active entity in which anaerobes predominate (*2*). A specific region of the intestine's duodenal-colonic axis will at any given time contain resident (autochthonous) bacterial species and a variable set of transient (allochthonous) species that temporarily occupy a functionally "empty" niche (*3*). Disruption of this open ecosystem—for example, with antibiotics—has revealed the essential role played by the microbiota in preventing infectious diseases (*4*).

The factors that allow components of this indigenous microbiota to establish and maintain their regional habitats are largely unknown. The stability of the ecosystem is all the more remarkable given that the intestinal epithelium is rapidly renewed throughout life (*5*). Integration of the ecosystem into the host may be achieved, at least in part, through dynamic interactions that allow microbes to modify cellular differentiation programs and thus create favorable niches. One way of identifying such programs is by comparing genetically identical mice that (i) have never been exposed to any microorganisms (germ-free, GF); (ii) have been raised with a "normal" functional microbiota (conventional, CONV), and

(Iii) have been raised in a germ-free state for a period of time and then exposed to an intact microbiota or a selected group of its component organisms (ex–germ-free, XGF) (6). Here we have used XGF mice to identify a single, genetically manipulatable resident bacterial species that reproduces an epithelial differentiation program normally specified at the completion of gut morphogenesis by the indigenous flora.

A panel of lectins (7) was used to define cell lineage–specific, spatial, and temporal patterns of glycoconjugate accumulation in the intestinal epithelium of immunocompetent inbred NMRI/KI mice raised under GF or CONV states (8–10). Glycoconjugates were examined because their production provides a sensitive marker of gut epithelial differentiation (7, 11) and can be modified by exogenous factors (12). Glycoconjugates are also used by bacteria as a source of nutrients (13) and can serve as receptors for their adhesins (14).

A comparison of postnatal day 1 (P1) to P90 GF and CONV mice (15) revealed that sustained production of fucosylated intestinal glycoconjugates after P21 requires components of the microbiota. Proliferation in the intestinal epithelium is confined to the crypts of Lieberkühn (5). Each villus is supplied by several crypts that surround its base. Four principal lineages arise from a multipotent crypt stem cell. Paneth cells differentiate as they move down to the base of the crypt where they produce antimicrobial peptides (16). Absorptive enterocytes, enteroendocrine cells, and mucus-producing goblet cells differentiate as they migrate up from each crypt to the apex of an associated villus where they undergo apoptosis and exfoliation, a sequence that takes 3 to 5 days (5). At P21, lineage-specific changes in fucosylation begin to occur in both GF and CONV mice (17) (Fig. 1, A to D). Paneth cells acquire fucosylated glycoconjugates as do cells in the upper half of some crypts. These fucose$^+$ crypts occur as multicrypt patches. Typically, only a small subset of villi/patch have fucose$^+$ enterocytes and goblet cells (Fig. 1, C and D). The number and size of patches expand between P21 and P28 in CONV mice (Fig. 1, E to G). In GF animals, Paneth cell staining expands to include all crypts, but fucosylated epitopes are lost from the middle and upper portion of crypts and their associated villi (Fig. 1, I and K). The rate of clearance of fucose$^+$ cells from P21 to P28 GF crypt-villus units equals the rate of cell migration as measured by pulse labeling with 5-bromo-2′-deoxyuridine, indicating that loss of fucosylated glycoconjugates reflects cellular replacement rather than suppression of production within an existing population. Adult CONV animals express fucosylated epitopes in their enterocytic, goblet, and Paneth cell lineages with a prominent duodenal-ileal gradient of increasing levels and extent of fucosylation (Fig. 1J). In contrast, adult GF mice express fucosylated glycoconjugates in their Paneth cells only (Fig. 1K). The cecal and colonic epithelium of CONV and GF mice have an identical fucose$^+$ phenotype.

