How glycan metabolism shapes the human gut microbiota

Nicole M. Koropatkin, Elizabeth A. Cameron and Eric C. Martens

Abstract | Symbiotic microorganisms that reside in the human intestine are adept at foraging glycans and polysaccharides, including those in dietary plants (starch, hemicellulose and pectin), animal-derived cartilage and tissue (glycosaminoglycans and *N*-linked glycans), and host mucus (*O*-linked glycans). Fluctuations in the abundance of dietary and endogenous glycans, combined with the immense chemical variation among these molecules, create a dynamic and heterogeneous environment in which gut microorganisms proliferate. In this Review, we describe how glycans shape the composition of the gut microbiota over various periods of time, the mechanisms by which individual microorganisms degrade these glycans, and potential opportunities to intentionally influence this ecosystem for better health and nutrition.

Inflammatory bowel disease
A group of pathologies that are characterized by inflammation in the gut; the most notable examples are Crohn's disease and ulcerative colitis, which involve inflammation in the distal small intestine or the colon. These diseases are thought to stem from a congruence of host susceptibility factors (such as a genetic predisposition to uncontrolled inflammatory responses or reduced mucosal immunity) and stimulation by environmental or microbiological (bacterial and viral) triggers.

Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, Michigan 48109, USA.
Correspondence to E.C.M.
e-mail: emartens@umich.edu
doi:10.1038/nrmicro2746
Published online
11 April 2012

The dense microbial community (the microbiota) that is established in the human intestine shortly after birth has a profound effect on human health and physiology, providing benefits such as modulation of immune development[1], digestion of recalcitrant dietary nutrients[2] and inhibition of pathogen colonization[3]. However, abnormalities in the composition of the microbiota (dysbiosis) have been implicated in several disease states, including inflammatory bowel disease (IBD)[4], colon cancer[5], antibiotic-associated colitis[6] and obesity[7]. Dysbiosis is postulated to occur when a typically healthy microbial community becomes unbalanced owing to either increased abundance of potentially harmful microorganisms[8–10] or increased flux through harmful metabolic pathways. The normal composition of the gut microbiota, both at single time points and over longer periods of human life, has been deeply probed in the past few years[11–15]. Current investigations seek to define the dominant forces shaping the microbiota in order to better understand the causes of dysbiosis and to develop strategies to restore a healthy community.

One major factor shaping the composition and physiology of the microbiota is the influx of glycans into the intestine, mostly from diet and host mucosal secretions. Humans consume dozens of different plant- and animal-derived dietary glycans, most of which cannot be degraded by enzymes encoded in the human genome. Microbial fermentation transforms these indigestible glycans into short-chain fatty acids (SCFAs), which serve as nutrients for colonocytes and other gut epithelial cells. Gut microorganisms therefore have a pivotal symbiotic role in helping humans access calories from otherwise indigestible nutrients[16]. Microbial species differ in their glycan preferences. Thus, selective consumption of these nutrients can influence which microbial groups proliferate and persist in the gastrointestinal tract, implicating dietary glycans as a non-invasive strategy by which humans can directly influence the balance of species in the gut.

In addition to dietary glycans, which fluctuate in composition and abundance, some members of the microbiota can degrade the glycans found in host mucous secretions or shed epithelial cells. These endogenous glycans provide consistent sources of nutrients to the microbiota, despite potentially drastic changes in the host diet. Endogenous host glycans are presented to bacteria in the intestinal lumen as *O*-linked glycans attached to secreted or cell-associated mucin glycoproteins (the major component of mucus) or as *N*-linked glycans present in shed epithelial cells. Some proportion of the endogenous glycans is likely to be concentrated directly adjacent to host tissue, in the protective mucous layer. The ability of certain microorganisms to penetrate and degrade mucus as a nutrient source positions them in close proximity to host cells. As a consequence, species that are adept at using these endogenous glycans may exert a disproportionate effect on colonic health, especially during states of dysbiosis.

This Review explores the role of glycans in shaping the gut microbiota. We first consider the assembly of this microbiota from birth to adulthood and how this process is catalysed by changes in glycan availability.

Glycans
Polymers of multiple simple sugars connected by covalent linkages. Glycans may be attached to other molecules such as lipids (forming glycolipids) and proteins (forming glycoproteins). Like nucleic acids, glycans have polarity: a linear molecule has one reducing end and one non-reducing end. Here, the term glycan is used synonymously with polysaccharide.

Short-chain fatty acids
Linear and branched fatty acids that contain six or fewer carbon atoms and are produced in addition to lactic and formic acids as end products of bacterial fermentation. These molecules are also referred to as volatile fatty acids. Examples include acetic, propionic and butyric acids.

Mucus
A viscous mixture consisting predominantly of mucin glycoproteins, which may be either attached to cell membranes or secreted from the cell in soluble form. Mucus frequently contains other secreted host compounds, such as secretory immunoglobulin A and antimicrobial peptides.

Glycosidic linkages
Chemical connections that occur between numbered carbon atoms in two sugar monomers, mediated by a shared oxygen atom. These bonds can be in the α- or β-conformation, and multiple linkages may be connected in linear or branched chains to construct more complex glycan structures.

Hemicellulose
A heterogeneous class of glycans that is found associated with cellulose in the matrix of plant cell walls. Unlike highly insoluble cellulose, hemicelluloses have more amorphous and flexible structures that help bind cellulose to pectin fibrils. The type and amount of hemicellulose in the plant cell wall is dependent on the botanical origin and includes molecules such as xylan, xyloglucan, galactomannan and glucomannan.

We then consider the mechanisms of glycan acquisition that have evolved in some of the most abundant (and therefore successful) members of the human gut microbiota. Finally, we consider how the spatial abundance and diversity of glycans in different gut regions (that is, the lumen versus the mucosa, and the proximal versus the distal intestine) may select for regional microbial subpopulations, some of which may be of particular interest in pathologies resulting from dysbiosis.

Glycans shape the gut microbiota

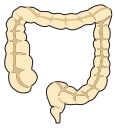
The glycan landscape in the human gut. The biochemistry of the various host and dietary glycans that enter the gut is exceptionally diverse. Many different glycosidic linkages may be incorporated into a single polymer, so degradation of these polymers requires several linkage-specific degradative enzymes. The human genome is capable of fully degrading a small subset of glycans — namely, starch, lactose and sucrose, each of which contains only one or two different linkages. By contrast, some microorganisms in the intestinal tract target dozens of glycans and possess the corresponding enzymatic tools for depolymerizing each of these molecules into their component sugars. Gut microorganisms vary widely in the number of different glycans that they are capable of targeting[17,18]. For example, the human gut symbiont *Bacteroides thetaiotaomicron* can degrade more than a dozen types of glycan[17,19], whereas some species are restricted to one or a few types[18]. From an ecological perspective, species with broad glycan-degrading abilities can be thought of as 'generalists' that shift their metabolism from meal to meal, whereas species with narrower glycan-degrading potential can be considered 'specialists' that focus on one or a few glycans. Specialists run the risk of becoming extinct in a host if their preferred nutrients wane for too long, so such microorganisms would most probably evolve to degrade ubiquitously abundant dietary glycans or host-derived mucins.

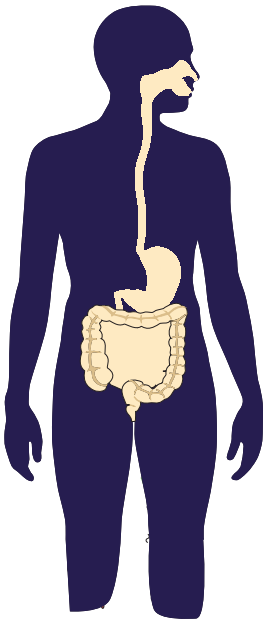
The task of degrading glycans in the gut is further complicated by the fact that many of these substrates are sequestered in larger structures such as the plant cell wall or in regional microhabitats such as the mucous layer, which may be difficult for some species to access (FIG. 1). Plant cell wall glycans (cellulose, hemicellulose and pectin) are intertwined in a polysaccharide matrix in many foods. In addition, hemicelluloses and pectins vary substantially in their fine-level structure between plant sources[20,21]. Thus, the dietary glycans available in wholewheat bran differ from those available in a potato skin or an apple. Intracellular plant glycans such as starch may be contained either in insoluble granules or as chemical forms that are resistant to degradative enzymes (BOX 1). Cooking, milling and other food preparation processes can influence the abundance of these 'resistant starch' (RS) forms as well as the availability of other plant glycans to intestinal microorganisms. Finally, the chemical diversity of endogenous *O*- and *N*-linked glycans (hundreds of different structures may be attached to a single mucin glycoprotein[22]) requires that mucosal bacteria produce many different degradative enzymes (which is a substantial metabolic investment) to effectively use these heterogeneous polymers. Indeed, one reason why such glycan diversity exists in secreted mucus could be to deter microbial species from becoming too efficient at harvesting these structures, thus protecting the integrity of this important barrier.

Regardless of the particular glycan substrate degraded by gut microorganisms, the colonic epithelium benefits from the products of this microbial metabolism by absorbing SCFAs such as butyrate, propionate and acetate. The butyrate that is produced in the colon exerts local effects on the colonic epithelium because it is a preferred energy source of colonocytes and has also been associated with suppressed growth of colonic tumours[23]. Acetate and propionate are absorbed into the bloodstream and travel to the liver, where they are incorporated into lipid and glucose metabolism, respectively[24]. In addition to being absorbed by the host, acetate in the colonic environment may augment the production of butyrate by some species[25,26] and prevent colonization of some enteric pathogens[27].

Glycan-catalysed changes in the infant gut microbiota. The human gut microbiota is established in the first few days of life and is initially seeded from the microorganisms that are encountered during passage through the birth canal and during incidental environmental exposures. After this initial colonization, the host experiences a series of progressive changes (ecological successions) in the richness and diversity of its gut inhabitants[28,29]. Early in life, few types of glycan transit the gut, as diet is restricted to mother's milk or formula. Many more glycans become available to the microbiota post-weaning, as a diet rich in plant and animal matter is introduced. Despite potential fluctuations in the intake of dietary carbohydrates, endogenous host glycans represent a stable source of nutrients for the microbiota throughout the host's lifespan. However, during both dietary phases (pre- and post-weaning), the carbohydrate composition of the gut is one important factor that guides the establishment of the microbial community.

