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The Critical Roles of Polysaccharides in Gut Microbial Ecology and Physiology

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Abstract

The human intestine harbors a dense microbial ecosystem (microbiota) that is different between individuals, dynamic over time, and critical for aspects of health and disease. Dietary polysaccharides directly shape the microbiota because of a gap in human digestive physiology, which is equipped to assimilate only proteins, lipids, simple sugars, and starch, leaving nonstarch polysaccharides as major nutrients reaching the microbiota. A mutualistic role of gut microbes is to digest dietary complex carbohydrates, liberating host-absorbable energy via fermentation products. Emerging data indicate that polysaccharides play extensive roles in host–gut microbiota symbiosis beyond dietary polysaccharide digestion, including microbial interactions with endogenous host glycans and the importance of microbial polysaccharides. In this review, we consider multiple mechanisms through which polysaccharides mediate aspects of host-microbe symbiosis in the gut, including some affecting health. As host and microbial metabolic pathways are intimately connected with diet, we highlight the potential to manipulate this system for health.

Contents

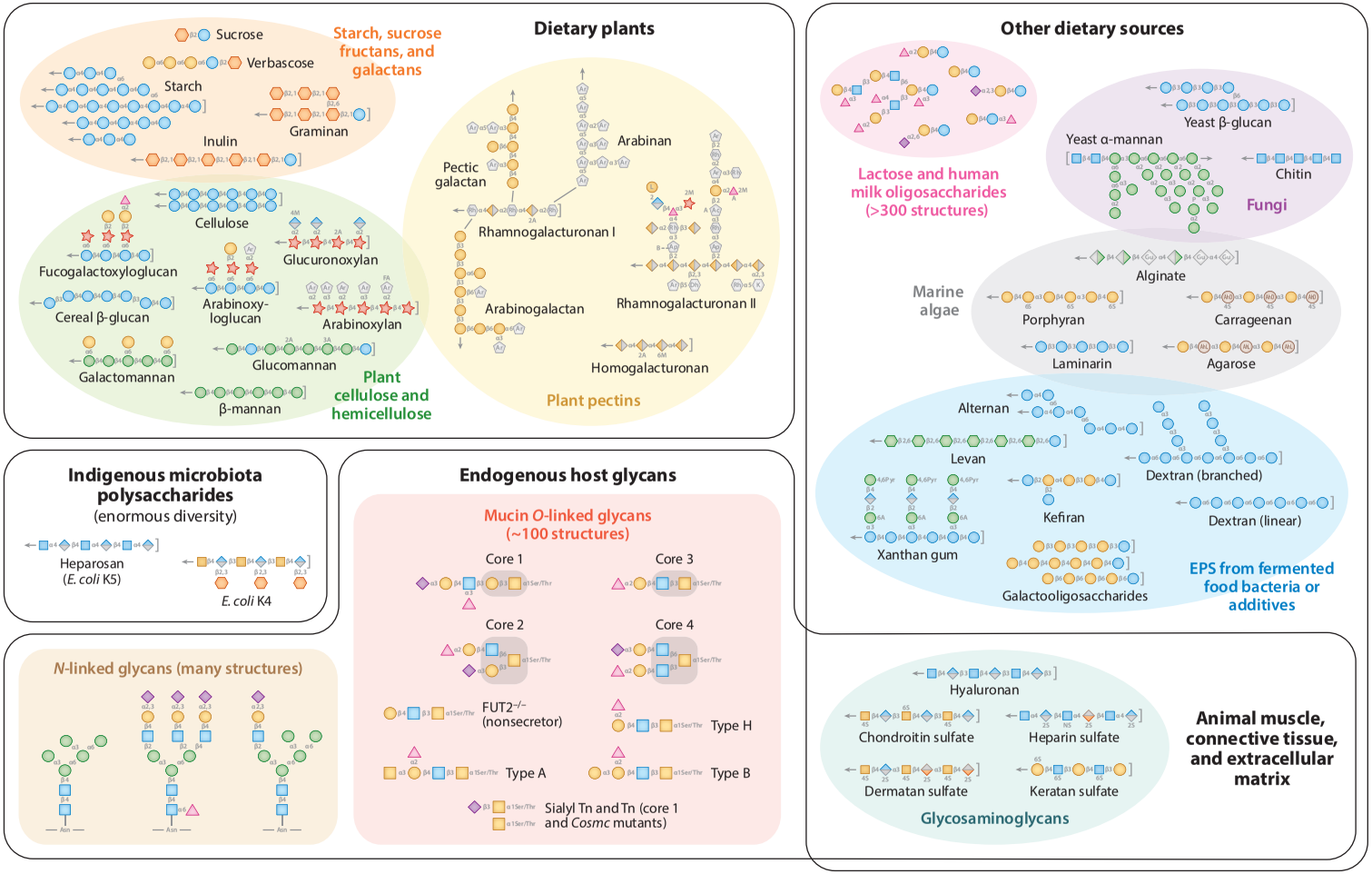
INTRODUCTION

Polysaccharides are the most chemically diverse molecules in living systems (14). This is due partly to the fact that they are composed of more than 20 common substituent sugars when one counts different monosaccharides plus their variable pyranose (six-atom) and furanose (five-atom) ring forms (**Figure 1**). However, unlike nucleic acids and proteins, which are linked by uniform phosphodiester and peptide bonds, polysaccharides can be extensively diversified by their linkage patterns, which may be either α or β and can be branched at more than two positions on a single substituent. Finally, carbohydrates can be covalently coupled to other common biological molecules, such as proteins and lipids (i.e., glycoconjugates), adopt secondary structure similar to proteins, and even exist in crystalline form, such as cellulose and some forms of starch.