These findings indicate that initiation of fucosylated glycoconjugate production in the small intestine occurs independently of its microbiota but that the microbiota is required to complete the fucosylation program between P21 and P28. The patterns of lectin binding in GF and CONV epithelium suggest

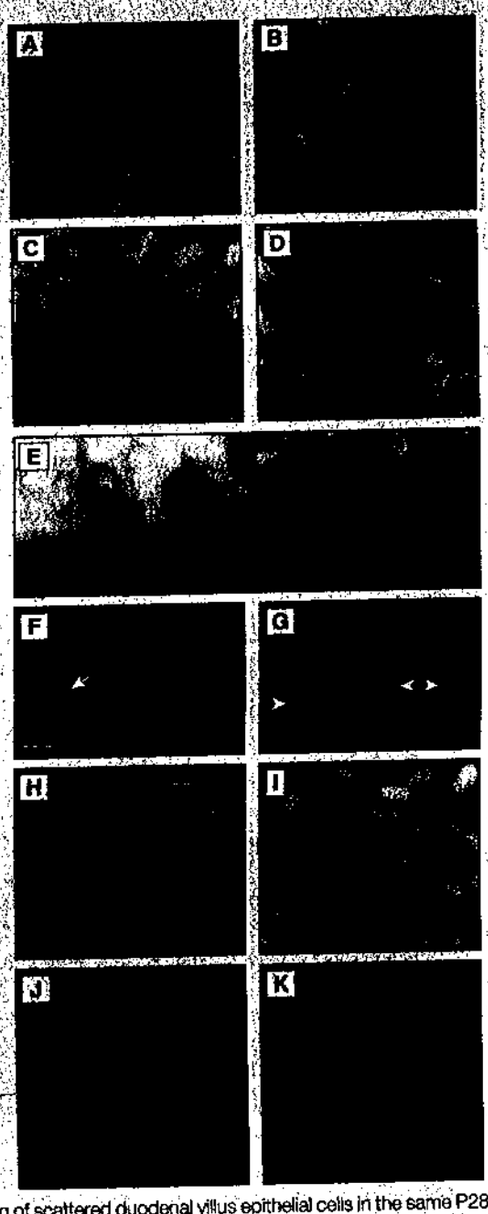


Fig. 1. The microbiota is required to sustain production of α1,2-linked fucosylated glycoconjugates in the ileal epithelium. (**A** and **B**) Wholemounts showing villi from the distal third of the small intestine (ileum) of P17 GF and CONV animals, stained with peroxidase-conjugated UEA1 (17). At this developmental stage, there are no differences in the patterns of fucosylation between CONV (A) and GF (B) animals. Fucose$^+$ goblet cells (brown, arrow) are more numerous in the distal than in the proximal half of the small intestine. Villus enterocytes (white) and crypt Paneth cells (not visible) are fucose$^-$ throughout the duodenal-ileal axis. (**C** and **D**) By P21, isolated fucose$^+$ villi (detected with UEA1) are apparent in CONV and GF mice. In CONV animals (C), fucosylated glycoconjugates are present in crypts surrounding the base of the positive villus (arrow). This is not the case in GF mice (D), where fucosylation is being extinguished in the crypts. (**E**) Side view of an ileal wholemount from a P23 CONV mouse, stained with peroxidase-UEA1. Acquisition of the fucose$^+$ phenotype involves a progressive upward migration of a band of positive cells that encircles the villus. (**F** and **G**) Section from a fucose$^+$ patch similar to that shown in (E), incubated with tetramethylrhodamine isothiocyanate (TRITC)–UEA1 and digoxigenin-conjugated OPA (detected with FITC–conjugated sheep antibody to digoxigenin). (F) UEA1 staining alone and (G) double exposure showing colocalization of the two lectins, establishing that there is an induction of α1,2- and α1,6-linked fucosylated glycoconjugates in villus enterocytes [closed arrow in (F)] and goblet cells [open arrow in (F)]. Arrowheads in (G) point to the leading edges of columns of upwardly migrating fucose$^+$ epithelial cells in adjacent villi. (**H** and **I**) Ileal wholemount from P25 CONV (H) and GF (I) mice. Without the microbiota, fucosylation is extinguished. (**J** and **K**) Ileal wholemounts from P28 CONV and GF mice. The fucosylation program is fully expressed in CONV mice (J) where all of the patches of UEA1$^+$ ileal crypt-villus units have coalesced. In GF animals (K), only Paneth cells located at the base of crypts are UEA1$^+$. Inset in (J) shows UEA1 staining of scattered duodenal villus epithelial cells in the same P28 CONV wholemount, emphasizing the marked duodenal-ileal gradient of fucosylation. All wholemounts were photographed under a 6.3× objective. Bar in (F), 25 μm.