Immediately after birth, infants consume a steady diet of breast milk or infant formula. Several hundred different glycan structures have been identified in human breast milk[30,31] with the primary components being lactose, glucose, galactose, *N*-acetylglucosamine, fucose, sialic acid and a mixture of complex human milk oligosaccharides (HMOs). These HMOs, which are highly diverse glycans that seem to be abundant in the breast milk of humans but not that of other mammals[32], are composed of repeating and variably branched lactose or *N*-acetyl-lactosamine units that are often decorated with sialic acid and fucose monosaccharides[30,31] (FIG. 1). HMOs have structural similarities to human blood group antigens and the *O*-linked structures present in mucus. In contrast to the simpler lactose, most HMOs are not digested by human enzymes, suggesting that they have evolved as natural prebiotics to guide the development of the infant gut microbiota by selectively feeding certain species[33–35].





Box 1 | **Starch in the human diet**

Starch is a plant storage glycan that consists of glucose monomers joined via α1,4 glycosidic linkages, with possible α1,6 branching. Two common forms of starch are defined on the basis of their molecular structure: amylose is an unbranched α1,4-linked polymer that tends to form helices which further aggregate into crystalline structures, and amylopectin is a less dense form containing variable amounts of α1,6-linked branches. Mammals secrete several amylase and glucoamylase enzymes in the upper gastrointestinal tract; these enzymes degrade some parts of ingested starch, and the resulting glucose is directly absorbed in the small intestine. Dietary starches are often subdivided into rapidly digesting, slowly digesting and resistant forms[109,110]. Resistant starch (RS) is the portion of dietary starch that cannot be accessed by human intestinal enzymes and therefore transits to the colon undigested. The properties of RS can be altered by cooking and subsequent food-handling steps: gelatinization occurs when starch-containing foods are heated in the presence of water, forcing the hydration of starch granules and subsequent leaking of solubilized starch chains, and retrogradation occurs when a gelatinized product is cooled for sufficient time to allow amylose regions to recrystallize. Four types of RS are recognized: RS1 is physically inaccessible starch that is present in seeds or partly milled whole grains, RS2 is naturally granular starch similar to that contained in uncooked potato, RS3 is retrograded starch (composed largely of recrystallized amylose regions) and RS4 is starch that has been chemically or enzymatically modified to avoid digestion. Many studies suggest that supplementation of the diet with RS improves colonic health and stimulates the production of short-chain fatty acids, particularly butyrate, which has been linked to a range of colonic health benefits. In addition, creating foods from RS eliminates the amount of glucose that is directly liberated during digestion, which changes the impact on human physiology and makes RS more acceptable in a diabetic diet.

found measurable differences in the bacteria that were present in adult mice several weeks after weaning, suggests that some members of the mammalian microbiota that have the capacity to affect host health can be selected by early nutritional conditions and persist in the gut after these conditions have been removed.

The ability of some members of the microbiota to access the glycans attached to mucus may also have a role in early colonization by providing some bacteria with a source of endogenous nutrients during a period when dietary glycans are absent. Owing to the chemical similarity of HMOs and *O*-linked mucin glycans (FIG. 1), bacterial strategies for degrading these polymers are likely to overlap. It was recently reported that, in the Gram-negative human gut symbiont *B. thetaiotaomicron*, the outer-membrane enzyme systems that are used to degrade *O*-linked glycans in host mucus[51–53] are also deployed during metabolism of HMOs[19]. An additional study used a germ-free female mouse colonized with a competing population of wild-type, mutant and complemented-mutant *B. thetaiotaomicron*; the mutant lacked five gene clusters that have been implicated in host glycan foraging in the adult gut, and the complemented mutant carried chromosomal copies of the deleted gene clusters. Although all three strains successfully colonized the mother to similar levels, in a model of natural intergenerational transmission the deletion mutant was outcompeted >200-fold relative to the wild-type and the complemented-mutant bacteria[53]. In this study, pre-weaned pups were exposed to similar amounts of each strain from their mother's faecal microbiota, but selectively retained the mucin-degrading wild-type and complemented strains. Thus, the ability to forage host glycans in the neonatal gut before the introduction of a more complex diet may be one key parameter that helps a species to establish colonization.

Post-weaning and adulthood. The carbohydrate composition of the human diet undergoes a somewhat abrupt change when the infant is ~6 months old, when complex foods such as cereals, fruits and vegetables are introduced. When such complex plant glycans enter the gut, the composition of the microbiota shifts, and microorganisms that prefer these glycans, such as members of the Gram-negative phylum Bacteroidetes and other species of the phyla Firmicutes amd Actinobacteria (that is, other than *Lactobacillus* and *Bifidobacterium* spp.), become more prevalent[17,28,29]. Recent culture-independent metagenomic studies have characterized the functionality of the microbial genes that are present at various time points in the developing human microbiota and noted that genes for plant carbohydrate degradation are present before the introduction of solid food[29,49]. These genes may be harboured in the genomes of glycan generalists such as *B. thetaiotaomicron*, which degrade milk oligosaccharides or host mucin glycans before weaning and shift their metabolism to dietary glycans as these polymers are introduced. The presence of glycan-adaptable species before weaning suggests that the gut microbiota is primed for the dietary change after weaning, perhaps because the cyclical faecal–oral transmission of microorganisms from parent to child selects for species that can target the glycans which are present in both the infant and adult gut.

As a fully omnivorous diet is achieved after weaning, the composition of the microbiota (as measured by the abundance of broad taxonomic groups) stabilizes and experiences fewer temporal changes[54–56]. Studies using culture-independent techniques to enumerate the human gut microbiota have found that two bacterial phyla, Firmicutes and Bacteroidetes, are numerically dominant in the adult microbiota[11,13,57]. However, it has been documented that the contribution made by a third phylum, Actinobacteria, is frequently underestimated by molecular approaches and the use of 'universal' primers for the 16S ribosomal RNA gene; therefore this phylum is likely to be more abundant than reported[58]. Firmicutes are usually the most abundant members; however, the ratio of bacteroidetes and firmicutes can change over time and be influenced by different diets, especially those that promote changes in host adiposity[7,57], although a mechanistic explanation for these changes remains to be found. Another human study examined the differences between the gut microbiota of African children who consumed a predominantly vegetarian, fibre-rich diet and European children who consumed a lower-fibre diet that is more typical of the diet of Western societies. This study found a higher prevalence of bacteroidetes and actinobacteria than of firmicutes and proteobacteria in the African children, and the opposite trend in European children, suggesting that the higher-fibre African diet is conducive to the growth of specific fibre-degrading species[59]. Interestingly, the

Germ-free mice
Mice that are raised in the complete absence of microbial colonization, usually following aseptic delivery by caesarian section and by housing the animals in sterile isolators that exclude access of environmental microorganisms. Other animal species such as rats, pigs and chickens have also been reared under germ-free conditions.

genera of the bacteroidetes were different between the two groups: the microbiota of the African children contained members of the genera *Prevotella* and *Xylanibacter*, the latter being a genus that is very rarely, if ever, detected in Western samples[7,11,60]. By contrast, the European children harboured *Bacteroides* and *Alistipes* as the dominant genera of bacteroidetes. In light of these clear differences at the genus level, an interesting approach for future work will be to measure the glycan-degrading abilities of these different bacteroidetes to determine whether they have evolved to specifically utilize the different glycans that are contained in each diet.

Beyond the influence of certain types of diet in shaping the composition of the microbiota, supplementing the diet with particular glycans can affect species abundance. Not all species that possess the potential to degrade a given glycan will do so successfully *in vivo*. For example, inulin and smaller fructo-oligosaccharides selectively increase the abundance of *Bifidobacterium* spp.[61], although many *Bacteroides* spp. are also able to use these glycans[62]. More recently, attention has focused on the ability of RS to direct changes in the composition of the microbiota (BOX 1). According to studies of human and animal feeding, some microbial species may be more adept than others at degrading various forms of RS and are responsive to diets augmented with this nutrient. Human consumption of some RS forms preferentially results in increases in the SCFA butyrate, which has been reported to exert anti-inflammatory[23,63–66] as well as antitumorigenic effects[23,67–70], and it has been suggested that this dietary intervention could be used as a therapeutic strategy for IBD[71]. Butyrate is produced by firmicutes but is rarely associated with the SCFA profiles of bacteroidetes[72,73]. Human volunteers who consume RS2 (see BOX 1) experience increases in the firmicutes *Ruminococcus* spp. and *Eubacterium rectale*[74]; likewise, overweight individuals who consume a diet high in RS3 exhibit increases in *E. rectale*, *Roseburia* spp. and *Ruminococcus bromii*[75]. These findings are consistent with *in vitro* observations which saw that these species bind directly to insoluble starch particles and may be the primary components of bacterial food chains that target starch[76,77].

Responses to rapid diet changes. In contrast to long-term changes between infancy and adulthood, our diets can elicit rapid changes in the composition of the microbiota as dietary glycans and other nutrients fluctuate from meal to meal[62,78–80]. Studies using germ-free mice that were colonized with a transplanted human microbiota demonstrated that a rapid shift from a high-fat diet to a high-carbohydrate diet results in community changes that are observable after just 1 day but take several days to stabilize[78]. In addition, a recent study of ten human subjects who were fed either high-fat, low-fibre or low-fat, high-fibre diets in a controlled setting demonstrated that detectable changes in the microbiota are observable within 24 hours of a dietary shift[81]. Observations such as these underscore the relationship between the microbiota and host diet, suggesting that some proportion of our gut microbiota is constantly fluctuating in its abundance as a result of meal-to-meal variations. In contrast to protein and fat, which are readily targeted by human absorptive systems, non-starch dietary glycans have a low digestibility, so changes in their abundance may exert a major effect on the microbiota. With this in mind, high-fat diets could, by reducing dietary fibre, enrich the microbiota with species that can digest host mucosal glycans. Indeed, in one study involving germ-free mice that were colonized with a simplified microbiota consisting of just *E. rectale* and *B. thetaiotaomicron*, when the colonized mice were switched to a high-fat, low-fibre diet *B. thetaiotaomicron* increased its expression of genes encoding enzymes that degrade host glycans[82]. Much work is still needed to determine the precise relationships governing these diet–microbiota interactions, the locations along the length and width of the gut that are influenced by different dietary glycans, and the microbial populations that should be targeted for enrichment or depletion during certain states of dysbiosis.