Despite the astronomical diversity of polysaccharide combinations that may be synthesized from common component sugars, relatively little of this theoretical diversity has been explored by natural biological systems. A substantial portion of the known diversity exists in plants, fungi, and marine algae, including the variable pectins, hemicelluloses, α-mannans, and α-/β-galactans (e.g., agarose and carrageenan) that are essential for the physiology and structural integrity of these organisms (2, 19, 52, 91). It is likely that even more diversity exists in the *O*- and *N*-linked

Figure 1

Representative diversity of polysaccharides derived from various dietary, host, and microbial sources. Gray brackets indicate reducing ends of polysaccharides and gray arrows indicate the possibility of extended polymer length, which may be very long in some cases (e.g., corn starch with up to 10^6 glucose units per chain). Most of the polysaccharides shown are known to be degraded by human gut bacteria. The three major sources of polysaccharides (diet, endogenous host glycans, and microbially produced glycans indigenous to the microbiota) are arranged in order of increasing diversity, beginning in the upper left and proceeding clockwise through indigenous microbiota polysaccharides. For the latter, only two *Escherichia coli* capsule (CPS) structures are shown, which overlap with the host glycosaminoglycans heparin and chondroitin sulfate. However, the diversity of microbiota CPS structures is likely astronomical and is still largely unexplored. Linkage types (α or β) between sugars are indicated, and where the donor sugar is linked via carbon 1 to another monosaccharide, this number is not indicated (e.g., β1,4 linkage between two sugars is written as β4).



Sugar symbols:

- Glucose
- Mannose
- Galactose
- Fructose
- *N*-acetylgalactosamine
- *N*-acetylglucosamine
- *N*-acetylneuraminic acid (sialic acid)
- Fucose
- Galacturonic acid
- Glucuronic acid
- Iduronic acid
- Mannuronic acid
- Arabinose
- Xylose
- Rhamnose
- Guluronic acid
- Acerose
- Apiose
- 2-Keto-3-deoxy-D-*manno*-octulosonic acid
- 2-Keto-3-deoxy-D-*lyxo*-heptulosaric acid
- 3,6-Anhydro-D-galactose
- 3,6-Anhydro-L-galactose

Other modification symbols:

M	Methyl	Asn	L-asparagine
A	Acetyl	Ser	L-serine
S	Sulfate	Thr	L-threonine
FA	Ferulic acid		
P	Phosphate		
Pyr	Pyruvate		

glycolipid and glycoprotein conjugates found in mammalian tissues and secreted products, such as mucus and shed host cells (55, 122, 124). Finally, microbes may be the most diverse and underexplored repositories of glycobiological content, given their ability to build not just essential structural polysaccharides (peptidoglycan and lipopolysaccharide) but also an extensive array of polysaccharide capsules and exopolysaccharides, many of which contain rare component sugars. All three of the above sources of glycobiological diversity (plant, animal, and microbial) converge in the human distal gut, where a dense and dynamic community of symbiotic microbes (microbiota) exists in homeostasis (from a microbial perspective, perhaps turbulent disequilibrium) with the host. The gut microbiota plays many important roles in health and disease and is, in turn, influenced by the dynamic compositional and physiological changes that are exerted in this ecosystem by diet. As such, dietary polysaccharides are an obvious focus of rational interventions involving the microbiota. However, other host and microbial carbohydrate-based physiological pathways have also emerged as important factors in health and disease, and some of these are influenced by diet. In this review, we consider the many interconnected carbohydrate-based transactions that take place in the gut and involve the microbiota. We focus on the sources and chemical details of the complex carbohydrates that mediate these interactions, as well as emerging frontiers in understanding and manipulating these complex relationships to promote health.

INFLUENCE OF DIETARY POLYSACCHARIDES ON THE MICROBIOTA-HOST SYMBIOSIS

The genomic era, coupled to reinvigorated culturing efforts for human gut microorganisms, has accelerated mechanistic research toward understanding a mutualistic behavior that has long been attributed to human gut microbes: the critical importance of these organisms in digesting dietary fiber polysaccharides (47). While the human genome only encodes a handful of gastrointestinal enzymes that mostly target starch—thereby releasing glucose for direct absorption in the small intestine—some individual human gut bacteria produce hundreds of individual enzymes with collective catalytic specificities ranging far beyond starch (42). For example, the gram-negative symbiont *Bacteroides thetaiotaomicron*, one of the first common gut bacteria after *Escherichia coli* to have its genome sequenced, encodes over 300 enzymes in the glycoside hydrolase, polysaccharide lyase, carbohydrate esterase, and sulfatase families (11, 135). Despite this impressive armament of degradative enzymes, the sequenced type strain of this species is still incapable of utilizing a number of common polysaccharides as nutrient sources, most notably plant cell wall hemicelluloses that are abundant in grains, nuts, fruits, and vegetables (75). However, systematic investigations of a few additional *Bacteroides* species (*B. ovatus*, *B. cellulosilyticus*, *B. xylanisolvens*), each with even larger polysaccharide-degrading enzyme repertoires than *B. thetaiotaomicron*'s, revealed abilities to degrade hemicellulosic polysaccharides and, like studies of *B. thetaiotaomicron*, identified the genes involved (36, 37, 75, 81). Even more intriguingly, studies focusing on the ability of human gut bacteria to catabolize relatively rare dietary polysaccharides, such as carrageenan, agarose, porphyran, and alginate polymers found in edible seaweed, have revealed the presence of these abilities in geographically and culturally distinct populations (53, 54, 125).

An impressive series of follow-up studies have targeted select multiprotein catabolic systems in some of the species noted above for in-depth biochemical and molecular genetic studies of the enzymatic, regulatory, and transport mechanisms involved (5, 31, 66, 97, 100, 104, 114, 115). Although many of these detailed efforts have focused on members of the gram-negative *Bacteroidetes*, one of the dominant phyla colonizing the gut, similar studies are emerging for members of genera belonging to the two abundant gram-positive phyla, *Firmicutes* and *Actinobacteria* (10, 26, 40, 41, 50, 83). The known mechanisms through which members of all three of these phyla

degrade dietary polysaccharides, ranging from milk oligosaccharides to plant and fungal cell walls, have recently been reviewed in detail (25) and therefore are not covered in depth here. These mechanisms mostly involve either energy-dependent Sus-like transport systems coupled to outer membrane–associated and periplasmic enzymes in the *Bacteroidetes* (48, 74) or ABC-transport systems coupled to degradative enzymes in the *Firmicutes* and *Actinobacteria* (26, 40, 50). In addition, a third mechanism, cellulosome-like scaffolded enzyme systems, has recently been discovered in some human gut members of *Firmicutes* (namely members of the genus *Ruminococcus*) that so far have been found to use these systems to target cellulose and resistant starch (10, 137).

