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Minireview

Importance of Glycans to the Host-*Bacteroides* Mutualism in the Mammalian Intestine

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Much of the mutualistic relationship between humans and their resident intestinal *Bacteroides* species is founded on glycans. The host provides plant polysaccharides and host-derived glycans and, in return, receives beneficial end products of bacterial fermentation. Glycans from the bacteria themselves are required for the establishment and survival of these organisms in the colonic ecosystem and provide immunomodulatory properties to the host. Coordinated synthesis and catabolism of bacterial glycans is likely to contribute to the host-bacterial mutualism.

The Human Intestinal Ecosystem

The human digestive tract is specifically designed to obtain nutrients from ingested food, with compartmentalized regions performing distinct yet concerted functions. Digestion begins in the mouth with the breakdown of starch by amylase in the saliva. As the food passes to the stomach, acid is released, along with enzymes that aid in protein degradation. The chyme that enters the small intestine is mixed with bile to emulsify lipids for breakdown and absorption. The small intestine is also the region where macromolecules are broken down to smaller components with subsequent absorption of vitamins, small protein fragments, monosaccharides, and degraded lipids. The material that enters the large intestine (colon) is relatively devoid of dietary proteins, lipids, and simple sugars. Although starch is mostly degraded in the small intestine, some may also transit to the colon. The majority of the undigested macromolecules that arrive in the colon are complex nonstarch polysaccharides (dietary fiber) that the host does not have the enzymatic capability to degrade. Digestion in the colon is greatly aided by the trillions of microbes that inhabit this niche. This consortium of microorganisms produces an enormous enzymatic repertoire with the capacity to break down an extensive array of complex polysaccharides that the host is unable to process. In this regard, the intestinal microbiota has been referred to as a metabolic organ (Martens et al., 2008).

The numerically abundant microbial species of the human colonic ecosystem have evolved features that allow them to dominate this niche. Like the food's journey through the digestive chambers, the microbes that inhabit the colon are acquired orally and therefore are exposed to the harsh conditions of the digestive system en route to their niche. Organisms that successfully reach the colon must have survived the low pH of the stomach, the bile that is secreted into the small intestine, and intestinal innate immune factors designed to kill ingested microbes. Once in the colon, the abundant commensal microbes must establish themselves in a manner that does not deleteriously affect the host. In this regard, most resident colonic bacteria do not intimately interact with the intestinal epithelial cells but rather remain on the outer mucus layer overlying the epithelial surface (Johansson et al., 2008). In addition, the abundant Gram-negative bacteria of this ecosystem typically lack some of the molecules that stimulate innate host immunity, such as a potent endotoxin and enteric type flagella. To establish long-term colonization of this niche, these bacteria must have mechanisms to defend against attacks from other microbial members of the ecosystem and from phage. Of utmost importance for survival is that the bacteria are able to utilize the nutrients that are available in the ecosystem and do so in a manner that is efficient, sufficiently diverse, and responsive to nutritional changes.

An individual's intestinal microbiota is comprised of hundreds of different species of bacteria; however, there are relatively few that predominate. In general, bacteria from two phyla comprise the numerical majority: the Gram-positive Firmicutes and the Gram-negative Bacteroidetes (Eckburg et al., 2005). Within the Bacteroidetes phylum, the human gut organisms fall within the order Bacteroidales with two predominant genera, the *Bacteroides* and the *Parabacteroides*. *Bacteroides* species are among the most studied of the abundant gut bacteria, and much of their ability to thrive in this ecosystem directly pertains to their use and production of glycans. Host, dietary, and bacterial glycans play essential roles in the mutually beneficial (mutualistic) host-*Bacteroides* relationship. *Bacteroides* species have extensive machinery to utilize the complex polysaccharides present in the colon as a source of carbon and energy. In doing so, they produce fermentative end products in the form of short-chain fatty acids (SCFAs) that provide nutrition and other beneficial properties to the host. The production of several different types of glycans by the *Bacteroides* also aids the mutualism, as their production is essential for bacterial survival and provides beneficial immunomodulatory properties to the host.