Microbial strategies for harvesting glycans

Sus-like systems of the phylum Bacteroidetes. Given the broad diversity of glycans that enter the gut, the microorganisms that colonize this habitat must possess efficient strategies for competing for these nutrients. The best studied strategy for glycan acquisition by human gut bacteria is one that is used by members of the phylum Bacteroidetes. Work performed by Salyers and colleagues[83,84] provided the first mechanistic insight into how the prototypical symbiont, *B. thetaiotaomicron*, approaches starch metabolism and revealed a glycan acquisition paradigm that is universal among bacteroidetes[85,86]. To metabolize starch, *B. thetaiotaomicron* requires a single eight-gene locus encoding the starch utilization system (Sus)[87]. The products of this system are located in the outer membrane and the periplasm of the bacterium and work to sequentially bind starch to the cell surface, degrade it into oligosaccharides and transport these pieces into the periplasmic space, where they are degraded to even simpler sugars such as glucose and imported into the cell (FIG. 2).

Subsequent sequencing of the *B. thetaiotaomicron* genome revealed the presence of as many as 88 related gene clusters, each of which contain homologues of at least two of the genes (*susC* and *susD*) that are present in the *sus* locus[53,88]. These loci are termed polysaccharide utilization loci (PULs), and their products, which collectively encompass ~18% of all genes in *B. thetaiotaomicron* str. VPI-5482 (the type strain), have been termed Sus-like systems because they function by a similar mechanism as Sus but harbour enzymes that are predicted to target glycans other than starch. These Sus-like systems are widespread among the Bacteroidetes members in the human gut and are unique to this phylum, with many species dedicating around one-fifth of their genomes to encoding Sus-like pathways, similarly to *B. thetaiotaomicron*[52,85,89]. A combination of whole-genome transcriptional profiling, gene disruption experiments and analysis of purified enzymes has been used to determine the cognate glycan substrates for the

Food chains
Arrangements of multiple species in space and time that allow some members to feed either directly on others or on their by-products. Keystone members, which act first in a food chain, are particularly important because their absence also influences the status of the dependent species that are downstream in the food chain.

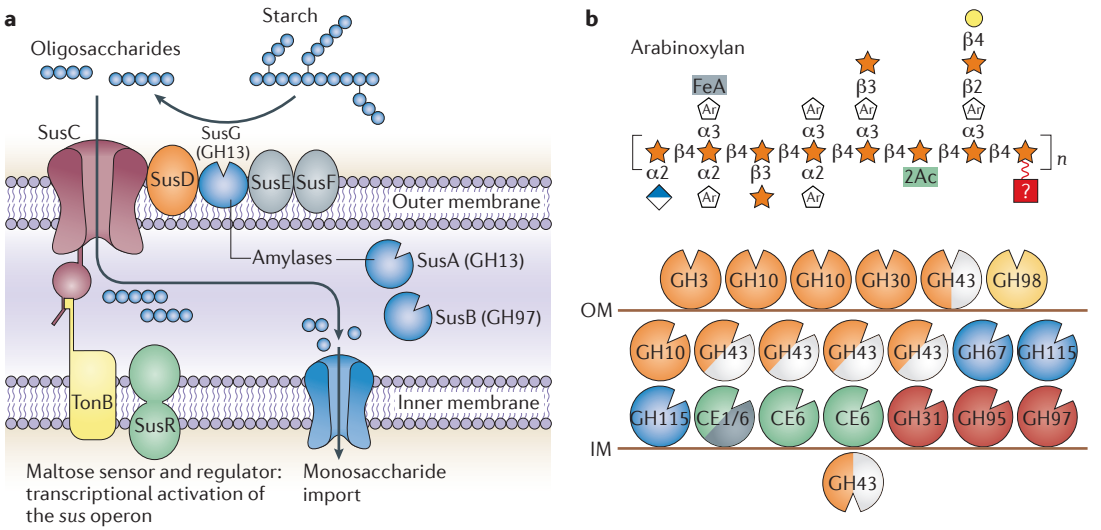


Figure 2 | **Variations in functional complexity among starch utilization system (Sus)-like systems.** Two different representations of starch utilization system (Sus)-like systems in *Bacteroides* spp. that inhabit the human gut. **a** | A model of the *Bacteroides thetaiotaomicron* Sus. The TonB-dependent transporter SusC works in concert with the starch-binding lipoproteins SusD, SusE, SusF and SusG; SusG is a glycoside hydrolase family 13 (GH13) α-amylase. Starch binding is initiated by SusD, SusE and SusF, initial degradation is carried out by SusG, and oligosaccharides are transported into the periplasm via SusC in concert with the inner-membrane protein TonB. In the periplasm, malto-oligosaccharides are further degraded to glucose by another GH13 enzyme, SusA (also known as neopullulanse), and a GH97 enzyme, SusB (an α-glucosidase). The presence of liberated maltose is sensed in the periplasm by the inner-membrane-spanning regulator SusR, which activates expression of the other Sus proteins, and simple sugars are imported across the inner membrane by an undefined permease (or permeases). Homologues of SusC and SusD are a hallmark of every Sus-like system, but carbohydrate-binding proteins akin to SusE and SusF, as well as glycoside hydrolases, vary substantially between Sus-like loci. **b** | The enzymes encoded in two polysaccharide utilization loci (PULs) from *Bacteroides ovatus*, a species that targets the hemicellulose arabinoxylan, a heteropolymer with multiple monosaccharides and glycosidic linkages[21]. Only the glycoside hydrolases encoded in these PULs are depicted, along with their predicted cellular locations: the extracellular lipoproteins are thought to be located above the outer membrane (OM), periplasmic enzymes are shown between the OM and the inner membrane (IM), and a cytoplasmic enzyme is shown below the IM. Maize arabinoxylan[21] is depicted using the same monosaccharide scheme as used in FIG. 1. The various *B. ovatus* glycan-degrading enzymes are colour-coded according to the monosaccharide linkages that they are predicted to hydrolyse in maize arabinoxylan; labelling with two colours means that the enzyme family includes members that are capable of degrading two linkages present in arabinoxylan. The target linkages of enzymes in red are unknown. Ac, acetyl group; CE, carbohydrate esterase.

Sus-like systems encoded by PULs and to establish how glycan recognition and specificity are achieved[53,62,89]. Investigations of just a few bacteroidetes from the human gut have identified Sus-like systems for all the glycans — with the exception of cellulose — that are common in plant and animal tissue and would be expected to enter the human gut (TABLE 1)[53,62,89,90]. Moreover, many Sus-like systems seem to be devoted to the breakdown of the *O*-linked glycans found in host mucosal secretions. Additional Sus-like systems with substrate specificities for chitin, cellular *N*-linked glycans or algal polysaccharides have been identified in bacteroidetes that do not inhabit the human gut but are found in soil, the canine mouth and the ocean, respectively, indicating that this glycan acquisition strategy is not specific to those bacteroidetes that are part of the human gut microbiota[91–95].

The examination of individual Sus-like systems provides a portrait of how broadly they have evolved. The number of enzymes in a given system is directly correlated with the complexity of the target glycan (TABLE 1). The prototypical system, Sus, is among the simplest of these systems, with only three enzymes, whereas a pair of *Bacteroides ovatus* systems for processing branched arabinoxylans together contain 21 annotated enzymes (FIG. 2). Most of these enzymes are predicted to target glycosidic linkages or chemical substituents that are present in arabinoxylans from various cereal plants, although some of the enzymes do not target known linkages (FIG. 2), suggesting that they target variations in glycan structures that have not yet been discovered.

Given the range of complexity among individual Sus-like systems, an important question is how the individual components of each system function together. Structural biology and protein biochemistry studies have provided substantial insight into the molecular mechanism of two of the outer-membrane components of Sus, each of which makes direct binding interactions with starch[96,97]. In addition, crosslinking and protein stability experiments with the *B. thetaiotaomicron* Sus and with Sus-like systems in other species suggest that some of the Sus components interact directly with each other, but the stoichiometry and specific interactions in these potential complexes remain unclear[84,95]. Given the abundance of these systems in the human gut microbiome,

Table 1 | **Relationship between glycan complexity and the enzyme content in Sus-like systems**

Glycan	Unique linkages*	Degrading species	Number of PULs in the species	Number of enzymes in the system
Pectic galactan (β1,4-galactan)[20]	1	*Bacteroides thetaiotaomicron*	1	2
Levan (β2,6-fructan)[68]	1	*B. thetaiotaomicron*	1	3
Inulin (β2,1-fructan)[68]	1	*Bacteroides ovatus* and *Bacteroides caccae*	1	4
Starch[89]	2	*B. thetaiotaomicron*	1	3
Barley β-glucan[20]	2	*B. ovatus*	1	3
Galactomannan and glucomannan[20]	3	*B. ovatus*	1	4
Homogalacturonan[20]	4	*B. thetaiotaomicron*	1	7
Arabinan[20]	4	*B. thetaiotaomicron*	2	6
Xyloglucan[20]	4	*B. ovatus*	1	8
Arabinogalactan[20]	4	*B. thetaiotaomicron*	2	8
Yeast α-mannan[60]	4	*B. thetaiotaomicron*	3	12
Heparin[60]	5	*B. thetaiotaomicron*	1	5
Hyaluronan, dermatan and chondroitin sulphates[60]	7	*B. thetaiotaomicron*	1	5
Xylan[20]	11	*B. ovatus*	2	21
Mucin *O*-linked glycans[60]	12	*B. thetaiotaomicron*	15	17
Rhamnogalacturonan I[20]	13‡	*B. thetaiotaomicron*	1	20
Rhamnogalacturonan II[20]	22	*B. thetaiotaomicron*	1	32

PULs, polysaccharide utilization loci. *Includes all potential glycosidic linkages, plus methyl, acetyl, sulphate or ferulic acid linkages.‡Linkages for rhamnogalacturonan I include side chains that are typically attached to a backbone chain[25].

ABC transporters
(ATP-binding cassette transporters). A protein superfamily that is found in almost every form of life from bacteria to humans. These systems are typically composed of three main components: a solute-binding protein that binds ligands and dictates specificity, a membrane transporter through which the ligand passes, and an ATPase that provides the energy to drive ligand transport. Bacteria use ABC importers to take up nutrients such as iron, peptides or sugars, and ABC efflux transporters to pump toxic compounds out of the cell.