While substantial need for mechanistic investigation remains, especially in gram-positive gut bacteria, it has become clear that most of the known dietary fiber polysaccharide structures expected to be introduced into the gut (**Figure 1**), even insoluble cellulose, can be utilized by some gut bacteria. Two open questions are, how much variation exists across individuals, and how much redundancy exists among individual bacterial members of a microbiota? The answer to each of these questions will be important for understanding how individual members of the microbiota metabolize various dietary polysaccharides, whether they do so mostly alone or as consortia, and how resilient communities are to loss of species that conduct key digestive functions. With respect to the latter point, it was recently shown in gnotobiotic mice colonized with a human microbiota that prolonged dietary fiber starvation is capable of catalyzing irreversible extinction of some microbial groups that are more consistently supported by a fiber-rich diet (118). In this context, it has been hypothesized that the gut microbiota of modern humans—especially those living in industrialized nations where fiber consumption has diminished in recent decades—has lost carbohydrate-metabolizing bacteria over multiple generations (119). If this is true, we may need to look to human populations that have retained high-fiber, unprocessed diets for repositories of microorganisms that can eventually be reintroduced (i.e., as probiotics) into humans with low digestive diversity.

HOST GLYCOCONJUGATES SHAPE AND ARE SHAPED BY THE MICROBIOTA

Changes in Host Intestinal Glycosylation in Response to Symbiont Colonization

A second major source of glycans present in the gut is the pool of endogenous molecules attached to cell surfaces, shed epithelial cells, and secreted mucus. The structures of these glycans, typically conjugated to either lipids or proteins (*O*-linked to serine or threonine or *N*-linked to asparagine), are distinct from those of dietary polysaccharides other than milk oligosaccharides, which are themselves produced by host mammals and can overlap substantially with *O*-linked glycans (**Figure 1**). The interactions between gut microbes and host glycans are already known to be manifold and can contribute to beneficial or detrimental outcomes for both the host and gut microbes. Interestingly, the glycobiology of the intestinal surface has been shown to be both temporally and geographically dynamic and responsive to microbiological signals, leading to the idea that changes in intestinal glycosylation occur in part to influence the microbiota. One of the first examples of this phenomenon is α-fucosylation of ileal epithelial cells during development of the murine gut (17). In weaned mice with a conventional microbiota, there is heavy fucosylation of the ileal surface. In genetically identical mice raised germfree, this fucosylation is absent but can be restored (even in adult germfree mice), if a single species (*B. thetaiotaomicron*) or a conventional microbiota is introduced. Even more interesting, *B. thetaiotaomicron* mutants that cannot utilize L-fucose as a nutrient source fail to elicit ileal fucosylation in germfree mice, suggesting the presence of a

two-way signaling pathway by which microbial foraging of fucose from host glycans triggers increased production by the host (17, 56). This phenomenon of commensal-mediated cell surface fucosylation was subsequently recapitulated in cultured murine ileal organoids injected only with *B. thetaiotaomicron* culture. As observed in vivo (17), inoculation correlates with transcriptional activation of murine *FUT2* (α−1,2-fucosyltransferase), which encodes the enzyme responsible for building this host glycan linkage (43).

More recently, increased small intestinal fucosylation has also been linked to systemic signals associated with bacterial infection (92). Intraperitoneal injection into mice of Toll-like receptor 2, 4, or 9 ligands that signal the presence of various bacterial molecules (triacylated lipoprotein, lipopolysaccharide, or CpG DNA, respectively) triggers a rapid increase in small intestine fucosylation that is also dependent on Fut2 activity. While the small intestine harbors a relatively low-density microbial community, newly fucosylated glycoproteins are degraded as they transit to the colon in a microbiota-dependent fashion. Thus, it has been hypothesized that such a response exists to provide endogenous sources of glycan-derived nutrients to the microbiota during times of stress due to bacterial infection (92). Such a response may suppress the activity or proliferation of potential microbiota-resident pathogens, either by selectively feeding nutrients to indigenous mutualists or by altering host glycan structures to make them less accessible to opportunistic pathogens. In the context of these studies highlighting microbiota-mediated intestinal glycan fucosylation, ~20% of North Americans and Europeans exhibit the nonsecretor blood group phenotype (64), attributed to loss of *FUT2* (**Figure 1**); the effect of this sequence variation has been shown to directly affect the composition and physiology of the microbiota in a diet-dependent fashion (62).

Host- and Microbiota-Catalyzed Changes During Disease

The studies described above examined changes in fucosylation of glycans secreted in the small intestine, a site of lower microbial colonization that may pass these nutrients to the distal gut. Glycoconjugates in the colon, a site with high colonization density, are also critical to the diet-microbiota-host axis. One of the most prominent sources of colonic glycans is secreted mucus, a mixture of mucin glycoproteins (Muc2 and Muc5ac are abundant in human and mouse colon) and other molecules, such as immunoglobulins (80). The most abundant form of glycan nutrients attached to mucins are *O*-linked glycans (**Figure 1**), composing 50–80% of secreted mucin mass (4, 58). A number of taxonomically diverse gut bacteria have been shown to utilize mucus, including *B. thetaiotaomicron* (73, 121), although clearly not all species possess this ability (57, 93).

Mucus is secreted by goblet cells as a gel-forming layer overlying colonic epithelial cells. It functions, in part, as a physical barrier that protects the host from the dense microbiota (located just microns away in the gut lumen), as well as from invading pathogens (80). A number of important microbiota-mucus interactions have been revealed in recent years. Near-complete elimination of this barrier through deletion of the murine *Muc2* gene results in the microbiota living immediately adjacent to host tissue, causing inflammation and eventual colorectal cancer (59, 129). While mutations this severe are not known to naturally exist in humans, more subtle mucin glycosylation defects have been created in mouse models that also lead to increased susceptibility to either spontaneous or chemically induced inflammation resembling inflammatory bowel disease (IBD). Some of these mutations involve elimination of core glycosyltransferase enzymes (**Figure 1**), which like Fut2 mentioned above are involved in synthesizing host glycoconjugates (13, 49). Whereas Fut2 adds terminal fucose to existing chains, core 1 and core 3 glycosyltransferases build broad sets of common base structures that are subsequently extended and diversified. Thus, eliminating these key activities results in broad reductions in *O*-glycan diversity, potentially making it easier for colonizing microbes to degrade these protective structures and erode the function of this barrier.

