Trends in Microbiology

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Opinion

Nutritional strategies for mucosal health: the interplay between microbes and mucin glycans

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Many aspects of the mechanisms underlying the symbiosis between humans and gut microbes remain unknown and encompass some of the most intriguing questions in microbiome research. An important factor in this symbiosis is the interplay between microbes and human-produced glycans in mucin and breast milk. In this Opinion paper, I propose a synergy between the structural diversity of human mucin glycans and the enzymatic repertoire of the gut microbiome. The contribution of microbes to mucosal health is discussed, and the role of breast milk glycans in mucosal colonization by microbes is explained. The use of prebiotic mucin glycans in general, and specialized infant and medical nutrition in particular, should be considered as the field of interest to modulate the microbiota and improve mucosal health.

Highlights

The gut mucosa forms a protective barrier for epithelial cells but also serves as an ecological niche for specific nonharmful members of the microbiota.

The degradation of mucin glycans by the microbiome leads to a pool of microbial products that are beneficial for host mucus production and for immune and metabolic responses.

Mucin glycans are catabolized by microbes through a sequential action of different microbial enzymes, and cross-feeding between microbes is taking place while scavenging the mucin glycan structures leading to a microbial ecological network.

Human milk and mucin glycans show similar molecular characteristics and sugar code.

A proper seeding of the gut mucosa with microbes in early life will positively stimulate host immune and metabolic health in later life.

The use of food containing synthetic glycans designed to target microbial activity at the mucosa will enhance colonization with beneficial microbes, and their presence and glycan-degrading activity will stimulate mucosal health.

The gut microbiome and mucin glycans

Over the past decades changes in birth mode and infant feeding, antibiotic use, and overall nutrition have impacted the establishment of **host microbial symbioses** (see Glossary) and this is hypothesized to be associated with the dramatic global rise in immune-related disorders. The gut **mucosa** plays a crucial role in host microbial interactions. **Mucin glycans** are important drivers of the composition and functionality of the gut **microbiota**. In turn, the mucosal microbiota has a pervasive impact on mucus composition and thickness as well as immune and metabolic health (Figure 1, Key figure). In this Opinion paper I propose that synthetic glycans can be used as nutritional ingredients to promote a healthy mucosa through the microbiota. The role of breast milk and mucin glycans for a healthy mucosal colonization is discussed. I postulate how mucosal colonizers can play a key role in the intestinal microbial ecological network and how I think mucosal colonizers may offer powerful tools to modulate health. I also describe how a possible optimum could be reached between the structural diversity of mucin glycans and the gut microbiome. Finally, I present new insights on how prebiotic mucin-like glycans can be used for nutritional strategies to foster mucosal microbes and to modulate mucosal health.

The sugar code of human mucin glycans

The gut mucosa forms a protective barrier for epithelial cells but also serves as an **ecological niche** for specific members of the microbiota. Mucins are glycoproteins that provide mucus with functional properties [1]. Human glycosyltransferases assemble these polysaccharides that comprise a broad range of structurally diverse sugar chains [2]. The sugar code of mucin is composed of *N*-acetylgalactosamine (GalNAc), *N*-acetylglucosamine GlcNAc, galactose, fucose, and sialic acid linked through different types of bond [3,4] (Figure 2). The oligosaccharide chains contain approximately 2–20 monosaccharides in both linear and moderately branched structures, and the expression of combinations of the glycosyltransferases with different substrate specificities leads to an immense diversity of glycan chains [1]. The composition of mucin glycans differs even along the length of the gut [5], and between individuals, because it

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is partly determined by genetic factors. For example, the presence or absence of **fucosylated** oligosaccharides depends on **secretor status** (Se) and Lewis (Le) genes [1]. These individual differences can impact health outcomes and microbiota composition both during infancy and adult life. As such, fucose nonsecretors have an increased risk for diseases such as Crohn's disease and celiac disease [6,7], and they have gut microbial communities that are distinct from those of fucose secretors [8]. The absence of fucosylated oligosaccharides in breast milk is related to the reduced abundance of *Bifidobacterium* spp. in the infant gut and a higher risk of diarrhea and allergic disease [9–11]. Some members of the human gut microbiota are highly adapted to the mucosal niche within the intestine. This microbial niche is especially present at the mucosal interface in the colon, where the mucus layer is the thickest and is, in turn, divided into an inner and outer layer. The inner mucus layer is described to be devoid of microbes while the outer mucus layer of the colon harbors a stable community of mucosa-colonizing microbes that form an **ecological network** based on niche-specific conditions, such as low levels of oxygen and the availability of mucin proteins and glycans [12]. Apart from the mucus that covers the gut epithelial cells, fecal pellets are coated with a layer of mucus that creates an additional barrier for microbes to reach epithelial cells by entrapping them in the fecal pellet [5].