The *Bacteroides*' Extensive Glycan Utilization Machinery

The polysaccharide composition of the colonic ecosystem is comprised not only of the complex plant polysaccharides, which the host lacks the enzymatic capacity to degrade, but also of host-derived mucopolysaccharides contained in the mucus layer overlaying the intestinal epithelial surface. The glycoprotein mucins are diverse in both their monosaccharide composition and glycan linkages. Both dietary and host glycans provide a

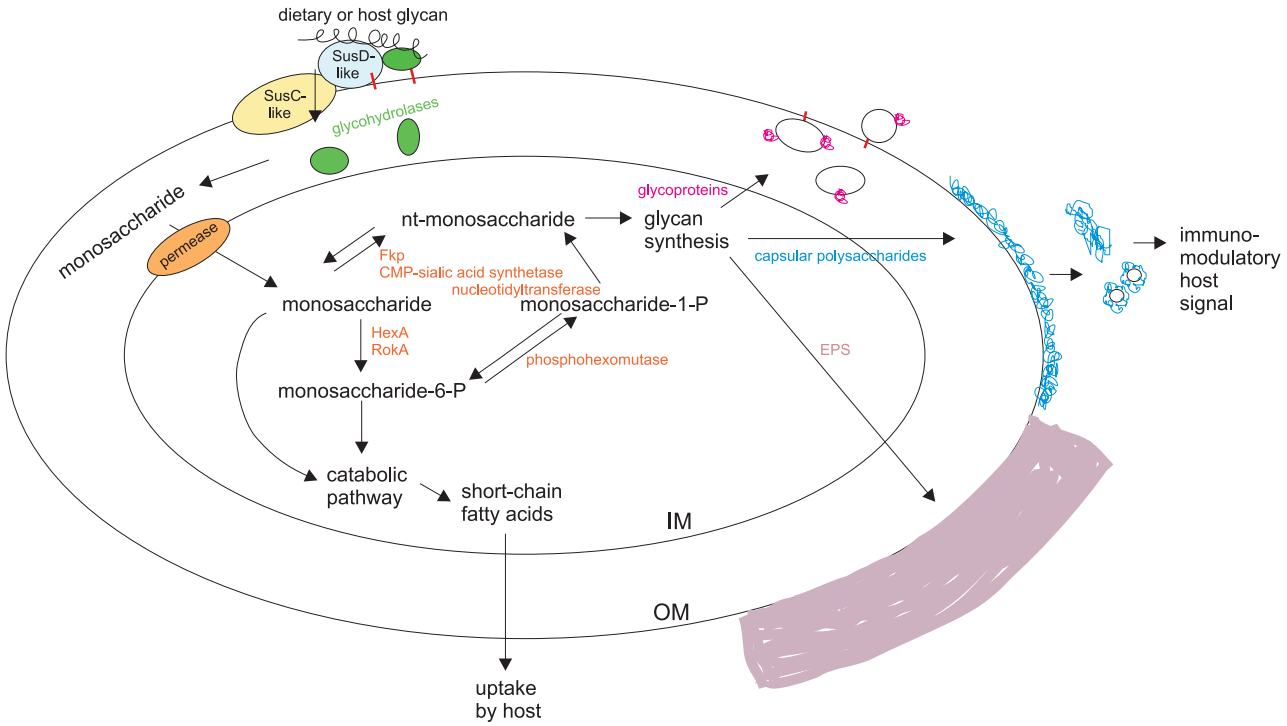

Figure 1. Overview of the Glycan Utilization and Synthesis Pathways in *Bacteroides fragilis*