Cellulosome
An extracellular multienzyme complex that is formed in some Gram-positive bacteria and fungi. Cellulosomes bind and degrade plant cell wall polysaccharides that are otherwise resistant to degradation, including cellulose. Scaffoldin, the major non-enzymatic structural component, connects the enzymes via interactions between dockerin domains in the enzymes and cohesin modules in scaffoldin.

it is essential that we understand how the individual components of the more simple systems work together, both before and after an encounter with an extracellular glycan. This knowledge could then be extrapolated to investigate more complex systems such as those involved in degrading plant cell wall glycans.

Glycan acquisition strategies of Gram-positive bacteria. Bacteroidetes constitute only a fraction of the human gut microbiota. The glycan acquisition strategies of firmicutes, which are typically more prominent in microbiota samples from individuals in Western societies, are less well defined. On average, sequenced firmicutes encode fewer carbohydrate-degrading enzymes than bacteroidetes but possess more ABC transporters (ATP-binding cassette transporters) that transport carbohydrates[82]. ABC transporters are frequently encoded adjacent to genes encoding glycoside hydrolases, suggesting that these two functions are co-regulated and function together[98]. Similar ABC transporter systems are also used by actinobacteria, such as *B. infantis*, the genome of which encodes many family 1 solute-binding proteins (extracellular adaptors that work with ABC transporters) that bind HMOs and mucin glycans[41]. The discovery of glycan-specific ABC transporter systems in many of the firmicutes and actinobacteria inhabiting the human gut suggests that these systems represent a second general paradigm for carbohydrate degradation and uptake by human gut bacteria that is worthy of attention in future studies.

Another well-studied glycan acquisition paradigm of Gram-positive species, the cellulosome, has been conspicuously absent to date from studies of the human gut microbiome. Cellulosomes are present in carbohydrate-degrading microorganisms from the bovine rumen and soil, and, as their name implies, are especially important in cellulose degradation[99,100]. At least one metagenomic study of the human gut microbiota revealed the presence of protein components (dockerins and cohesins) that are signatures of cellulosomes; these functions were binned with genomes from the phylum Firmicutes, suggesting the genera *Faecalibacterium*, *Eubacterium* and *Ruminococcus* as their possible genomic sources[12]. This exciting finding implies that at least some firmicutes in the microbiota have evolved to use the cellulosome strategy in the human gut.

Glycan gradients in the gut

Glycan microhabitats. No gut microorganism characterized to date is capable of tackling all of the glycan structures that enter the human intestine. Rather, individual species and strains typically display a subset of glycan-degrading phenotypes that equip them to target just part of the overall glycan repertoire present at certain times or locations[18,41,101]. As dietary glycans vary in solubility and in their accessibility within the digesting food particles, and mucus glycans are enriched in a layer overlying the epithelium, particular glycans are likely to be concentrated in regional microhabitats depending on

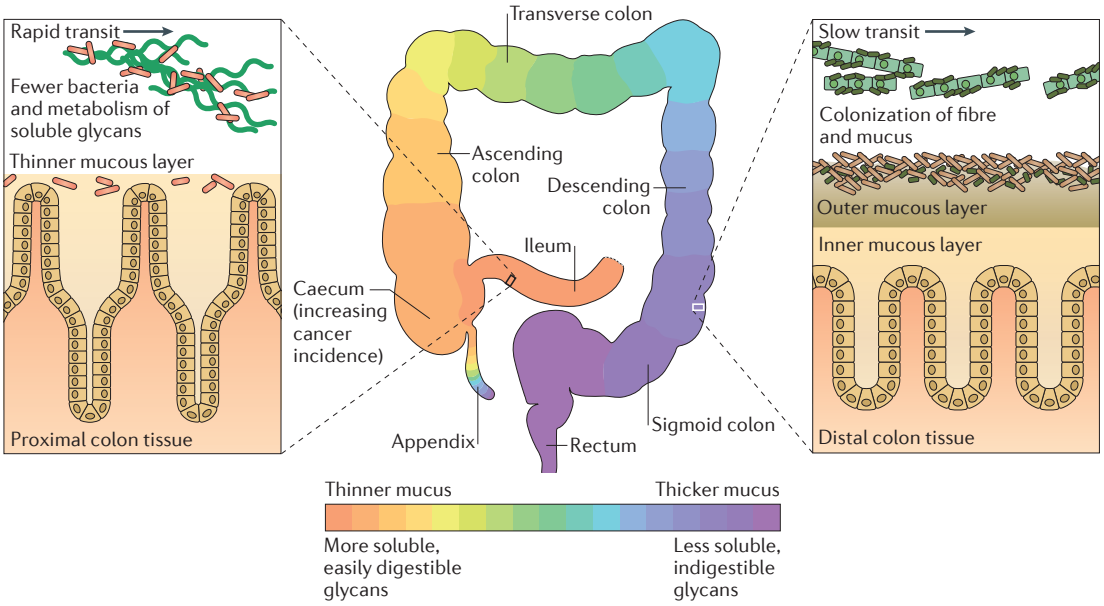


Figure 3 | **Glycan utilization along the length of the human gut and its potential health effects.** A schematic of the human ileum and colon that is colour coded to reflect potential glycan gradients. The solubility and digestibility of dietary glycans that transit the lumen are variable, and therefore each glycan is likely to be digested at a different rate. The thickness of the intestinal mucus also follows a longitudinal gradient along the gut, but this gradient may be reciprocal to that of glycan digestibility, with greatest thickness in the sigmoid colon and rectum, where mostly insoluble or indigestible glycans are likely to be present[121]. The left and right panels show the lumenal and mucosal niches in the ileum and distal (sigmoid) colon, respectively. In the ileum, the mucous layer is thin, the transit time of gut contents is faster, and bacteria are likely to target more soluble and rapidly digestible glycans, such as inulin and the different oligosaccharide side chains (for example, α-arabinans and β-galactans) that are commonly attached to pectin (specifically, rhamnogalacturonan) backbones. By contrast, the distal colon has a much thicker mucous layer, transit time is slower, and the residual glycans that fuel bacterial growth are likely to be less soluble and therefore take longer to degrade. Note the presence of inner and outer mucous layers, with bacterial colonization largely occurring in the outer layer only[122]. A possible reason for the increased mucus thickness in the distal gut may be that it shields the epithelium from extended exposure to larger numbers of bacteria, which have more time to proliferate given the slower transit rate. It is widely accepted that increased intake of dietary fibre is beneficial for colon health. In light of this, it is interesting that the incidence of colon cancers in several developed countries in North America, Europe and Asia has increased in more proximal colonic regions over the past few decades[123–126]. One explanation offered for this phenomenon involves changing dietary habits in these societies — specifically, reduced fibre intake and increased consumption of fat and animal protein. This trend could alter the microbiota or its metabolism in more proximal regions, leading to carcinogenesis by several possible mechanisms (reduced transit time, increased production of toxic metabolites or decreased production of protective metabolites such as butyrate).

their location and the variable rates at which they are depleted (FIG. 3).

Regional variations in microbial colonization of the gut have been investigated in a few studies using human and animal samples. One seminal culture-independent study of three healthy adult humans found that bacteroidetes were enriched in colonic mucosal biopsies relative to faecal samples[11]. This study also noted a "patchy and heterogeneous" abundance of mucosal species among different biopsy sites along the colon, suggesting that there is variation in the community structure along the length of the gut. Studies in mice using fluorescence *in situ* hybridization also revealed an enrichment of bacteroidetes in the mucous layer[102], although in neither mice nor humans were the taxonomic identities of mucus-specific bacteria defined to lower-level taxa. A recent study using germ-free mice colonized with just two intestinal symbionts, *E. coli* and *Bacteroides fragilis*[103], revealed that only *B. fragilis*, which is known to degrade mucin glycoproteins[104], penetrates the mucous layer in the mouse colon. Thus, the ability to metabolize the *O*-linked oligosaccharides and, possibly, the underlying peptide backbone that compose the mucus is likely to be a key factor in determining which microorganisms physically associate with this layer. Future work will be required to determine how widespread this ability is among bacteria. Given the link between the microbiota and some IBD states[4,10], the mucosal subpopulation may be of particular interest because of its close proximity to host tissue.

In addition to the lumen–mucus gradient, the longitudinal length of the gastrointestinal tract represents a much longer distance (~8 metres in humans, from stomach to anus) along which the microbial composition varies. In the proximal small intestine both microbial diversity and bacterial density are low, with only a few species predominating. Colonization density and diversity increase in the distal small intestine (ileum) and colon, the site of fermentation for most of the dietary glycan, but the effect of glycan in shaping the local

Box 2 | **'Prescription' diets: prebiotics for treating disease**

The microbiota of the human gut responds to dietary changes, so this community can be manipulated to promote host health through the addition of specific dietary components (prebiotics) to the existing diet. The long-chain β-fructan inulin, as well as smaller fructo-oligosaccharides, are well-studied prebiotic fibres, and their ability to promote the growth of *Bifidobacterium* spp. is well documented[61], although many other beneficial species from the human gut, such as the *Roseburia* spp.[111] and *Faecalibacterium prausnitzii*[112], also increase in abundance in response to dietary inulin. Dietary supplementation with inulin and fructo-oligosaccharide has been shown to curb the metabolic effects of a high-fat diet, specifically by leading to improved glucose tolerance, a reduction in colonic inflammation and endotoxaemia, and improved colonic barrier function[113,114]. Inulin may also be useful as a preventive prebiotic for colon cancer; in rats with chemically induced colon cancer, tumour incidence decreased by up to 70% when the diet was supplemented with a combination of inulin and soybean meal[115]. In this therapy, fructan stimulated microbial metabolism, which is thought to have enhanced the bioavailability of soybean isoflavones. In another study, microbial metabolites from inulin fermentation stimulated increased apoptosis in human colon cancer cell lines, especially in early adenoma cells[116]. Prebiotics involving resistant starch (see BOX 1 and main text) and butyrylated starch[70] may also have anticancer and anti-inflammatory properties in the colon, as they stimulate the production of the short-chain fatty acid butyrate by some microbiota members. More recently, prebiotic formulations involving arabinoxylans and chitin–glucan have shown promise for their potential to restore compositional balance (that is, the bacteroidetes/firmicutes ratio) to the microbial community and to improve colonic function even when a high-fat diet is consumed[117,118]. These prebiotics may also help lower cholesterol, as they stimulate the microbiota to produce propionate, a short-chain fatty acid that is absorbed into the bloodstream and travels to the liver, where it can inhibit cholesterol synthesis[119].

microbial composition along this length has not been extensively explored. A recent study using germ-free mice that were colonized with a human faecal microbiota found that the proportion of bacteroidetes in the microbiota increased from ~14% in the small intestine to ~42% in the caecum–colon[78], suggesting that the slower rate of glycan transit after the ileocaecal transition provides a more optimal habitat for these glycophilic species. The wide variation in the solubility and accessibility of glycans that transit the gut probably affects the species composition of the microbiota along the intestinal tract. For example, highly soluble glycans such as inulin might be metabolized more rapidly by bacteria and may be processed in proximal regions of the gut, whereas insoluble or complicated glycans may take longer to degrade and may thus reach more distal regions (FIG. 3). A better understanding of the relationships between dietary glycans and the regional or temporal composition of the microbiota may lead to more precise prebiotic strategies for manipulating the region-specific microbiota to improve intestinal health (BOX 2).