An increase in knowledge about the mucin sugar code has enabled us to decipher more precisely the interplay between human physiology, mucosal health, and the microbiota. Insight into the enzymatic capacity of the microbiota to utilize host-produced mucin glycans is essential to understand how microbes have adapted to specific niches in the human intestinal **glycan** landscape. This knowledge allows us to predict how these glycans contribute to the assembly of the microbiota and to host–microbe symbiosis, and how we might be able to use them to our advantage in innovative nutritional strategies using synthetic **prebiotic** glycans aimed at improving mucosal health.

Microbial adaptation to mucin glycans

Microbes interact with host-secreted mucin glycans, and this interaction drives the composition and functionality of the gut microbiota [13]. The microbiome encodes numerous **carbohydrate-active enzymes** (**CAZymes**) with the potential to degrade the complex polysaccharide chains of mucin glycans [14]. To degrade the sugar code of mucin glycans, a sequential action of different microbial enzymes is needed [15,16]. As only short glycans can be transported into the microbial cell, it requires several extracellular secreted and membrane-bound enzymes to predigest the large mucin glycans. The main enzymatic activity needed is that of **glycosyl hydrolases** (**GHs**), such as endo-β-*N*-acetylgalactosaminidase, fucosidases, *N*-acetyl-β-hexosaminidases, β-galactosidases, and sialidases [17] (Figure 2). The complete degradation of mucin polysaccharides can be done by a combination of enzymes that can be expressed by a diverse range of gut microbes [15,16]. The highly diverse gut microbiota expresses different enzymes with similar mucus-degrading capabilities. Available genomes from gut microbiota members indicated that a majority of the organisms have the capability to cleave, and catabolize, at least one of the **mucin O-glycan** monosaccharides [13,18], and more data are becoming available on the characterization of mucin-degrading CAZymes from gut mucosal microbes [13,19–22]. For example, the large and diverse glycoside hydrolase 16 (GH16) family were recently characterized as endoacting enzymes and were described to target the polyLacNAc structures within the oligosaccharide side chains of mucins. When expressed by prominent mucin-degrading microbes, the GH16 enzymes can thus be involved in the initial step in mucin breakdown [23]. The fact that microbes use extracellular degradation enables microbial communities to share carbon sources and collaboratively break down mucin glycans. The first targets for GH enzymes are the terminal residues on the O-glycans such as sialic acid, fucose, and glycosulfate. This can be done by microbes expressing fucosidases, sialidases, and mucin-desulfating sulfatases or

Glossary

CAZymes: carbohydrate-active enzymes which can break down a complex and diverse range of glycan structures from both dietary and host sources. These enzymes can be produced by the gut microbiome – for example, to facilitate food and mucin degradation and contribute to microbial growth in the gut mucosal environment.
Commensal microbes: microbes that colonize the gut without benefitting or harming the human host.
Ecological network: the complex network of living organisms, their physical environment, and their interactions in a particular unit of space. In this article: a host, its associated microbiome, and all potential interactions between microorganisms and their interactions with the gut environmental conditions.
Ecological niche: in the present context, a region providing a specific set of conditions in the gut environment.
Fucosylated: refers to the presence of fucose residue(s) in a mucin glycan.
Glycan: a chain of mono-sugar residues. Such chains can consist of similar mono-sugars or a diversity of mono-sugars. In the context of this article, the chains contain a sugar code that is typical for the host's gut mucin glycans or human milk glycans.
Glycoproteins: proteins that contain oligosaccharide chains (glycans) covalently attached to amino acid side-chains.
Glycosyl hydrolases (GHs): enzymes that catalyze the hydrolysis of glycosidic bonds in complex mucin sugars chains.
Host microbial symbioses: the associations, of any type, between microbes that reside in the gut and the human host; such associations involve close and long-term biological interactions (mutualism, commensalism, or parasitism).
Human milk oligosaccharides (HMOs): also called human milk glycans, these are sugar molecules that are a part of a group of oligosaccharides of which the sugar code can be found exclusively in human breast milk.
Metagenomic: refers to genetic material recovered directly from samples – in the context of this article, from a fecal sample that contains mainly the DNA of microbes.
Microbial cross-feeding: the use, by a microbe, of a metabolic product