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that the microbiota affects expression of host fucosyltransferases (FT) that add L-fucose in α1,2 and α1,6 linkages (*18*) (Fig. 1G).

XGF experiments ($n = 3$) demonstrated that the fucosylation program could be reinitiated and completed in the adult intestine by inoculation of GF mice with gut flora from CONV animals (*19*). Quantitation of anaerobe colony-forming units (CFU) (*20*) established that 48 hours after inoculation, the density of viable organisms in the feces of XGF mice was equivalent to that of identically aged CONV mice. Fucosylation progressed in the same manner as observed in P21 to P28 CONV mice whether GF mice were conventionalized at P28 or P70; fucose$^+$ patches first appeared 7 days after conventionalization, and the CONV pattern was fully recapitulated within 14 days (Fig. 2, A to C). Reverse transcriptase–polymerase chain reaction (RT-PCR) of total cellular duodenal, ileal, and colonic RNA from GF and XGF animals (*21*) confirmed that production of fucosylated glycoconjugates is associated with accumulation of a host α1,2-FT mRNA (Fig. 2D).

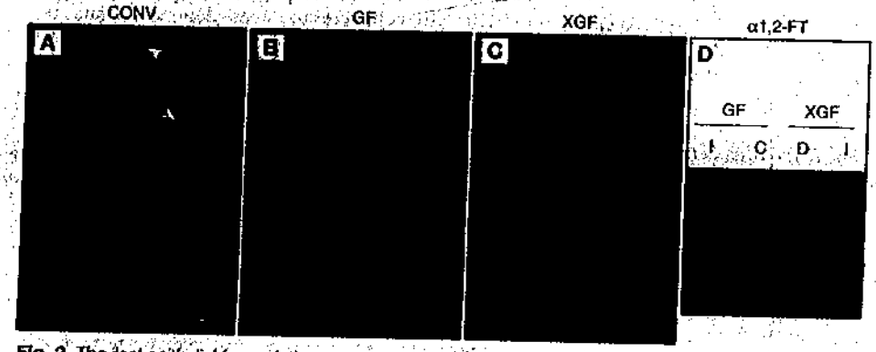


Fig. 2. The ileal epithelial fucosylation program can be reinitiated and completed in the adult intestine by inoculation of GF NMRI mice with a CONV microflora. (**A** to **C**) Sections of ileal crypt-villus junctions from P42 CONV and GF mice, and a P42 XGF mouse exposed to a CONV flora at P28. Sections were stained with TRITC-UEA1 and a FITC-conjugated goat antibody to mouse immunoglobulin A (IgA). (A) CONV mice express fucosylated glycoconjugates (red) in Paneth cells at the crypt base (open arrow) and in villus enterocytes (closed arrow; note Golgi and brush border staining) and goblet cells (closed arrowhead). The lamina propria contains numerous IgA$^+$ cells (green). (B). GF mice express fucosylated glycoconjugates in their Paneth cells only (open arrow). IgA$^+$ cells are rare. (C) XGF mice recapitulate the CONV pattern of fucosylation within 7 days after initial exposure to bacteria. (**D**) Representative RT-PCR analysis of duodenal (D), ileal (I), and colonic (C) RNA prepared from a P42 GF mouse and a P42 XGF animal inoculated 7 days earlier with a CONV microflora. A 230-bp fragment derived from a mouse α1,2-FT mRNA (*21*) is present in XGF ileum but not duodenum. The band is absent in GF ileum but present in GF colon. The presence of this mRNA correlates with the distribution of fucosylated glycoconjugates in GF and XGF animals. Actin mRNA levels were shown by RT-PCR to be equivalent in the RNA samples. Bar, 25 μm.