Microbial food chains. Microbial species interact *in vivo* to form complicated food chains, and some of these relationships centre on glycan metabolism (FIG. 4). Species in food chains may be dependent on other species for partial glycan degradation or for the production of certain metabolites (such as SCFAs, vitamins and gases). One example of a glycan-driven bacterial food chain, which has been suggested on the basis of *in vitro* faecal batch fermentation, is the initial processing of insoluble plant cell wall fibres by certain firmicutes and actinobacteria. These organisms adhere most tightly to dietary fibres, a trait that may allow them to carry out the initial glycan digestion steps to liberate shorter, more soluble polysaccharides for other bacteria[76]. Additional interactions in the gut are demonstrated by co-colonization of germ-free mice with *B. thetaiotaomicron* and *E. rectale*[82], or *B. thetaiotaomicron* and *Bifidobacterium longum*[105], which leads to different bacterial glycan degradation profiles to those obtained from monoassociation experiments. These studies suggest that bacteria adjust their metabolism according to both the glycans present and other microorganisms in the community, perhaps in an attempt to either synergize or avoid competition. Metabolic cross-feeding (syntrophy) between two or more species also serves to augment the efficiency of glycan metabolism. Several bacterial sugar fermentation pathways generate hydrogen gas (H_2) as a mechanism to recover NAD^+ (REF. 106). Accumulated H_2 inhibits fermentation efficiency but can be removed by methanogenesis, acetogenesis or sulphate reduction, which are catalysed by different groups of microorganisms (FIG. 4). The presence of H_2-scavenging microorganisms in the same microhabitat or vicinity as sugar-fermenting bacteria benefits both types of organism and increases the overall metabolic efficiency.

In food chains such as those described above, the presence or absence of certain keystone members could influence the abundance of other dependent species. Interestingly, a recent metagenomic study of the microbiomes of 33 humans revealed the presence of just three different human 'enterotypes' (REF. 14). An enterotype is defined as a microbial community structure that is similar to communities from other individuals on the basis of the presence and relative abundance of detectable species. Each enterotype contains a subset of all possible gut microbiota species in a signature combination that may have evolved to function optimally as a community. If this proposal is true, then each enterotype should hold clues about functional food chains between the microorganisms of the human gut, including those related to glycan metabolism. For example, in one enterotype the abundance of *Bacteroides* spp. is positively correlated with the abundance of bacteria from two other taxa (the genus *Parabacteroides* and the order Clostridiales) and negatively correlated with that of several other bacterial groups. By contrast, a second enterotype is defined by an abundance of members of the bacteroidetes genus *Prevotella*, which exhibit positive and negative correlations with different groups relative to the first enterotype. These correlations may provide insight into which species are able to thrive together in a microbial food chain and which are potentially competing with each other for nutrients. Additional questions include how different dietary components, including various glycans, interact with the microbiota, and whether these compounds contribute to enterotype formation. These questions were addressed in a recent study that involved 98 human subjects and examined the connections between long- and short-term dietary habits and microbiota enterotypes[81]. This study found that long-term consumption of a high-fibre diet is associated with an enterotype that is enriched for *Prevotella* spp. By

Human Microbiome Projects
Several ongoing efforts to sequence the microbial communities that are associated with various human body sites, including the gut. A major component of these projects is to sequence cultured 'reference' organisms. However, because many human-associated microorganisms have not yet been isolated in laboratory culture, a second approach is to directly sequence DNA extracted from microbial community samples (metagenomics).

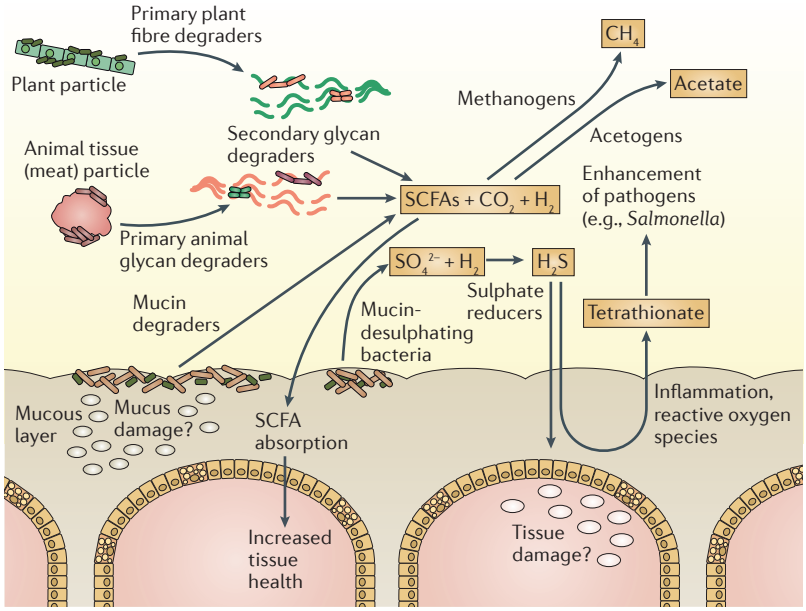


Figure 4 | **Glycan microhabitats and food chains in the gut.** An illustration of the ways in which different gut microorganisms are thought to interact during processing of various glycan substrates. Dietary material derived from plant cell wall or meat particles will be rich in source-specific glycans, such as cellulose, hemicellulose and pectin (plant material) or glycosaminoglycans and cellular glycoproteins (meat sources). These types of nutrients are likely to enter the distal gut as particulate forms that will be attacked by primary glycan degraders (for example, *Roseburia*, *Eubacterium*, *Clostridium*, *Ruminococcus* and *Bifidobacterium* spp.), which are capable of directly binding to these insoluble particles and digesting their glycan components[76]. After this initial degradation of glycan-containing particles, more-soluble glycan fragments can be digested by secondary glycan degraders, which contribute to the liberated pool of short-chain fatty acid (SCFA) fermentation products that is derived from both types of degraders. A similar food chain of primary and secondary degraders has been proposed to occur in the mucous layer[127]; primary species are capable of directly degrading high-molecular-mass mucin glycoproteins, whereas secondary species are optimized to target the resulting oligosaccharide products. Bacterial fermentation of glycan is enhanced by the removal of downstream hydrogen gas (H_2) consumers, which convert this gas to methane (CH_4), acetate or hydrogen sulphide (H_2S) depending on the types of microorganism present. The H_2S pathway also requires free sulphate (SO_4^{2-}), which can be derived from many food products but also from the degradation of animal proteins and of sulphated glycans that are abundant in animal tissue (for example, chondroitin sulphate) or in mucus. The resulting H_2S is toxic to host cells but is readily metabolized and detoxified by colonic tissue to form thiosulphate[128]. In the context of mucosal inflammation, thiosulphate can be converted to tetrathionate via reactive oxygen species, an event that has been recently tied to metabolic enhancement of the intestinal pathogen *Salmonella enterica* subsp. *enterica* serovar Typhimurium[129].

Koch's postulates
Guidelines that are used to establish causality between a potential microbial pathogen and a disease, as published by Robert Koch in 1890. The postulates state that a microorganism that causes a disease should be abundant in animals suffering from that disease, isolated from diseased specimens, able to be introduced into healthy animals to cause disease and able to be re-isolated from newly infected hosts.

contrast, long-term consumption of a diet that is rich in protein and animal fat is correlated with an enterotype containing more *Bacteroides* and *Ruminococcus* spp. As mentioned above, the absence of dietary fibre in high-fat or high-protein diets could select for species that are capable of resorting to degradation of mucus glycans as an alternative nutrient base.

Glycan microhabitats and bacterial genome evolution. Microbial species in the gut evolve as a result of selective pressures that shape the behaviour (phenotype) and underlying genome architecture (genotype) of each species. When the first genomes of broadly saccharolytic human gut microorganisms were completed, it was observed that some species encode a diverse array of glycan-degrading enzymes. For example, *B. thetaiotaomicron* str. VPI-5482 is predicted to encode 296 different glycan-degrading enzymes, as currently defined in the Carbohydrate-Active Enzymes (CAZy) database[107]. Many of these enzymes are not represented in the human genome, highlighting the complementarity between human and microbial carbohydrate-degrading enzymes. Ongoing Human Microbiome Projects and other independent projects have collectively provided several hundred sequenced reference genomes of cultured human gut species[108]. The availability of genomic blueprints for so many gut microorganisms provides an unprecedented opportunity to compare the evolution of a large number of species that have evolved together in a similar environment. A recent study found that two closely related human symbionts, *B. thetaiotaomicron* and *B. ovatus*, which have 96.5% identity in their 16S rRNA gene sequences, differ widely in their glycan utilization phenotypes and corresponding genomic structures[89]. Each of these species dedicates ~20% of its genes to encoding Sus-like systems, but less than one-third of these genes are homologous between the two species. More strikingly, the Sus-like systems that are unique to each species are scattered throughout the respective genomes and correlate with a glycan utilization 'theme' — degradation of mucin *O*-linked glycans for *B. thetaiotaomicron*, and degradation of plant cell wall hemicellulose for *B. ovatus*. Thus, these two species seem to have acquired or retained niche-specific Sus-like systems that could direct them to distinct glycan microhabitats: the mucous layer and the cell walls of digesting plant matter. Future work will be needed to determine whether this theme extrapolates to other sequenced human gut bacteria and how the resulting phenotypic diversity among species and strains directs regional variation in microbial colonization.