While mutations in the core glycosyltransferases noted above have also not yet been directly associated with human intestinal disorders, mutations in the X chromosome–linked endoplasmic reticulum protein *Cosmc* (a chaperone for core 1 glycosyltransferase) have been associated with human IBD. A recent study found that *Cosmc*-deficient mice fail to synthesize normal mucin glycosylations, instead decorating these important barrier molecules with truncated *O*-linked glycans, such as Tn antigen and sialyl-Tn (**Figure 1**) (65). This defect in mucus glycosylation, in turn, leads to alterations in the colonic microbiota and spontaneous inflammation, underscoring the conclusion that genetic alterations in colonic glycoconjugates can predispose the host to disease.

While the examples discussed above outline paths by which gut microbes trigger host glycosylation or elicit disease in the absence of normal mucin glycan development, additional recent studies have revealed that the microbiota can be driven to erode the colonic mucous layer in the absence of dietary polysaccharides (35, 39). Because the dietary polysaccharides that collectively compose fiber from plants and other sources (**Figure 1**) are a predominant source of nutrition for the microbiota, the absence of these nutrients forces some members of the microbiota to rely instead on indigenous host glycans. A shift from utilizing dietary versus host polysaccharide nutrients has been observed in gnotobiotic mice colonized with just one bacterium, *B. thetaiotaomicron* (121), which is otherwise metabolically programmed to preferentially degrade dietary fiber polysaccharides before mucin glycans (72, 94, 103) and may otherwise avoid foraging on host carbohydrates in the presence of sufficient dietary input. An important feature of the study noted above (in which a low-fiber diet catalyzed microbiota-mediated erosion of the colonic mucous layer) is that the wild-type mouse host remained healthy despite its diminished mucous barrier. However, when mice fed high-fiber (thick mucus) and low-fiber (thin mucus) diets were challenged with the enteric pathogen *Citrobacter rodentium*, those with a microbiota-eroded mucous layer experienced accelerated disease progression that resulted in lethal colitis in some animals (35).

Collectively, the studies described above underscore that both mutualistic bacteria and pathogens may alter the glycan landscape at different regions in the intestine. Moreover, these studies suggest the existence of a very complex system in which both dietary polysaccharides and members of the gut microbiota influence the host's response (**Figure 2**). Other emerging literature has linked *Bacteroides* commensals to release of sugars (e.g., sialic acid) for other bacteria, including pathogens, and has begun to uncover polysaccharide-digesting food chains consisting of multiple bacterial species (85, 93, 138). Even bacteriophages, some of which have the ability to bind to mucous glycans via conserved lectin or immunoglobulin-like domains in their virion proteins, have entered the picture and have been hypothesized to provide a layer of adaptive immunity to the mucous layer by retaining high populations of phages that have recently killed the most successful colonizers (6, 7). Still, an even more complex and only lightly explored horizon exists in the suite of polysaccharide capsules, exopolysaccharides, and cell walls that are produced by the many different symbiotic bacteria that are indigenous to the gut microbiota or that are present in fermented foods (**Figure 1**). Emerging roles for polysaccharides in this final category are considered in the next section of this review.

BIOLOGICAL EFFECTS OF MICROBIALLY PRODUCED GLYCANS

An Underexplored Repository of Polysaccharide Diversity and Function

In addition to facilitating dietary polysaccharide degradation, many gut microbes also possess the ability to synthesize a wide variety of glycan structures. These include exopolysaccharides (EPS), microbial glycans that are loosely associated with the cell surface and may easily slough off into the extracellular environment; and capsular polysaccharides (CPS), glycans that are more firmly attached to the cell surface. However, whether a glycan is attached to the cell surface or