Glycan fermentation and synthesis are a foundation for the bacteria's mutualistic relationship with the host. The upper left of the bacterial cell shows products encoded by a model PUL. The dietary or host glycan first binds to a glycan-specific SusD-like outer surface lipoprotein. In some cases, outer surface glycohydrolases can begin to degrade the polymer to smaller oligosaccharides that are then transported into the periplasm by the outer membrane TonB-dependent β-barrel SusC-like proteins. In the periplasm, additional glycohydrolases degrade the molecule to monosaccharide components that are transported to the cytoplasm by sugar-specific permeases. *B. fragilis* and other *Bacteroides* species do not have phosphotransfer sugar transport systems (Brigham and Malamy, 2005), and monosaccharides are therefore imported into the cytoplasm uncharged. At this point, the monosaccharides can either be destined for incorporation into bacterial glycans or for catabolism. The *B. fragilis* genome encodes at least two enzymes that can directly convert unphosphorylated monosaccharides (fucose and sialic acid) into their nucleotide-activated forms for incorporation into bacterial glycans. Three of the glycan types synthesized by *B. fragilis* are shown. The EPS is a large polymer that extends far from the bacterial surface. The capsular polysaccharides are more closely associated with the cell surface, but they can be released either by sloughing or in the form of outer membrane vesicles (Patrick et al., 1996), where they provide immunomodulatory properties to the host. The glycoproteins are essential for the bacteria's normal functioning and localize to the outer surface, outer membrane, and periplasm. Alternative to use in glycan synthesis, the uncharged monosaccharide can be acted upon by different enzymes. The cytoplasmic hexokinases, RokA and HexA, collectively phosphorylate most hexoses and *N*-acetylated hexoses on the sixth carbon. At this point, the hexose-6-P can serve as a substrate for catabolism or as a substrate for phosphohexomutases. These proteins catalyze the reversible synthesis of hexose-1-P, which are the substrates for nucleotidyltransferases that convert these sugars to their nucleotide-activated forms. These simple nucleotide-activated hexoses then serve as substrates for the synthesis of more complex nucleotide-activated di- and trideoxysugars that are incorporated into bacterial glycans. Alternatively, the catabolism of these monosaccharides provides SCFAs that benefit the host.

rich nutrient source for those colonic organisms that are equipped with the enzymatic arsenal to harvest them.

Early studies of *Bacteroides thetaiotaomicron* demonstrated its ability to utilize a variety of plant and host polysaccharides for its metabolism (reviewed in Salyers, 1990), but not until the publication of the first *Bacteroides* genome sequence was the extent of this capacity appreciated (Xu et al., 2003). The *B. thetaiotaomicron* VPI-5482 genome contains 88 polysaccharide utilization loci (PULs) (Martens et al., 2008), each of which encodes sets of proteins involved in sensing, importing, and degrading specific glycans of the colonic ecosystem. The first characterized PUL of *B. thetaiotaomicron* was the *sus* locus involved in starch utilization, which is comprised of eight genes encoding SusR and SusA–SusG. The Sus products include a regulatory protein; five outer membrane proteins involved in starch binding, degradation, and import into the periplasm (Reeves et al., 1996); and two periplasmic glycohydrolases with neopullulanase and α-glucosidase activity (D'Elia and Salyers, 1996).

Although different PULs encode different repertoires of functional products involved in the utilization of specific polysaccharides, there are many commonalities between these loci. PULs often contain genes encoding hybrid two-component histidine kinase response regulators, ECF-type sigma factors and anti-sigma factors, outer membrane proteins involved in nutrient binding and import (SusD and SusC paralogs, respectively), and glycohydrolases that enzymatically cleave the glycosidic linkages of specific glycans (Martens et al., 2008). Transcriptional profiling of bacteria grown on media containing single polysaccharides is allowing assignments of specific glycans utilized by specific PULs. The cellular localizations of representatives of some of the products encoded by PULs are shown in Figure 1.

The genome sequences of other abundant human gut *Bacteroides* species demonstrate similar expansions of PULs, although there are some differences in the metabolic capacities of different species. For example, *B. thetaiotaomicron* VPI-8254

is able to efficiently utilize the host-derived glycans heparin, chondroitin, and hyaluronan, whereas other *Bacteroides* species lack the necessary enzymatic arsenal to harvest these molecules (Xu et al., 2007). Other *Bacteroides* species, however, have glycolytic capabilities absent in *B. thetaiotaomicron*, such as *B. vulgatus*'s large complement of enzymes that target pectin (Xu et al., 2007). Because humans are colonized with many different *Bacteroides* species simultaneously, the composite polysaccharide degradative abilities of the collective *Bacteroides* population within an individual are enormous.