Summary and future perspectives

The variations in microbiota composition that result from the differing abilities of gut microorganisms to metabolize glycans could have profound implications for our understanding of both how the microbiota assembles over a human lifetime and how short-term community variations influence human health. Owing to their proximity to host tissue, mucus-associated microorganisms may have a disproportionate impact on host responses and may therefore be a subpopulation that is of particular interest when investigating diseases that are thought to result from dysbiosis. As an example, a recent study using a mouse model of IBD successfully fulfilled Koch's postulates, finding that some individual *Bacteroides* spp., including *B. thetaiotaomicron*, were sufficient to elicit disease in the context of a natural microbiota[10]. One future goal is to develop strategies to manipulate the function of the microbiota using prebiotic, probiotic or pharmacological strategies. In order for this to be achieved, it is imperative that we gain deeper insight into the fine structure of the microbiota composition and the metabolism of its constituents in different gut regions. Glycan availability is likely to be a major factor in determining these relationships.

1. Round, J. L. & Mazmanian, S. K. The gut microbiota shapes intestinal immune responses during health and disease. *Nature Rev. Immunol.* **9**, 313–323 (2009).
2. Flint, H. J., Bayer, E. A., Rincon, M. T., Lamed, R. & White, B. A. Polysaccharide utilization by gut bacteria: potential for new insights from genomic analysis. *Nature Rev. Microbiol.* **6**, 121–131 (2008).
3. Wardwell, L. H., Huttenhower, C. & Garrett, W. S. Current concepts of the intestinal microbiota and the pathogenesis of infection. *Curr. Infect. Dis. Rep.* **13**, 28–34 (2011).
4. Packey, C. D. & Sartor, R. B. Commensal bacteria, traditional and opportunistic pathogens, dysbiosis and bacterial killing in inflammatory bowel diseases. *Curr. Opin. Infect. Dis.* **22**, 292–301 (2009).
5. O'Keefe, S. J. *et al.* Products of the colonic microbiota mediate the effects of diet on colon cancer risk. *J. Nutr.* **139**, 2044–2048 (2009).
6. Khoruts, A., Dicksved, J., Jansson, J. K. & Sadowsky, M. J. Changes in the composition of the human fecal microbiome after bacteriotherapy for recurrent *Clostridium difficile*-associated diarrhea. *J. Clin. Gastroenterol.* **44**, 354–360 (2010).
7. Ley, R. E., Turnbaugh, P. J., Klein, S. & Gordon, J. I. Microbial ecology: human gut microbes associated with obesity. *Nature* **444**, 1022–1023 (2006).
8. Sun, L., Nava, G. M. & Stappenbeck, T. S. Host genetic susceptibility, dysbiosis, and viral triggers in inflammatory bowel disease. *Curr. Opin. Gastroenterol.* **27**, 321–327 (2011).
9. Garrett, W. S. *et al.* Enterobacteriaceae act in concert with the gut microbiota to induce spontaneous and maternally transmitted colitis. *Cell Host Microbe* **8**, 292–300 (2010).
10. Bloom, S. M. *et al.* Commensal bacteroides species induce colitis in host-genotype-specific fashion in a mouse model of inflammatory bowel disease. *Cell Host Microbe* **9**, 390–403 (2011).
11. Eckburg, P. B. *et al.* Diversity of the human intestinal microbial flora. *Science* **308**, 1635–1638 (2005).
12. Turnbaugh, P. J. *et al.* Organismal, genetic, and transcriptional variation in the deeply sequenced gut microbiomes of identical twins. *Proc. Natl Acad. Sci. USA* **107**, 7503–7508 (2010).
13. Qin, J. *et al.* A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* **464**, 59–65 (2010).
14. Arumugam, M. *et al.* Enterotypes of the human gut microbiome. *Nature* **473**, 174–180 (2011).
A metagenomics-based analysis of a total of 272 new and published human faecal microbiomes, revealing the existence of just three dominant enterotypes.
15. Caporaso, J. G. *et al.* Moving pictures of the human microbiome. *Genome Biol.* **12**, R50 (2011).
The first long-term study of the dynamics of the human faecal microbiome, with a daily time point analysis.
16. McNeil, N. I. The contribution of the large intestine to energy supplies in man. *Am. J. Clin. Nutr.* **39**, 338–342 (1984).
17. Salyers, A. A., Vercellotti, J. R., West, S. E. & Wilkins, T. D. Fermentation of mucin and plant polysaccharides by strains of *Bacteroides* from the human colon. *Appl. Environ. Microbiol.* **33**, 319–322 (1977).
18. Salyers, A. A., West, S. E., Vercellotti, J. R. & Wilkins, T. D. Fermentation of mucins and plant polysaccharides by anaerobic bacteria from the human colon. *Appl. Environ. Microbiol.* **34**, 529–533 (1977).
19. Marcobal, A. *et al.* Bacteroides in the infant gut consume milk oligosaccharides via mucus-utilization pathways. *Cell Host Microbe* **10**, 507–514 (2011).
20. Mohnen, D. Pectin structure and biosynthesis. *Curr. Opin. Plant Biol.* **11**, 266–277 (2008).
21. Saulnier, L., Marot, C., Chanliaud, E. & Thibault, J.-F. Cell wall polysaccharide interactions in maize bran. *Carbohydr. Polym.* **26**, 279–287 (1995).
22. Larsson, J. M., Karlsson, H., Sjovall, H. & Hansson, G. C. A complex, but uniform *O*-glycosylation of the human MUC2 mucin from colonic biopsies analyzed by nanoLC/MS^n. *Glycobiology* **19**, 756–766 (2009).
23. Hamer, H. M. *et al.* Review article: the role of butyrate on colonic function. *Aliment. Pharmacol. Ther.* **27**, 104–119 (2008).
24. Rombeau, J. L. & Kripke, S. A. Metabolic and intestinal effects of short-chain fatty acids. *JPEN. J. Parenter. Enteral Nutr.* **14**, S181–S185 (1990).
25. Duncan, S. H., Barcenilla, A., Stewart, C. S., Pryde, S. E. & Flint, H. J. Acetate utilization and butyryl coenzyme A (CoA):acetate-CoA transferase in butyrate-producing bacteria from the human large intestine. *Appl. Environ. Microbiol.* **68**, 5186–5190 (2002).
26. Duncan, S. H. *et al.* Contribution of acetate to butyrate formation by human faecal bacteria. *Br. J. Nutr.* **91**, 915–923 (2004).
27. Fukuda, S. *et al.* Bifidobacteria can protect from enteropathogenic infection through production of acetate. *Nature* **469**, 543–547 (2011).
28. Palmer, C., Bik, E. M., Digiulio, D. B., Relman, D. A. & Brown, P. O. Development of the human infant intestinal microbiota. *PLoS Biol.* **5**, e177 (2007).
29. Koenig, J. E. *et al.* Succession of microbial consortia in the developing infant gut microbiome. *Proc. Natl Acad. Sci. USA* **108** (Suppl. 1), 4578–4585 (2010).
A study of microbiota formation in a single human child over the first 3 years of life, using 16S rRNA genes and metagenomic approaches to correlate changes in the microbiota with life events such as diet shifts, illness and antibiotics.
30. Kunz, C., Rudloff, S., Baier, W., Klein, N. & Strobel, S. Oligosaccharides in human milk: structural, functional, and metabolic aspects. *Annu. Rev. Nutr.* **20**, 699–722 (2000).
31. Ninonuevo, M. R. *et al.* A strategy for annotating the human milk glycome. *J. Agric. Food Chem.* **54**, 7471–7480 (2006).
32. Fuhrer, A. *et al.* Milk sialyllactose influences colitis in mice through selective intestinal bacterial colonization. *J. Exp. Med.* **207**, 2843–2854 (2010).
33. Chaturvedi, P., Warren, C. D., Buescher, C. R., Pickering, L. K. & Newburg, D. S. Survival of human milk oligosaccharides in the intestine of infants. *Adv. Exp. Med. Biol.* **501**, 315–323 (2001).
34. German, J. B., Freeman, S. L., Lebrilla, C. B. & Mills, D. A. Human milk oligosaccharides: evolution, structures and bioselectivity as substrates for intestinal bacteria. *Nestle Nutr. Workshop Ser. Pediatr. Program* **62**, 205–218; discussion 218–222 (2008).
35. Gnoth, M. J., Kunz, C., Kinne-Saffran, E. & Rudloff, S. Human milk oligosaccharides are minimally digested *in vitro*. *J. Nutr.* **130**, 3014–3020 (2000).
36. Favier, C. F., Vaughan, E. E., De Vos, W. M. & Akkermans, A. D. Molecular monitoring of succession of bacterial communities in human neonates. *Appl. Environ. Microbiol.* **68**, 219–226 (2002).
37. Sela, D. A. Bifidobacterial utilization of human milk oligosaccharides. *Int. J. Food Microbiol.* **149**, 58–64 (2011).
38. Miwa, M. *et al.* Cooperation of β-galactosidase and β-*N*-acetylhexosaminidase from bifidobacteria in assimilation of human milk oligosaccharides with type 2 structure. *Glycobiology* **20**, 1402–1409 (2010).
39. LoCascio, R. G., Desai, P., Sela, D. A., Weimer, B. & Mills, D. A. Broad conservation of milk utilization genes in *Bifidobacterium longum* subsp. *infantis* as revealed by comparative genomic hybridization. *Appl. Environ. Microbiol.* **76**, 7373–7381 (2010).
40. Turroni, F. *et al.* Genome analysis of *Bifidobacterium bifidum* PRL2010 reveals metabolic pathways for host-derived glycan foraging. *Proc. Natl Acad. Sci. USA* **107**, 19514–19519 (2010).
41. Garrido, D., Kim, J. H., German, J. B., Raybould, H. E. & Mills, D. A. Oligosaccharide binding proteins from *Bifidobacterium longum* subsp. *infantis* reveal a preference for host glycans. *PLoS ONE* **6**, e17315 (2011).
42. Martinez, R. C. *et al. In vitro* evaluation of gastrointestinal survival of *Lactobacillus amylovorus* DSM 16698 alone and combined with galactooligosaccharides, milk and/or *Bifidobacterium animalis* subsp. *lactis* Bb-12. *Int. J. Food Microbiol.* **149**, 152–158 (2011).
43. Rodriguez-Diaz, J., Monedero, V. & Yebra, M. J. Utilization of natural fucosylated oligosaccharides by three novel α-L-fucosidases from a probiotic *Lactobacillus casei* strain. *Appl. Environ. Microbiol.* **77**, 703–705 (2011).
44. Schwab, C. & Ganzle, M. Lactic acid bacteria fermentation of human milk oligosaccharide components, human milk oligosaccharides and galactooligosaccharides. *FEMS Microbiol. Lett.* **315**, 141–148 (2011).
45. Coppa, G. V. *et al.* Oligosaccharides in human milk during different phases of lactation. *Acta Paediatr. Suppl.* **88**, 89–94 (1999).
46. Favier, C. F., de Vos, W. M. & Akkermans, A. D. Development of bacterial and bifidobacterial communities in feces of newborn babies. *Anaerobe* **9**, 219–229 (2003).
47. Fallani, M. *et al.* Determinants of the human infant intestinal microbiota after the introduction of first complementary foods in infant samples from five European centres. *Microbiology* **157**, 1385–1392 (2011).
48. Harmsen, H. J. *et al.* Analysis of intestinal flora development in breast-fed and formula-fed infants by using molecular identification and detection methods. *J. Pediatr. Gastroenterol. Nutr.* **30**, 61–67 (2000).
49. Kurokawa, K. *et al.* Comparative metagenomics revealed commonly enriched gene sets in human gut microbiomes. *DNA Res.* **14**, 169–181 (2007).
50. Mariat, D. *et al.* The Firmicutes/Bacteroidetes ratio of the human microbiota changes with age. *BMC Microbiol.* **9**, 123 (2009).
51. Sonnenburg, J. L. *et al.* Glycan foraging *in vivo* by an intestine-adapted bacterial symbiont. *Science* **307**, 1955–1959 (2005).
The first *in vivo* transcriptomics-based study of a human gut symbiont (*B. thetaiotaomicron*) in the intestines of gnotobiotic mice consuming diets with varying glycan content.
52. Bjursell, M. K., Martens, E. C. & Gordon, J. I. Functional genomic and metabolic studies of the adaptations of a prominent adult human gut symbiont, *Bacteroides thetaiotaomicron*, to the suckling period. *J. Biol. Chem.* **281**, 36269–36279 (2006).
53. Martens, E. C., Chiang, H. C. & Gordon, J. I. Mucosal glycan foraging enhances fitness and transmission of a saccharolytic human gut bacterial symbiont. *Cell Host Microbe* **4**, 447–457 (2008).
A study that uses transcriptional profiling of *in vitro*-grown cultures to identify *B. thetaiotaomicron* genes that are involved in the degradation of host glycans. This study demonstrates a link between foraging for host glycans and intergenerational transmission of microbiota members.
54. Costello, E. K. *et al.* Bacterial community variation in human body habitats across space and time. *Science* **326**, 1694–1697 (2009).
55. Franks, A. H. *et al.* Variations of bacterial populations in human feces measured by fluorescent *in situ* hybridization with group-specific 16S rRNA-targeted oligonucleotide probes. *Appl. Environ. Microbiol.* **64**, 3336–3345 (1998).
56. Zoetendal, E. G., Akkermans, A. D. & De Vos, W. M. Temperature gradient gel electrophoresis analysis of 16S rRNA from human fecal samples reveals stable and host-specific communities of active bacteria. *Appl. Environ. Microbiol.* **64**, 3854–3859 (1998).
57. Ley, R. E. *et al.* Obesity alters gut microbial ecology. *Proc. Natl Acad. Sci. USA* **102**, 11070–11075 (2005).
58. Hill, J. E. *et al.* Improvement of the representation of bifidobacteria in fecal microbiota metagenomic libraries by application of the cpn60 universal primer cocktail. *Appl. Environ. Microbiol.* **76**, 4550–4552 (2010).
59. De Filippo, C. *et al.* Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc. Natl Acad. Sci. USA* **107**, 14691–14696 (2010).
An investigation that analyses the composition of the microbiota in two populations of children (one in Africa and the other in Europe) that consume different diets.
60. Tap, J. *et al.* Towards the human intestinal microbiota phylogenetic core. *Environ. Microbiol.* **11**, 2574–2584 (2009).
61. Meyer, D. & Stasse-Wolthuis, M. The bifidogenic effect of inulin and oligofructose and its consequences for gut health. *Eur. J. Clin. Nutr.* **63**, 1277–1289 (2009).
62. Sonnenburg, E. D. *et al.* Specificity of polysaccharide use in intestinal *Bacteroides* species determines diet-induced microbiota alterations. *Cell* **141**, 1241–1252 (2010).
A mechanistic study of fructan utilization by multiple *Bacteroides* spp. from the human gut microbiota, revealing that a single gene cluster can be evolutionarily altered between species to switch glycan substrate specificity.
63. Segain, J. P. *et al.* Butyrate inhibits inflammatory responses through NFκB inhibition: implications for Crohn's disease. *Gut* **47**, 397–403 (2000).
64. Luhrs, H. *et al.* Cytokine-activated degradation of inhibitory κB protein α is inhibited by the short-chain fatty acid butyrate. *Int. J. Colorectal Dis.* **16**, 195–201 (2001).