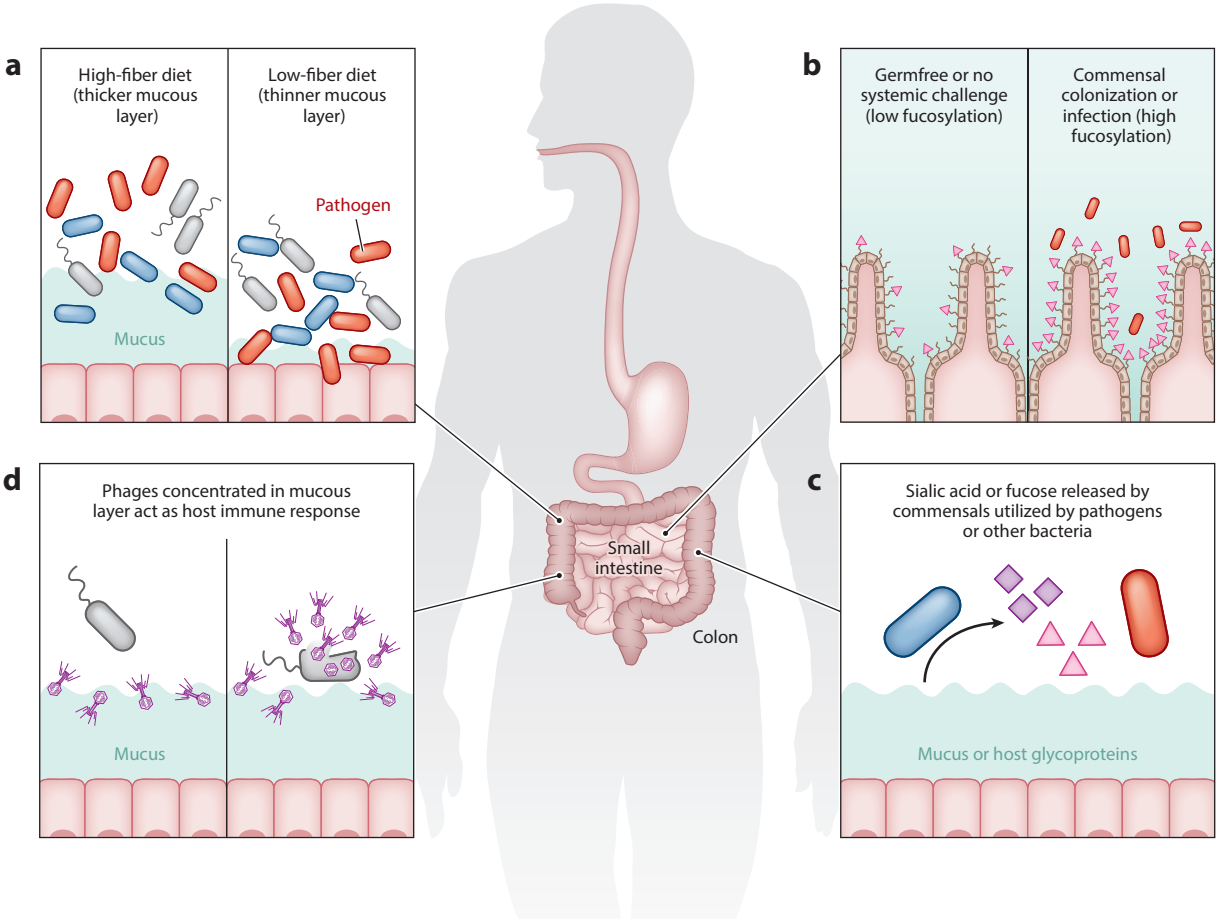


Figure 2

Examples of how polysaccharides mediate interactions between the gut microbiota and host. (*a*) On a high-fiber diet fewer bacteria access the colonic mucous glycoprotein layer for nutrients, but when dietary fiber is low, this barrier can be eroded by mucin-degrading bacteria, facilitating pathogen access to the host epithelium. (*b*) When germfree mice are colonized with a conventional microbiota or a single species such as *Bacteroides thetaiotaomicron* or when conventional mice are challenged with a systemic infection signal (bacterial TLR ligands), the small intestine epithelium increases the amount of surface and secreted fucosylated glycans (*pink triangles*). (*c*) The metabolic activity of some commensal bacteria (including *Bacteroides* spp.) may release free sugars (*purple diamonds*, *pink triangles*) that are accessible by pathogens (*Clostridium difficile* or *Salmonella* spp.; 85) or other members of the microbiota, such as *Escherichia coli* (92), that lack their own enzymes to liberate these sugars. (*d*) Bacteriophages that have been released from recently killed bacteria can remain bound in the colonic mucous layer, potentially functionalizing it with phage-based adaptive immunity that is specific for bacterial populations that have recently been killed by these viruses.

not is unclear in many gut microbes; thus, these terms are often used interchangeably. For the purposes of this review, we use the term microbially produced glycans (MPGs) to encompass both CPS and EPS. MPGs have been studied in many pathogenic microorganisms or their close symbiotic relatives, revealing extensive complexity, but unfortunately they have been studied far less in nonpathogenic microbes that compose most of the gut microbiota. Much of this research has focused on models like *Escherichia coli*, and inferences to symbiotic capsules are drawn from these systems and from studies on other pathogens. There is enormous diversity of capsule types

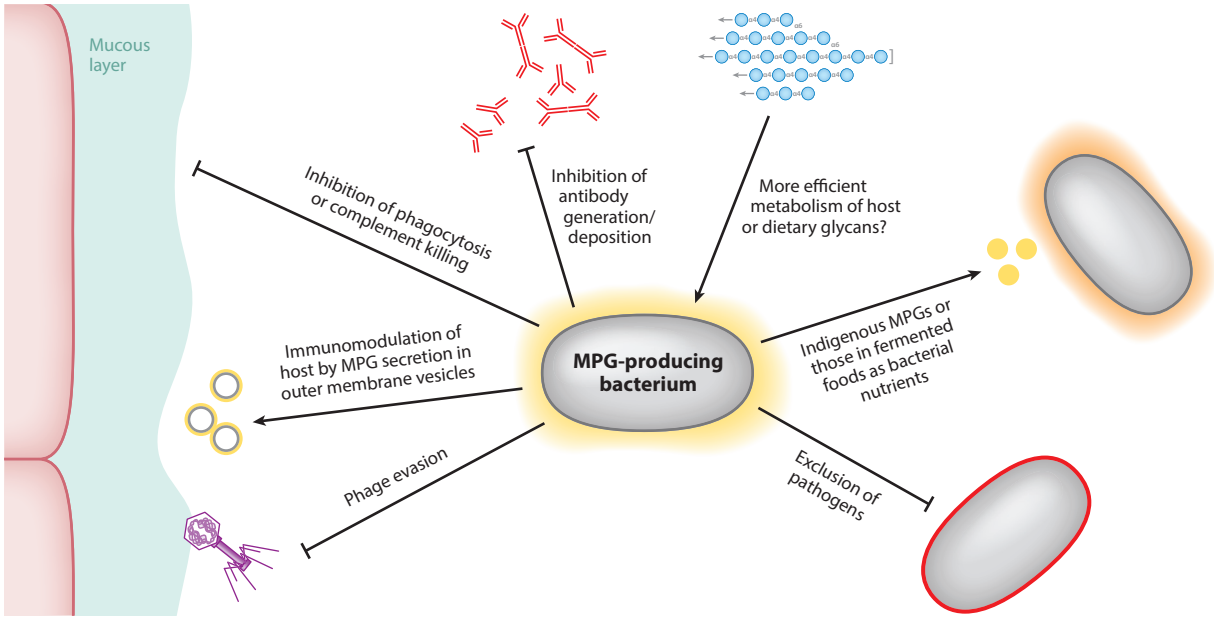


Figure 3

Multiple roles of microbially produced glycans (MPGs) in symbiotic bacterial fitness in the gut. Various mechanisms by which MPGs enhance bacterial fitness are shown. No single model system has demonstrated all of these mechanisms, indicating that the MPG structure and/or bacterial niche dictates the role of each polysaccharide.

displayed just by various *E. coli* strains (~80 known K antigens; 133), and emerging data from genomic sequencing efforts have revealed that polysaccharide synthesis genes are both highly abundant and overrepresented in gut bacteria relative to environmental samples (24, 38). Thus, gut bacterial MPGs are likely the most diverse group of polysaccharides relevant to host-microbiota interactions in the gut. Based on studies of just a handful of MPGs discussed below, it is likely that their biological effects are also broad (**Figure 3**), influencing host immunological responses, bacterial fitness, and other important aspects of the host-microbe relationship.

Synthesis

Multiple mechanisms exist for MPG synthesis, with the best-studied systems being in *E. coli* (133). These include the Wzy-dependent mechanism, where assembly of repeat unit oligosaccharides occurs via glycosyltransferases in the cytoplasm; these oligosaccharides are then polymerized into longer chains on the cell surface (gram-positive bacteria) or the periplasm, with subsequent export to the cell surface (gram-negative bacteria). This contrasts with other mechanisms such as ABC transporter–dependent synthesis, where the entire glycan chain is polymerized in the cytoplasm. Synthase-dependent and glucansucrase/fructansucrase-dependent pathways produce relatively less complex glycans, with one or two monosaccharides incorporated into the glycan. All four of these pathways have been reviewed extensively elsewhere (130, 133, 136).