Several studies of *B. thetaiotaomicron* show that this organism has evolved mechanisms to prioritize glycan usage depending on nutrient availability within the ecosystem. Analyses using gnotobiotic mice supplied with diets either devoid of or rich in dietary polysaccharides have revealed a preference for utilization of dietary polysaccharides over host-derived glycans (Sonnenburg et al., 2005). If dietary polysaccharides are scarce, there is an increase in the expression of PULs involved in utilizing host glycans (Sonnenburg et al., 2005). In the absence of dietary glycans, the types of host glycans that are utilized are further prioritized. When three of the prominent host glycans of the intestine are each provided to *B. thetaiotaomicron* in vitro, the organism preferentially uses glucosaminoglycans over mucin O-glycans and N-glycans (Martens et al., 2008). However, bacteria isolated from gnotobiotic mice monoassociated with *B. thetaiotaomicron* demonstrate that the PULs for glucosaminoglycans are not highly expressed compared to those involved in O-glycan utilization, and therefore glucosaminoglycans may not be a readily available nutrient source in the mouse cecum.

The question arises as to whether the intestinal *Bacteroides* are selfish with regard to their nutrient utilization or whether they share their degradative products with microbial members less adapted to utilize the available complex glycan pool, possibly forming symbiotic or mutualistic microbial relationships. The data suggest that although some glycohydrolases are present on the bacterial surface, the polysaccharide substrate is likely anchored to the outer membrane first via a SusD paralog and is subsequently transported into the periplasm for further degradation. It is unlikely that these bacteria release a large number of soluble extracellular glycohydrolases, and therefore they probably do not extensively share with their microbial neighbors nor harvest glycans that are not first associated with the bacterial surface.

The degradation of dietary polysaccharides by the *Bacteroides* does, however, contribute significantly to host nutrition. End products of fermentation of these complex polysaccharides include SCFAs that are excreted to the intestinal lumen and utilized by host cells as a source of energy (Bergman, 1990). Therefore, the utilization of dietary glycans by these resident bacteria is the basis of a nutritional mutualism between host and microbe. In addition, SCFAs are reported to be essential to the overall health of the colon and one in particular, butyrate, has been demonstrated to have anticancer properties (reviewed in Hamer et al., 2008), leading to the prediction by many that the production of butyrate by the intestinal microbiota as an end product of dietary polysaccharide fermentation may be the link between a high-fiber diet and the decreased incidence of colon cancer.

Extensive Glycan Synthesis by the Intestinal *Bacteroides*

The great expansion of loci involved in glycan utilization by the *Bacteroides* is mirrored by an expansion of loci involved in bacterial glycan synthesis. The intestinal Bacteroidales dedicate a large amount of their genetic material to the synthesis of glycosylated molecules. A single strain of *Bacteroides fragilis*, for example, synthesizes eight distinct capsular polysaccharides (Krinos et al., 2001), an extracellular polysaccharide (Chatzidaki-Livanis et al., 2008), and a large number of glycoproteins (Fletcher et al., 2009). The *B. fragilis* 9343 genome encodes ~80 predicted glycosyltransferases and dedicates approximately 215,000 kb of its genome to the synthesis and regulation of glycans (Coyne et al., 2008).

The expression of each of the eight capsular polysaccharides of *B. fragilis* is subject to phase variation dictated by DNA inversions of the promoter regions upstream of seven of the eight polysaccharide biosynthesis loci (Krinos et al., 2001), conferring great antigenic variability to these organisms. The synthesis of multiple phase-variable capsular polysaccharides is a conserved feature of the intestinal Bacteroidales species, but not of the closely related oral species of this order, suggesting that this property confers an advantage for intestinal survival. The biological significance of the synthesis of such a large repertoire of phase-variable capsular polysaccharides by these bacteria is not yet fully understood. Production of at least one capsular polysaccharide is absolutely essential for *B. fragilis* to competitively colonize the gnotobiotic mouse intestine; however, an acapsular mutant is able to colonize to the same density as wild-type bacteria in the absence of competition (Coyne et al., 2008). Although the acapsular bacteria are equally as resistant as the wild-type to conditions of low pH and to bile (Coyne et al., 2008), the acapsular bacteria may be less fit to survive transit through the upper digestive tract, possibly due to greater sensitivity to innate immune factors. Selective killing of the acapsular bacteria in the upper digestive tract would result in lower numbers to competitively colonize the colon. Unlike the acapsular mutant, a *B. fragilis* mutant able to express only a single capsular polysaccharide competitively colonizes the colon of gnotobiotic mice (Coyne et al., 2008).