65. Hamer, H. M. *et al.* Butyrate modulates oxidative stress in the colonic mucosa of healthy humans. *Clin. Nutr.* **28**, 88–93 (2009).
66. Pryde, S. E., Duncan, S. H., Hold, G. L., Stewart, C. S. & Flint, H. J. The microbiology of butyrate formation in the human colon. *FEMS Microbiol. Lett.* **217**, 133–139 (2002).
67. Avivi-Green, C., Polak-Charcon, S., Madar, Z. & Schwartz, B. Apoptosis cascade proteins are regulated *in vivo* by high intracolonic butyrate concentration: correlation with colon cancer inhibition. *Oncol. Res.* **12**, 83–95 (2000).
68. McIntyre, A., Gibson, P. R. & Young, G. P. Butyrate production from dietary fibre and protection against large bowel cancer in a rat model. *Gut* **34**, 386–391 (1993).
69. Dronamraju, S. S., Coxhead, J. M., Kelly, S. B., Burn, J. & Mathers, J. C. Cell kinetics and gene expression changes in colorectal cancer patients given resistant starch: a randomised controlled trial. *Gut* **58**, 413–420 (2009).
70. Clarke, J. M., Topping, D. L., Bird, A. R., Young, G. P. & Cobiac, L. Effects of high-amylose maize starch and butyrylated high-amylose maize starch on azoxymethane-induced intestinal cancer in rats. *Carcinogenesis* **29**, 2190–2194 (2008).
71. Van Immerseel, F. *et al.* Butyric acid-producing anaerobic bacteria as a novel probiotic treatment approach for inflammatory bowel disease. *J. Med. Microbiol.* **59**, 141–143 (2010).
72. Pan, N. & Imlay, J. A. How does oxygen inhibit central metabolism in the obligate anaerobe *Bacteroides thetaiotaomicron*. *Mol. Microbiol.* **39**, 1562–1571 (2001).
73. Sakamoto, M. *et al. Butyricimonas synergistica* gen. nov., sp. nov. and *Butyricimonas virosa* sp. nov., butyric acid-producing bacteria in the family '*Porphyromonadaceae*' isolated from rat faeces. *Int. J. Syst. Evol. Microbiol.* **59**, 1748–1753 (2009).
74. Martinez, I., Kim, J., Duffy, P. R., Schlegel, V. L. & Walter, J. Resistant starches types 2 and 4 have differential effects on the composition of the fecal microbiota in human subjects. *PLoS ONE* **5**, e15046 (2010).
75. Walker, A. W. *et al.* Dominant and diet-responsive groups of bacteria within the human colonic microbiota. *ISME J.* **5**, 220–230 (2011).
A human volunteer study that tracks the changes in the microbiota following a shift to a low-carbohydrate diet, then to an RS-containing diet and finally to a diet that is rich in non-starch polysaccharides.
76. McWilliam Leitch, E. C., Walker, A. W., Duncan, S. H., Holtrop, G. & Flint, H. J. Selective colonization of insoluble substrates by human faecal bacteria. *Environ. Microbiol.* **9**, 667–679 (2007).
77. Macfarlane, S. & Macfarlane, G. T. Composition and metabolic activities of bacterial biofilms colonizing food residues in the human gut. *Appl. Environ. Microbiol.* **72**, 6204–6211 (2006).
78. Turnbaugh, P. J. *et al.* The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. *Sci. Transl. Med.* **1**, 6ra14 (2009).
An investigation that uses culture-independent methods to monitor alterations in the microbiota of humanized mice in response to rapid diet shift.
79. Faith, J. J., McNulty, N. P., Rey, F. E. & Gordon, J. I. Predicting a human gut microbiota's response to diet in gnotobiotic mice. *Science* **333**, 101–104 (2011).
80. Goodman, A. *et al.* Extensive personal human gut microbiota culture collections characterized and manipulated in gnotobiotic mice. *Proc. Natl Acad. Sci. USA* **108**, 6252–6257 (2011).
81. Wu, G. D. *et al.* Linking long-term dietary patterns with gut microbial enterotypes. *Science* **334**, 105–108 (2011).
82. Mahowald, M. A. *et al.* Characterizing a model human gut microbiota composed of members of its two dominant bacterial phyla. *Proc. Natl Acad. Sci. USA* **106**, 5859–5864 (2009).
83. D'Elia, J. N. & Salyers, A. A. Contribution of a neopullulanase, a pullulanase, and an α-glucosidase to growth of *Bacteroides thetaiotaomicron* on starch. *J. Bacteriol.* **178**, 7173–7179 (1996).
84. Cho, K. H. & Salyers, A. A. Biochemical analysis of interactions between outer membrane proteins that contribute to starch utilization by *Bacteroides thetaiotaomicron*. *J. Bacteriol.* **183**, 7224–7230 (2001).
85. Xu, J. *et al.* Evolution of symbiotic bacteria in the distal human intestine. *PLoS Biol.* **5**, e156 (2007).
86. Martens, E. C., Koropatkin, N. M., Smith, T. J. & Gordon, J. I. Complex glycan catabolism by the human gut microbiota: the *Bacteroidetes* Sus-like paradigm. *J. Biol. Chem.* **284**, 24673–24677 (2009).
87. Tancula, E., Feldhaus, M. J., Bedzyk, L. A. & Salyers, A. A. Location and characterization of genes involved in binding of starch to the surface of *Bacteroides thetaiotaomicron*. *J. Bacteriol.* **174**, 5609–5616 (1992).
88. Xu, J. *et al.* A genomic view of the human-*Bacteroides thetaiotaomicron* symbiosis. *Science* **299**, 2074–2076 (2003).
89. Martens, E. C. *et al.* Recognition and degradation of plant cell wall polysaccharides by two human gut symbionts. *PLoS Biol.* **9**, e1001221 (2011).
The first transcriptomic and genetic study to link specific genes in *Bacteroides* spp. from the human gut microbiota with degradation of all major plant cell wall polysaccharides except cellulose.
90. Hehemann, J. H. *et al.* Transfer of carbohydrate-active enzymes from marine bacteria to Japanese gut microbiota. *Nature* **464**, 908–912 (2010).
This work identifies an enzyme system that is involved in degrading a polysaccharide present in seaweed. This system was transferred from the marine metagenome to the human microbiome as a response to seaweed consumption.
91. Dodd, D., Moon, Y. H., Swaminathan, K., Mackie, R. I. & Cann, I. K. Transcriptomic analyses of xylan degradation by *Prevotella bryantii* and insights into energy acquisition by xylanolytic bacteroidetes. *J. Biol. Chem.* **285**, 30261–30273 (2010).
92. Miyazaki, K., Hirase, T., Kojima, Y. & Flint, H. J. Medium- to large-sized xylo-oligosaccharides are responsible for xylanase induction in *Prevotella bryantii* B14. *Microbiology* **151**, 4121–4125 (2005).
93. Bauer, M. *et al.* Whole genome analysis of the marine *Bacteroidetes* '*Gramella forsetii*' reveals adaptations to degradation of polymeric organic matter. *Environ. Microbiol.* **8**, 2201–2213 (2006).
94. McBride, M. J. *et al.* Novel features of the polysaccharide-digesting gliding bacterium *Flavobacterium johnsoniae* revealed by genome sequence analysis. *Appl. Environ. Microbiol.* **75**, 6864–6875 (2009).
95. Renzi, F. *et al.* The *N*-glycan glycoprotein deglycosylation complex (Gpd) from *Capnocytophaga canimorsus* deglycosylates human IgG. *PLoS Pathog.* **7**, e1002118 (2011).
96. Koropatkin, N. M. & Smith, T. J. SusG: a unique cell-membrane-associated α-amylase from a prominent human gut symbiont targets complex starch molecules. *Structure* **18**, 200–215 (2010).