Diversity

Extensive genetic diversity of MPG biosynthetic loci in individual species of several common and abundant gram-negative and gram-positive genera of gut bacteria is well known, including

Escherichia (133), *Lactobacillus* (1, 12, 82, 96), *Bifidobacterium* (45), and *Bacteroides* (29, 90). Many gut bacteria encode only a single genomic locus for MPG synthesis, which may be variable among related strains or species. However, lactic acid bacteria often possess both a glucansucrase (or fructansucrase) for synthesizing a homopolysaccharide and a locus encoding a heteropolysaccharide, and *E. coli* strains often have a CPS biosynthetic locus as well as a locus for synthesis of the EPS colanic acid. Alternatively, members of some bacterial groups encode multiple gene clusters that enable them to produce larger numbers of MPGs (44, 130). Notably, some gut-associated *Bacteroidales* (*Bacteroides* and *Parabacteroides* genera) can encode as many as a dozen distinct CPS synthesis loci per genome, with expression of these factors being regulated in some species by a complex network of phase-variable promoters and transcription factors (21, 22, 30). These latter observations imply additional benefits, at least for some species, for producing multiple, diverse CPS types in the gut environment and consistently varying their expression (28).

Contributions to the Diet-Microbiota-Host Axis During Intestinal Colonization

To persist in competitive gut ecosystems, intestinal microbes must either adhere to the mucosal surface or grow faster than the rate at which they are killed or excreted from the gut. While production of MPGs could be viewed as a waste of resources that would otherwise be devoted to microbial growth, based on their presence in widely divergent gut bacteria these structures apparently offer substantial benefits. Many probiotic strains that produce EPS benefit by longer gut persistence times or higher titers in mice, at least when competed against isogenic EPS-deficient (EPS^-) strains (44, 67, 69, 117). However, this is not always the case. One study compared two *Lactobacillus* strains that differed in their ability to persist in the mouse gut and identified an EPS synthesis locus in the persisting strain that was also transcriptionally upregulated in the mouse gut. However, deletion of this locus did not affect gut persistence times (33). Additionally, strain-to-strain variation exists in the benefits conferred by producing MPGs. Although Sims and colleagues found an EPS-producing (EPS^+) strain of *Lactobacillus reuteri* to be more competitive than an isogenic EPS^- strain (117), this was not the case in a different strain of *L. reuteri* (131). However, the addition of *L. johnsonii* as another competitor gave an increased advantage to the wild type, indicating that capsules may provide an advantage against a broad range of microbial competitors beyond close relatives (131). Similar variability has been seen for *Bacteroides* spp., for which individual strains typically express multiple capsules. Whereas a *Bacteroides fragilis* mutant expressing just one of its eight CPS (PSH) outcompetes an acapsular mutant in vivo, the PSH-expressing strain competes equally with wild-type *B. fragilis* capable of expressing different capsules (individual capsules in this species are alphabetically named PSA through PSH) (27). However, other mutants expressing just PSA, PSB, or PSC are all outcompeted against wild type in vivo (70). Differences in competition in these studies likely point to mechanistic differences in capsules as fitness factors and underscore the need to better understand the precise roles these capsules play in symbiotic organisms.

Links between diet and MPG expression. There are multiple mechanisms by which MPGs may enhance bacterial survival in the gut (**Figure 3**). Since commensal gut microbes provide a critical capacity to digest dietary polysaccharides, capsules with specific structures or properties may provide optimal access to certain nutrients or facilitate the most efficient use of the bacterial cell's resources. When lactic acid bacteria are cultured on sucrose, for example, they can hydrolyze sucrose into its monosaccharide constituents, glucose and fructose, and utilize a portion of this energy pool to directly synthesize extracellular glucans or fructans (see EPS derived from fermented food bacteria in **Figure 1**) (130). The amount of EPS produced varies with carbohydrate growth

source (96). Moreover, in *Bacteroides thetaiotaomicron*, changes in the host diet alter expression of this species' eight CPS synthetic loci when it is the sole colonizer in vivo. Specifically, in low dietary fiber conditions in the mouse gut, where *B. thetaiotaomicron* can access only host-derived glycans as a main nutrient source, it shifts capsule expression away from CPS4 (the most preferred on a high-fiber diet) toward CPS5 and CPS6 (in this species capsules are named CPS1 through CPS8) (15, 121). Additionally, molecular genetics studies that either inappropriately activated polysaccharide-degrading systems in vitro or impaired their function in vitro and in vivo (76, 120) suggest that particular capsules equip this species to proliferate optimally in conditions where different dietary or host-derived nutrients are available. However, direct evidence connecting expression of any particular CPS to optimal use of a specific polysaccharide nutrient is still lacking.

Interactions with the immune system. The capsules produced by pathogenic microbes protect them in various ways from the host immune response (102), including from innate immune mechanisms, such as complement-mediated killing or phagocytosis. Although MPGs in symbiotic microbes do not generally appear to provide resistance to prominent environmental challenges like bile acids (although bile can increase EPS expression in one strain; 106), they may provide increased resistance to complement and/or antimicrobial peptides in certain bacteria (27, 67).

Nonpathogenic microbes can also avoid host recognition via MPG production. EPS^+ variants of one *Bifidobacterium breve* strain with two EPS loci elicit lower levels of inflammatory cytokines and inflammatory cell types than their EPS^- counterparts (which lack both EPS loci) (44). Higher levels of plasma cells, higher titers of fecal IgA, and increased levels of T cells producing inflammatory cytokines were seen in mice exposed to EPS^- strains. Additionally, the EPS^+ wild-type strain is able to persist longer than EPS^- strains in wild-type mice, but this difference is abolished in B cell–deficient mice, indicating a role for the EPS in evading adaptive immune responses. Serum against the EPS^+ wild-type strain was able to agglutinate wild-type cells but poorly agglutinated EPS^- cells and vice versa, indicating that the presence of EPS protects underlying cell surface antigens from detection (44). This is supported by work in *L. johnsonii* where antibodies to an EPS^+ strain bound to an EPS^- strain at higher titers than to wild type, suggesting that EPS in part acts to block antibody recognition. A mutant overexpressing EPS had similar levels of bound antibody compared to wild type, indicating that the EPS itself is a poor immunogen (34).