Differences in CFU per milliliter of ileal contents were detected among individual mice. We took advantage of this variability to show that the extent of fucosylation at 7 to 21 days correlated with the density of colonizing organisms (Fig. 3). A minimum of 10^4 bacteria per milliliter was required to elicit a "patchy" fucose$^+$ phenotype; 10^7 bacteria per milliliter fully recapitulated the CONV pattern.

Colonization of the duodenum was less efficient than in the ileum (CFU = two orders of magnitude lower). Nonetheless, levels of bacteria sufficient to induce fucosylation in the ileum (10^6 to 10^7 CFU/ml) were unable to do so in the duodenum, demonstrating the organism's capacity to reproduce the region-specific CONV pattern of fucosylated glycoconjugate production.

We examined whether the bacteria's ability to metabolize L-fucose correlates with its capacity to induce epithelial fucosylation. Fu-4 is an isogenic *B. thetaiotaomicron* strain that contains an uncharacterized insertion of *Tn4351* rendering it unable to use L-fucose or D-arabinose as carbon sources (*13*). Southern (DNA) blots established that this transposon was inserted into a single site within the genome (*13*). The insertion does not block the organism's ability to take up [^{3}H]L-fucose or to colonize and compete with the isogenic wild-type strain (VPI 5482) in adult GF Balb/c cecum (*13*). Fu-4 colonizes the NMRI ileum and cecum at levels equivalent to those of wild-type bacteria ($\leq 10^7$ CFU per milliliter of ileal contents) but is much less efficient at inducing epithelial fucosylation. 10^7 CFU of Fu-4 per milliliter produced the scattered pattern of fucose$^+$ villi seen with only 10^4 CFU of the

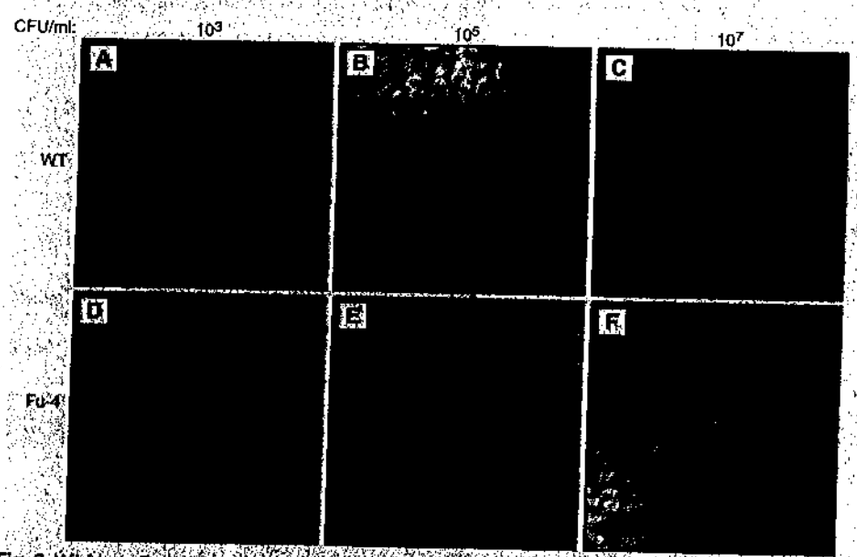


Fig. 3. Wild-type *B. thetaiotaomicron*, but not an isogenic strain that lacks the ability to utilize L-fucose, can signal ileal epithelial fucosylation. Ileal wholemounts, prepared 7 days after monocontamination of P28 male GF mice with VPI-5482 (wild-type) or Fu-4 strains and stained with peroxidase-UEA1. Results obtained from mice with 10^3 to 10^7 CFU per milliliter of ileal contents illustrate the correlation between ileal fucosylation and the density of colonizing organisms. A similar correlation was observed at 14 and 21 days (data not shown).

wild-type strain per milliliter (Fig. 3) (*24*).