97. Koropatkin, N. M., Martens, E. C., Gordon, J. I. & Smith, T. J. Starch catabolism by a prominent human gut symbiont is directed by the recognition of amylose helices. *Structure* **16**, 1105–1115 (2008).
A report of the structure of SusD, the *B. thetaiotaomicron* starch-binding protein, revealing a novel fold and the site of glycan interaction in this broadly expanded family of bacteroidetes proteins.
98. Rey, F. E. *et al.* Dissecting the *in vivo* metabolic potential of two human gut acetogens. *J. Biol. Chem.* **285**, 22082–22090 (2010).
99. Fontes, C. M. & Gilbert, H. J. Cellulosomes: highly efficient nanomachines designed to deconstruct plant cell wall complex carbohydrates. *Annu. Rev. Biochem.* **79**, 655–681 (2010).
100. Bayer, E. A., Lamed, R., White, B. A. & Flint, H. J. From cellulosomes to cellulosomics. *Chem. Rec.* **8**, 364–377 (2008).
101. Pokusaeva, K., Fitzgerald, G. F. & van Sinderen, D. Carbohydrate metabolism in bifidobacteria. *Genes Nutr.* **6**, 285–306 (2011).
102. Swidsinski, A., Loening-Baucke, V., Lochs, H. & Hale, L. P. Spatial organization of bacterial flora in normal and inflamed intestine: a fluorescence *in situ* hybridization study in mice. *World J. Gastroenterol.* **11**, 1131–1140 (2005).
103. Huang, J. Y., Lee, S. M. & Mazmanian, S. K. The human commensal *Bacteroides fragilis* binds intestinal mucin. *Anaerobe* **17**, 137–141 (2011).
104. Macfarlane, G. T. & Gibson, G. R. Formation of glycoprotein degrading enzymes by *Bacteroides fragilis*. *FEMS Microbiol. Lett.* **61**, 289–293 (1991).
105. Sonnenburg, J. L., Chen, C. T. & Gordon, J. I. Genomic and metabolic studies of the impact of probiotics on a model gut symbiont and host. *PLoS Biol.* **4**, e413 (2006).
106. Gibson, G. R. *et al.* Alternative pathways for hydrogen disposal during fermentation in the human colon. *Gut* **31**, 679–683 (1990).
107. Cantarel, B. L. *et al.* The Carbohydrate-Active EnZymes database (CAZy): an expert resource for glycogenomics. *Nucleic Acids Res.* **37**, D233–D238 (2009).
108. Nelson, K. E. *et al.* A catalog of reference genomes from the human microbiome. *Science* **328**, 994–999 (2010).
109. Zhang, G. & Hamaker, B. R. Slowly digestible starch: concept, mechanism, and proposed extended glycemic index. *Crit. Rev. Food Sci. Nutr.* **49**, 852–867 (2009).
110. Englyst, H. N., Kingman, S. M. & Cummings, J. H. Classification and measurement of nutritionally important starch fractions. *Eur. J. Clin. Nutr.* **46** (Suppl. 2), S33–S50 (1992).
111. Scott, K. P. *et al.* Substrate-driven gene expression in *Roseburia inulinivorans*: importance of inducible enzymes in the utilization of inulin and starch. *Proc. Natl Acad. Sci. USA* **108** (Suppl. 1), 4672–4679 (2011).
112. Ramirez-Farias, C. *et al.* Effect of inulin on the human gut microbiota: stimulation of *Bifidobacterium adolescentis* and *Faecalibacterium prausnitzii*. *Br. J. Nutr.* **101**, 541–550 (2009).
113. Cani, P. D. *et al.* Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia. *Diabetologia* **50**, 2374–2383 (2007).
114. Delzenne, N. M., Cani, P. D. & Neyrinck, A. M. Modulation of glucagon-like peptide 1 and energy metabolism by inulin and oligofructose: experimental data. *J. Nutr.* **137**, S2547–S2551 (2007).
115. Gourineni, V. P., Verghese, M., Boateng, J., Shackelford, L. & Bhat, K. N. Chemopreventive potential of synergy1 and soybean in reducing azoxymethane-induced aberrant crypt foci in fisher 344 male rats. *J. Nutr. Metab.* **2011**, 983038 (2011).
116. Munjal, U., Glei, M., Pool-Zobel, B. L. & Scharlau, D. Fermentation products of inulin-type fructans reduce proliferation and induce apoptosis in human colon tumour cells of different stages of carcinogenesis. *Br. J. Nutr.* **102**, 663–671 (2009).
117. Neyrinck, A. M. *et al.* Prebiotic effects of wheat arabinoxylan related to the increase in bifidobacteria, *Roseburia* and *Bacteroides*/*Prevotella* in diet-induced obese mice. *PLoS ONE* **6**, e20944 (2011).
118. Neyrinck, A. M. *et al.* Dietary modulation of clostridial cluster XIVa gut bacteria (*Roseburia* spp.) by chitin–glucan fiber improves host metabolic alterations induced by high-fat diet in mice. *J. Nutr. Biochem.* **23**, 51–59 (2011).
119. Van den Abbeele, P. *et al.* Arabinoxylans and inulin differentially modulate the mucosal and luminal gut microbiota and mucin-degradation in humanized rats. *Environ. Microbiol.* **13**, 2667–2680 (2011).
120. Varki, A. *et al. Essentials of Glycobiology* (Cold Spring Harbor Lab. Press, 1999).
121. Matsuo, K., Ota, H., Akamatsu, T., Sugiyama, A. & Katsuyama, T. Histochemistry of the surface mucous gel layer of the human colon. *Gut* **40**, 782–789 (1997).
122. Johansson, M. E. *et al.* The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. *Proc. Natl Acad. Sci. USA* **105**, 15064–15069 (2008).
123. Park, S. Y. *et al.* Proximal shift in the distribution of adenomatous polyps in Korea over the past ten years. *Hepato-gastroenterology* **56**, 677–681 (2009).
124. Larsen, I. K. & Bray, F. Trends in colorectal cancer incidence in Norway 1962–2006: an interpretation of the temporal patterns by anatomic subsite. *Int. J. Cancer* **126**, 721–732 (2010).
125. Toyoda, Y., Nakayama, T., Ito, Y., Ioka, A. & Tsukuma, H. Trends in colorectal cancer incidence by subsite in Osaka, Japan. *Jpn J. Clin. Oncol.* **39**, 189–191 (2009).
126. Singh, H., Demers, A. A., Xue, L., Turner, D. & Bernstein, C. N. Time trends in colon cancer incidence and distribution and lower gastrointestinal endoscopy utilization in Manitoba. *Am. J. Gastroenterol.* **103**, 1249–1256 (2008).
127. Png, C. W. *et al.* Mucolytic bacteria with increased prevalence in IBD mucosa augment *in vitro* utilization of mucin by other bacteria. *Am. J. Gastroenterol.* **105**, 2420–2428 (2010).
128. Furne, J., Springfield, J., Koenig, T., DeMaster, E. & Levitt, M. D. Oxidation of hydrogen sulfide and methanediol to thiosulate by rat tissues: a specialized function of the colonic mucosa. *Biochem. Pharmacol.* **62**, 255–259 (2001).

129. Winter, S. E. *et al*. Gut inflammation provides a respiratory electron acceptor for *Salmonella*. *Nature* **467**, 426–429 (2010).
This article demonstrates that tetrathionate, a metabolic by-product of the microbiota and human tissue combined, serves as an electron acceptor to enhance the physiology of a gut pathogen.

Acknowledgements
Work in E.C.M.'s laboratory is supported by the US National Institutes of Health (DK084214 and DK034933) and a Global Probiotics Council Young Investigator Grant. N.M.K. is supported by the University of Michigan (Ann Arbor, USA) Elizabeth M. Crosby faculty research grant. E.A.C. is supported by the University of Michigan Genetics Training Grant (GM07544).

Competing interests statement
The authors declare no competing financial interests.

FURTHER INFORMATION
Eric C. Martens's homepage: http://www.med.umich.edu/microbio/bio/martens
CAZy: http://www.cazy.org/

ALL LINKS ARE ACTIVE IN THE ONLINE PDF