While MPGs may conceal the microbe from the host immune system, some MPGs can directly modulate its effects. The best studied of these are termed zwitterionic polysaccharides (ZPS) and are capable of manipulating both the innate and adaptive immune systems (3, 78). ZPS contain alternating positive and negative charges, which are essential to their modulatory functions. The prototypical ZPS, PSA from *B. fragilis*, was first studied in a rodent model of anaerobic abscess formation. Treatment with sterile cecal contents along with *B. fragilis* or purified PSA (but not a PSA-deficient mutant) elicits disease in this model, whereas CPS from other pathogenic organisms were not capable of this effect (29, 89, 127). Chemically modifying PSA and other ZPS to neutralize their positive or negative charges reduced their ability to induce abscesses. Moreover, modification of a non-abscess-inducing capsule to contain both positively and negatively charged residues enabled it to elicit disease (abscess formation), indicating that it is the zwitterionic nature of the polysaccharides that allows them to induce this process (127).

Interestingly, without sterile cecal contents, prophylactic administration of purified PSA reduced abscess formation instigated by diverse pathogens, apparently working in a nonspecific fashion to suppress disease progression. Injection of naive mice with T cells from PSA-treated mice was sufficient to reduce abscess formation, pointing to a direct effect on the host immune system (126). PSA was later studied in the context of its role as a mutualistic factor influencing the

adaptive immune system, including $CD4^+$ T helper cells. PSA is capable of increasing $CD4^+$ T cell populations in germfree mice and altering the balance of Th1 and Th2 (subtypes of T helper cells that promote different immune responses to diverse pathogens) immune responses to levels that are similar to those in conventionally raised animals (77). Additionally, PSA stimulates Tregs (T helper cells that act to dampen or halt the immune response) to produce more anti-inflammatory IL-10 and reduce levels of inflammatory Th17 cells (T helper cells that instigate a proinflammatory immune response). Reducing levels of Th17 cells allows *B. fragilis* to reside in close association with the host epithelium, a niche preferred by this bacterium (105). Neff and colleagues (84) used the genes necessary for creating PSA's positively charged motif to probe genomes of phylogenetically diverse bacteria for potential ZPS synthesis loci. This motif is found widely throughout divergent bacterial isolates, and ZPS-encoding bacteria induce greater expression of IL-10 and Treg induction in human cells than those without a similar motif. Deletion of one ZPS synthesis locus from *Bacteroides cellulosilyticus* resulted in less IL-10 expression and fewer Tregs (84). These data support the idea that ZPS from diverse taxa can directly alter the host immune response.

Other MPGs may play similar roles as the ZPS previously described. For example, an EPS^- strain of *Bifidobacterium longum* elicits greater levels of proinflammatory cytokines than an isogenic EPS^+ strain. Addition of purified EPS can at least partially rescue wild-type levels of some of these cytokines, and EPS^+ (but not EPS^-) strains reduce inflammation in a mouse model of colitis (111). As this EPS does not contain zwitterionic charges, it may act through a different mechanism than the ZPS described above. In another model, intraperitoneal injection of purified *Bacillus subtilis* EPS reduces disease in a *C. rodentium* model of bacterial enteric infection and hyperplasia. Injection of naive mice with peritoneal cells isolated from the mice treated with EPS was sufficient to reduce disease, indicating a direct effect on host cells (61). Differences in induced immune cell and cytokine profiles exist for isogenic EPS^+ and EPS^- strains of other bacteria. For instance, treatment of macrophages with EPS^+ *Pediococcus parvulus* elicited lower inflammatory cytokine levels (and more anti-inflammatory IL-10) than treatment with an EPS^- strain (32). Moreover, mice colonized with EPS^+ *L. reuteri* had higher levels of Treg cells in the spleen compared to mice not colonized by *Lactobacillus* or colonized with an EPS^- strain (117). As with PSA and *B. subtilis* EPS, treatment of cultured cells or animal models with purified MPGs in these systems could isolate the effects of the bacterium from the direct effects of the capsule and establish whether these and other glycans directly affect the host immune response.

Exclusion of pathogens. While there is evidence that bacterial pathogens take advantage of indigenous microbes to establish infection via mechanisms that either directly involve carbohydrate metabolism or fermentative end products (20, 46, 85), mutualistic microbes may likewise influence pathogens via MPG-based mechanisms. For example, a recent study showed that treatment of mice with an EPS-producing *B. breve* strain (but not an isogenic EPS-deficient strain) prior to infection with the pathogen *C. rodentium* reduced pathogen levels in stool (44). One mechanism by which mutualistic bacteria may exclude intestinal pathogens is by inhibiting their attachment to host cells. Individual additions of several different *L. reuteri* EPS (including the starch-like polysaccharide reuteran; **Figure 1**) reduces hemagglutination of enterotoxigenic *E. coli* (ETEC) with red blood cells (a proxy assay for binding to mucus and other glycan-based host receptors) (132). Moreover, treatment of pig small intestine with reuteran in an ex vivo model reduces attachment of ETEC to host cells (23). Interestingly, not all EPS influence pathogen toxicity by reducing pathogen abundance and may therefore work via other mechanisms. For instance, in another model system, while treatment with EPS-producing *B. subtilis* did not reduce *C. rodentium* titers, treatment with either the EPS^+ strain or its purified EPS (but not with an isogenic EPS^- strain) did alleviate

disease symptoms (60, 61). As discussed above, such mechanisms could involve alterations to the host's immune or inflammatory state.

Degradation by other microbes. Several studies have identified changes in complex gut-derived communities upon treatment with MPGs. These studies typically involve the addition of various purified polysaccharides to a fecal slurry of bacteria followed by identification of changes in community structure, including increases in *Bifidobacterium*, *Bacteroides*, and other taxa (9, 107–109). However, these studies either suffer from low-throughput identification of individual taxa or rely on characterization of higher-order taxonomic groups via PCR-based techniques. Recent advances in sequencing technology have enabled higher-throughput assays to determine the microbial taxa most affected by MPG treatment and may point to mechanisms of community changes across individuals. Additionally, although effects on community structure may be due to MPG utilization as a nutrient source in some cases, not all MPGs are degraded by the gut microbiota. Degradation of several purified MPGs (but not many others) was demonstrated in a fecal slurry–based culture from one individual (107). Additionally, EPS from *Lactococcus lactis* that was fed to rats was recovered undigested from the stool (71). *B. thetaiotaomicron* illustrates both of these scenarios as it can degrade levan from *L. reuteri* but poorly degrades other glycans from *L. reuteri* (128). From the viewpoint of the microorganism producing the glycan, providing MPGs as a nutrient for neighboring bacteria may be one mechanism underlying a mutualistic relationship. For instance, cross-feeding of diet-based oligosaccharides has been established between strains of *B. ovatus* and *Bacteroides vulgatus*. Additional unidentified growth-promoting factors exist in this relationship and could be MPGs (98, 99). However, MPG degradation may also be detrimental to the producing organism, and this may be one way in which bacteria undermine competitors to increase their own gut fitness.