Two results suggest that *B. thetaiotaomicron* affects fucosylation without directly binding to the epithelium and that soluble mediators may be involved. Sections of unperfused CONV or XGF intestine were treated with Gram or Warthin-Starry silver stains. In CONV mice, or GF mice rendered XGF with a CONV flora, components of the indigenous microflora were readily seen bound to ileal villus epithelial cells. No binding was evident throughout the duodenal-ileal axis at any time point after inoculation of GF mice with the wild-type or mutant strains, even when their CFU per milliliter of ileal contents was equivalent to that in XGF animals colonized with a CONV flora ($n = 5$ per group per time point). Wild-type and Fu-4 strains were also labeled with fluorescein isothiocyanate (FITC) after growth to mid-log phase in thioglycollate medium or after they were physically removed from the cecum of XGF mice (in vivo selection) and then applied to sections of duodenum, jejunum, ileum, and cecum. Neither strain, harvested under these two conditions, bound to CONV, GF, or XGF gut epithelium (*25*, *26*).

Fucosylated glycoconjugates mediate attachment of pathogens to epithelial surfaces (*14*) and also provide a source of nutrients for members of the indigenous flora (*13*). The ability of *B. thetaiotaomicron* to regulate fucosylation programs in the distal intestine could affect the ability of other components of the normal flora to establish a stable niche or could affect the vulnerability of the intestine to colonization by pathogens.

Identifying molecular interactions between indigenous microbes and host cells that confer stability to their ecosystems should help our appreciation of what goes wrong when pathogens gain control and may provide new strategies for preventing or treating infections. However, members of the microflora rarely produce the dramatic and distinctive phenotypes seen in models of pathogenesis. We have reduced the intestine's ecosystem to a simplified model in which a single, genetically manipulatable, nonpathogenic member of the normal flora induces a specific and easily identifiable response. Features of this model include an apparent association between *B. thetaiotaomicron*'s capacity to utilize L-fucose and its ability to induce fucosylated glycoconjugate production in its host and the fact that a wild-type fucose-utilizing strain must achieve a critical density in the distal intestine to trigger epithelial fucosylation. The density dependence may reflect either a microbial signal that must reach a critical threshold before an epithelial response can be induced or a density-dependent change in the bacteria's metabolic properties. The integrity of the locus disrupted by the transposon appears to be necessary for the bacteria's contribution to the cross-talk, although this hypothesis will have to be confirmed by further genetic analyses; as will its contribution to fucose utilization. The induction of an α1,2-FT provides both a marker and a mediator of the fucosylation program. This bacterial locus and mammalian gene constitute the starting point for further dissection of a dialogue symbolic of the ongoing interaction between indigenous organisms and their host.