The synthesis of multiple phase-variable capsular polysaccharides is likely essential for the long-term colonization of these species in the normally complex human colonic microbiota. Capsular polysaccharides are often the targets of deleterious products of the colonic ecosystem such as phage adhesins and phage glycohydrolases. The ability of these bacteria to express subpopulations of organisms expressing distinct capsular polysaccharide surfaces would ensure that there is always a population of bacteria able to withstand an assault. The eight capsular polysaccharide biosynthesis loci are heterogenous within the species, meaning that different *B. fragilis* strains express compositionally and structurally distinct capsular polysaccharides. Therefore, it is unlikely that each capsular polysaccharide has a distinct function, per se; rather, the conserved and selected feature is the ability to express a large and varied repertoire of distinct surface polysaccharide structures.

In addition to the benefit afforded the bacteria by their ability to successfully colonize their niche, the capsular polysaccharides of *B. fragilis* also provide a beneficial property to the host. One

of the eight capsular polysaccharides synthesized by *B. fragilis* 9343, PSA, is an immunomodulatory molecule that has been demonstrated to prevent the development of colitis in an experimental animal model (Mazmanian et al., 2008). The immunomodulatory properties of this molecule lie in its zwitterionic nature, having both positive and negative charges on each repeat unit of the polymer. Although there is great variability in the PSA molecules synthesized by distinct *B. fragilis* strains, the last two genes of the PSA biosynthesis locus are conserved in the species and are involved in the synthesis and addition of the positively charged monosaccharide of PSA. Analysis of the genome sequences of numerous *Bacteroides* species reveals that many of the polysaccharide biosynthesis loci contain genes encoding both amino transferases and dehydrogenases (M. Coyne and L.C., unpublished data), which confer positive and negative charges to polysaccharides, respectively. Therefore, the production of immunomodulatory zwitterionic polysaccharides may be a common property of intestinal *Bacteroides* species.

The glycan synthesis machinery of *B. fragilis* also extends to glycoproteins and an exopolysaccharide (EPS). As yet, the functional significance of the phase-variable EPS has not been elucidated, and its importance may be confined to specific in vivo conditions. The glycoproteins, however, are critically important not only for in vivo survival of the bacteria, but also for the normal functioning of the organism. *B. fragilis* and other human intestinal *Bacteroides* species are some of the few bacteria demonstrated to have a general protein glycosylation system and the first O-glycosylation system shown to use a defined glycosylation motif (Fletcher et al., 2009). Although the number of proteins glycosylated in *B. fragilis* is unknown, it is likely extensive and includes molecules that localize to the periplasm, outer membrane, and cell surface. Many of these molecules are predicted to have roles in fundamental processes of the organism, including protein folding, protein-protein interactions, and protein degradation. It is not yet known if the glycoproteins of *Bacteroides* species are "symbiotic" molecules that provide beneficial properties to the host. Construction of defined mutants not only of general glycosylation functions but also of individual glycoproteins will aid in determining the contribution of these molecules to human health.

The plethora of polysaccharides and glycans that these bacterial species supply to the intestinal milieu raises the question of whether bacterial species can catabolize their own glycans or those of other microbial members. The types of monosaccharides that are components of bacterial glycans in general are extremely diverse compared to the relatively few monosaccharides that comprise eukaryotic glycans. *Bacteroides* species in particular synthesize many complex di- and trideoxy sugars that are incorporated into their capsular polysaccharides (Coyne et al., 2008). Production of complex polysaccharide structures by intestinal *Bacteroides* may have evolved because the complex polysaccharides are likely resistant to the large arsenal of bacterial glycohydrolases these bacteria produce. Although it is unlikely that most of the complex *Bacteroides* glycans are utilized as a nutrient source by the resident bacteria, this may not be true of all bacterial polysaccharides of this ecosystem. A recent study has shown that Gram-positive *Bifidobacterium* strains of the human colonic ecosystem produce EPSs that are fermentable substrates for various intestinal bacteria (Salazar et al., 2008). Therefore, glycan-mediated symbioses may also occur between microbial members of the human intestinal ecosystem.