Protection from bacteriophages. Bacteriophages are ubiquitous on the planet, and the gut is no exception. While studies focused on the taxonomic assembly of the human gut microbiome (the collection of organisms living in the gut), usually centered on bacteria, now abound thanks to next-generation sequencing, little is known about intestinal viruses, including bacteriophages (88). Bacteriophages isolated from human stool samples can target bacteria in model gut communities in mice, but few phage receptors on gut bacteria have been identified (101). MPGs provide one way for bacteria to shield cell surface receptors from phage recognition and adsorption. For instance, one group compared the formation of spontaneous phage-resistant mutants in isogenic CPS^+ and CPS^- *E. coli* strains (95). Whereas the wild-type CPS^+ strain produced mucoid (indicating increased capsule production) phage-resistant mutants, abolishing CPS production eliminated generation of phage-resistant mutants, implicating capsules as a key factor in mediating phage resistance (95). Moreover, experimental production of K1 CPS in a hybrid *E. coli* strain renders the cell resistant to infection by T7 phage (112).

Unfortunately for the bacterium, phages have at least two mechanisms to circumvent this protective layer. First, the MPG itself may provide a receptor for the phage, as has been well described for various *E. coli* capsules (51, 86, 134). Second, phages often encode depolymerases (hydrolases or lyases) capable of degrading the capsule, allowing the phage to tunnel through the thick CPS layer and bind to receptors on the bacterial surface (8, 86). At least some phages are able to infect strains of more than one capsule type—these produce two or more depolymerases (86, 113, 116).

The great diversity in CPS types in *E. coli* and in other species may be, at least in part, an arms race between phage and bacterium, as bacteria gradually develop new surface capsules and phage adapt to bind to or depolymerize these layers. Interestingly, phages with depolymerases acting

on the same CPS substrate may be genomically divergent from each other (and most similar to other phages that do not encode these same depolymerases) (123). Additionally, adaptor proteins can connect various depolymerases to the outer surface of the phage virion, allowing a more facile acquisition and incorporation of new, diverse degradative enzymes and likely explaining the tropism of coliphages discussed above (68, 123). These data indicate a role for horizontal gene transfer in tailoring a phage to its host capsule type.

While CPS-binding phages are relatively well studied in *E. coli*, little is known about phage-MPG dynamics in other commensal organisms. EPS may play a role in blocking phage infection of *L. lactis*, as an EPS$^-$ strain was less sensitive to phage infection, although the difference was marginal (71). Additionally, one study noted the inability of a phage to infect encapsulated strains of *L. lactis* while the phage was still able to degrade their EPS (110). Finally, one group identified unique EPS structures produced by different subtypes of an EPS synthesis locus containing variable glycosyltransferases (1). Altering a locus to change its subtype (and EPS structure) also yielded a corresponding change in phage specificity from one subtype to the other, providing direct evidence for phage susceptibility and EPS type (1). Whereas phages for many bacteria would only have to target a single capsule on an individual cell, switching between synthesis of multiple capsule types provides a potential mechanism for *Bacteroides* to evade its viral predators. Previously one group noted an unstable CPS phenotype in a *B. thetaiotaomicron* strain (in hindsight, likely due to capsule switching) that correlated with productive phage infection (18). Others noted similar phenotypes in other *Bacteroides* strains that correlated with differences in susceptibility to phage infection (16, 63). Experiments controlling for MPG expression are needed to better isolate the role of MPG in blocking/facilitating phage infection in symbiotic organisms other than *E. coli*.

CONCLUSION AND PROSPECTUS

It is clear that polysaccharides play many central roles in the human-microbe symbiosis that occurs in the distal gut. The accessibility of dietary polysaccharides to the distal gut microbiota—due to limitations of host enzymatic potential—has been known for a long time. Still, the precise impacts of dietary polysaccharides on microbiota physiology and subsequent effects on host health are just now being unraveled in molecular mechanistic detail. The landscape of host-derived glycans, such as those attached to mucin glycoproteins and other glycoconjugates, may be even more complex than those of dietary glycans. These molecules change dynamically due to host status (microbial colonization or infection) and vary in composition along the length of the gastrointestinal tract. Moreover, because lack of dietary fiber forces some members of the microbiota to utilize host-derived glycans as a nutrient source, the status of host glycan pools, such as the mucous layer, is directly coupled to diet. Finally, the vast array of different MPGs that are either produced by microbes used in food production/fermentation or indigenous to our gut microbiota play several roles, some of which (e.g., immune modulation by some bacterial MPGs) directly affect human health. While gut bacteria arguably produce most MPGs for their own immediate benefit, such as surviving attacks from the host immune system, phages, or environmental challenges, it is perhaps not surprising that the large number of glycobiological configurations that have been explored by gut bacteria have produced other biological effects.

MPGs could be harnessed to benefit human health in several ways. First, purified immunomodulatory glycans, such as *B. fragilis* PSA, could be used to directly treat inflammatory disease. Such approaches have been successful in the treatment of diseases of the nervous system and colon in mouse models (79, 87). An alternative approach might be to introduce species that naturally produce, or are engineered to produce, biologically active MPGs as probiotics, perhaps even coupled to feeding of dietary polysaccharides that are specifically chosen to support the producing

strain. Given the potentially enormous, yet still mostly unexplored, number of connections between dietary, host, and microbial polysaccharides, it is likely that these molecules share more than a chemical lexicon. Indeed, the effects of polysaccharides from each of these three sources are intrinsically linked, raising the possibility that with better understanding of these manifold interactions we will be able to easily manipulate the effects of these molecules (for example, through changes in diet or microbiota) to improve human health.

DISCLOSURE STATEMENT

E.C.M. is a member of the scientific advisory boards of Kaleido Biosciences and Isothrive, LLC.

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