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18. Sections prepared along the duodenal-colonic axis of P28 to P90 CONV and GF NMRI/Kl gut were stained with UEA1 (specificity: Fucα1,2Galβ), OPA (Fucα1,6/3*N*-acetylglucosamine (GlcNAc)], AAA (α-L-fucose), the H2 mAb (Fucα1,2Galβ1,4GlcNAc), *Arachis hypogae* agglutinin (Galβ1,3GalNAc), *Dolichos biflorus* agglutinin (GalNAcα1,3Gal), *Maakia amurensis* agglutinin (NeuAcα2,3Gal), and *Griffonia simplicifolia* type II agglutinin (GlcNAcα1,3Gal/Glc). Only the fucose-specific markers showed differences in binding. These differences were limited to the small intestinal epithelium.
19. The intact cecum and colon from CONV P42 mice were homogenized in 10 ml of sterile 0.9% NaCl. Samples (0.5 ml) were spread on the fur and inoculated into the mouth and rectum of P28 or P70 GF animals ($n = 5$ mice per time point per experiment). XGF mice were housed with CONV animals.
20. Three samples of luminal contents, recovered from duodenum, jejunum, ileum, and cecum of each animal, were inoculated individually into thioglycollate medium. Serial dilutions were made from this inoculum, samples plated onto blood agar, and CFU counted after a 3-day incubation at 37°C under anaerobic conditions.
21. Total cellular RNA was extracted with RNAzol B (Tel-Test, Houston, TX). RT-PCR reactions were carried out with 250 ng of RNA, and primers designed from a mouse α1,2-FT cDNA [P50 (5′-GCGAATATGCCACGCTGTTTGC-3′ and 5′-GCACGGGTATCCTGTGAAGCGC-3′)], under conditions suggested by the manufacturer of the GeneAmpThermostable *rTth* kit (Perkin-Elmer). Actin primers were used as internal controls to detect actin mRNA and to exclude any genomic DNA contamination [5′-TGGAATCCTGTGGCATCCATGAAAC-3′ and 5′-TAAAACGCAGCTCAGTAACAGTCCG-3′; actin mRNA, 320–base pair (bp) product; actin gene, 470-bp product].
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23. P28 GF animals were inoculated with *B. thetaiotaomicron* strain VPI-5482 (wild-type) or Fu-4 (isogenic with VPI-5482) (*13*) and housed in GF isolators until they were killed 2, 5, 7, 14, and 21 days later (four to five mice per time point per experiment; $n = 3$ independent experiments). The gastrointestinal tract was removed en bloc under sterile conditions. Luminal contents were recovered from a 1-cm segment located in the mid-jejunum, a 1-cm segment located 1 cm proximal to the ileal-cecal valve, and from a 0.5-cm segment of the proximal cecum. CFU were determined (*20*). The identity of the recovered organisms was verified by Gram stain, by their failure to grow aerobically, and by their antibiotic resistance phenotype (VPI 5482 is Kan^RErm^S; Fu-4 is Kan^RErm^R) (*13*).
24. Chi-square analysis was used to test the following hypotheses: (*i*) animals with 10^4 to 10^6 CFU/ml will exhibit an intermediate fucosylation phenotype, defined as 5 to 75% of ileal villi being fucose$^+$; and (*ii*) animals with $\geq 10^7$ CFU/ml will produce the CONV phenotype, defined as >95% of ileal villi being fucose$^+$. χ^2 test value = 3.841 ($\alpha = 0.05$; df = 1). Six out of six mice with 10^4 to 10^6 CFU of wild-type bacteria per milliliter of ileal contents had an intermediate phenotype ($\chi^2 = 0$)—accept hypothesis (*i*); 0/13 mice with 10^4 to 10^6 CFU of Fu-4 per milliliter had an intermediate phenotype ($\chi^2 = 13$)—reject hypothesis (*i*). Six out of six mice with $\geq 10^7$ CFU of wild-type bacteria per milliliter exhibited a CONV phenotype ($\chi^2 = 0$)—accept hypothesis (*ii*); 0/10 mice with $>10^7$ CFU of Fu-4 per milliliter had a CONV phenotype ($\chi^2 = 10$)—reject hypothesis (*ii*).
25. Bacteria were labeled with FITC as described (*26*) with the following modification: cecal contents were washed five times in 0.2 M sodium carbonate buffer (pH 9) and allowed to settle for 5 min between washes. The supernatant was taken for labeling. Methods for incubating Bouin's- or formalin-fixed or frozen sections of intestinal Swiss rolls (*17*) with labeled bacteria for in situ binding assays are described in (*26*).
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