Catabolism versus Glycan Synthesis

The fact that *Bacteroides* species can collectively reach densities of greater than 10^{10} bacteria per gram of human colonic contents necessitates an extreme demand for the harvest of carbon from the ecosystem and the concomitant production of energy. The carbon demand is further augmented as it is required for the synthesis of the numerous glycosylated molecules that are essential to the survival of these bacteria in their ecosystem. The degradation of host and dietary glycans by the numerous PULs of *Bacteroides* species results in the release of single monosaccharide components in the periplasmic space. The transport of these monosaccharides to the cytoplasm involves sugar-specific permeases that import monosaccharides without the simultaneous phosphorylation (Brigham and Malamy, 2005) that occurs in many other bacteria. The majority of these monosaccharides are then converted to their hexose-6-P forms via the action of two cytoplasmic sugar kinases—RokA and HexA—that collectively phosphorylate a broad range of hexoses and *N*-acetylated hexoses (Brigham and Malamy, 2005). Many of the hexose-6-P molecules can then be directed into either of two pathways, one for the synthesis of bacterial glycans and the other for catabolism (Figure 1).

At least two monosaccharides, fucose and sialic acid, have alternative pathways for both catabolism and incorporation into bacterial glycans. *B. fragilis* synthesizes two different enzymes, Fkp (Coyne et al., 2005) and a putative CMP-sialic acid synthetase (BF3831), which allow for the direct nucleotide activation of each of these monosaccharides, respectively, directing them for glycan synthesis. *fkp* is present in the genomes of all intestinal Bacteroidales species analyzed; however, it is rarely present in the genomes of other bacteria. The conservation of this gene in the intestinal Bacteroidales may be due to a large fucose requirement by these species, as this monosaccharide is a component of both capsular polysaccharides and glycoproteins. An alternative fate for the cytoplasmic fucose and sialic acid is that they can be utilized for catabolism. Whether these molecules are used for glycan synthesis or catabolized likely depends on the amounts of competing enzymes for these pathways. The intestinal Bacteroidales species *Parabacteroides distasonis*, which produces a tremendously large number of fucosylated glycans compared to *Bacteroides* species (Coyne et al., 2005), lacks a fucose utilization system (Xu et al., 2007). Therefore, in this organism, all the acquired fucose from the ecosystem can be shunted to the glycan synthesis pathway via Fkp.

Conclusions

Much of the mutualistic relationship that exists between humans and their resident intestinal *Bacteroides* species is founded on a glycan-based interplay between these organisms. The host provides the *Bacteroides* with nutrients in the form of plant polysaccharides and host-derived glycans that these microbes are exquisitely adept at sensing and utilizing accordingly. In return, the host receives the augmented metabolic capacity contributed by the *Bacteroides* and the beneficial end products of bacterial

fermentation. Bacterial glycans are also important players in this mutualism, as they are required for the establishment and survival of these organisms in the colonic ecosystem and also provide important immunomodulatory properties to the host.

There is still much work to be done defining which polysaccharides are utilized by each of the PULs of *Bacteroides* species. Although there are likely many overlapping glycan utilization profiles between different *Bacteroides* species, the data are also revealing that there may be species-specific PULs, which may help explain why different *Bacteroides* species coexist within an individual's colonic microbiota. Studies of glycan synthesis by the intestinal *Bacteroides* have mainly been directed at genetic and enzymatic analysis of biosynthesis and regulation, and few studies have analyzed the synthesis of these bacterial polysaccharides in vivo. There are many open questions regarding the interplay between glycan hydrolysis and glycan synthesis and whether glycan availability impacts the synthesis of specific polysaccharides. It will be interesting to determine if the orientations of the invertible capsular polysaccharide promoters are influenced by differing environmental conditions, especially the availability of specific nutrients in the ecosystem. Such analyses may demonstrate a correlation between the sensing and utilization of a specific polysaccharide from the ecosystem and the concomitant alteration of the bacteria's polysaccharide surface.

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