glycosulfatases [19]. Interestingly the terminal sugars are not always used as substrates by the microbial species that release them [24]. As an example, a limited group of gut microbes are able to express sialidases, which enables the release of sialic acid from mucin. However, not all of these microbes are able to catabolize the released sialic acid [25] (Figure 2). Interestingly, sequential removal of released sialic acid from mucin by **cross-feeding** microbes can protect against an infection caused by microbial pathogens that use these sugar groups as binding sites [18]. This might also explain the protective effect of sialidated **human milk oligosaccharides** (**HMOs**) [24,26–28]. This opens up possibilities for synthetic **sialidated** glycans for treating infectious diseases. Furthermore, the release of this terminal sialic acid can lead to the sequential degradation of the mucin glycans by other microbes, and sialidase activity might therefore be a key feature within the ecological network [25]. When peripheral residues have been removed, the core of the O-glycan chains can be further hydrolyzed by the microbiota. This can result in the release of monosaccharides, such as *N*-acetylglucosamine, *N*-acetylgalactosamine, and galactose, used by the microbial degrader itself or by other resident microbes [29,30]. Hence, cross-feeding actions enhance the ecological fitness of a specific species and often also have a disproportionate effect on overall microbiota function and metabolism. Mucin degradation by specific microbes is essential for building a stable microbial network [30–32]. The adaptation of specialized microbes that are capable of digesting host mucin glycans, and the dynamic expression of mucin glycans by the host upon microbial stimuli, implies coevolution of a symbiotic relationship [15,17]. As the degradation of synthetic glycans by a microbial network will lead to a pool of host modulatory products, I believe that proper seeding of the gut and mucosa with microbes will positively stimulate host immune and metabolic health in early and later life [33,34].

Breast milk and mucin glycans for a healthy mucosal colonization

The development of the intestinal microbiota in the first 1000 days of life is a dynamic process influenced by early-life nutrition [35]. Pioneer bacteria colonizing the infant gut and gradual diversification towards a stable ecosystem play a crucial role in establishing stable host–microbe interactions and an optimal symbiosis between them [6]. Immediately after birth, infants consume an exclusive diet of breast milk or infant formula or a combination of those two. The glycan landscape presented in human milk offers protection to the neonate, either by directly blocking binding of pathogens to intestinal cells, or indirectly, serving as an energy source for microbes that can protect against infection [36]. Therefore, milk serves as both infant and microbial nutrition, which, in concert, promote host–microbe symbiosis. Although HMOs are among the most abundant components in breast milk, they do not have any direct energetic value for the nursing infant. Instead, they are described to act as **prebiotics** selecting for specific microbial populations in the infant gut and have been postulated to exert antiadhesive or anti-inflammatory effects [36]. Human milk and mucin glycans show similar molecular characteristics as both are composed of comparable monosaccharide building blocks and linkages, which together comprise their sugar code. HMOs consist of a lactose core, which may be elongated by *N*-acetylglucosamine (GlcNAc), galactose, fucose, and/or sialic acid. Both HMOs and mucin glycans have large structural diversity with over 200 glycan structures identified in a single donor [37,38]. Due to the chemical similarity of HMOs and *O*-linked mucin glycans, microbes have developed homologous strategies for degrading these complex carbohydrates [24,26,39,40]. For example, *Akkermansia muciniphila* uses similar GHs to degrade glycan structures in either milk or mucin [24]. In my perspective, the ability of mucin-degrading microbes to forage on milk glycans present in the infant gut may have a role in early colonization of the mucosal layer with beneficial microbes [24,41]. Examples of such early seeding of the mucosal layer include *Bifidobacterium* spp., *Bacteroides* spp., and *A. muciniphila*. These microbes can use their mucus-utilization pathways for survival within the infant gut, which is rich in HMOs that contain mucin-like structures [24,26,41]. Microbial glycan

produced by a different microbe. Also termed metabolic cross-feeding.
Microbiome: a community of microbes, including their molecules and products, that are active and inhabit a particular environment.
Microbiota: a community of microbes that inhabit a particular environment.
Mucin glycans: sugar side-chains attached to a mucin protein backbone.
Mucin O-glycan: a sugar molecule attached to the oxygen atom of serine or threonine residues in a mucin protein.
Mucins: glycoproteins that can form a gel.
Mucosa: a region that provides physical separation between the lumen and the body. It consists of physical, biochemical, and immune elements.
Mucosal barrier: the physical barrier that separates the lumen and the body. It consists of physical, biochemical, and immune elements.
Pili: long, thin surface appendages found on many bacteria. They play major roles in colonization by facilitating adhesion, motility, DNA exchange, and protein uptake and secretion.
Prebiotics: compounds, such as glycans, that induce the growth or activity of beneficial microorganisms in the gut.
Probiotics: live microorganisms that provide health benefits when consumed.
Secretor status: refers to the presence or absence of blood-group antigens in, for example, mucus. People who secrete these antigens in their bodily fluids are referred to as secretors, while people who do not are termed nonsecretors. Secretor status is controlled by the FUT2 gene (also called the Se gene).
Sialidated: in the present context, refers to a mucin glycan containing the residue of a sialic acid sugar.
Sugar code: in a glycan, the pattern of glycosylation made up of different mono-sugars combining the parameters anomeric status, linkage positions, ring size, and addition of branches.
Synbiotics: food ingredients or dietary supplements combining probiotics and prebiotics.

Key figure

The interplay between microbes and mucin glycans

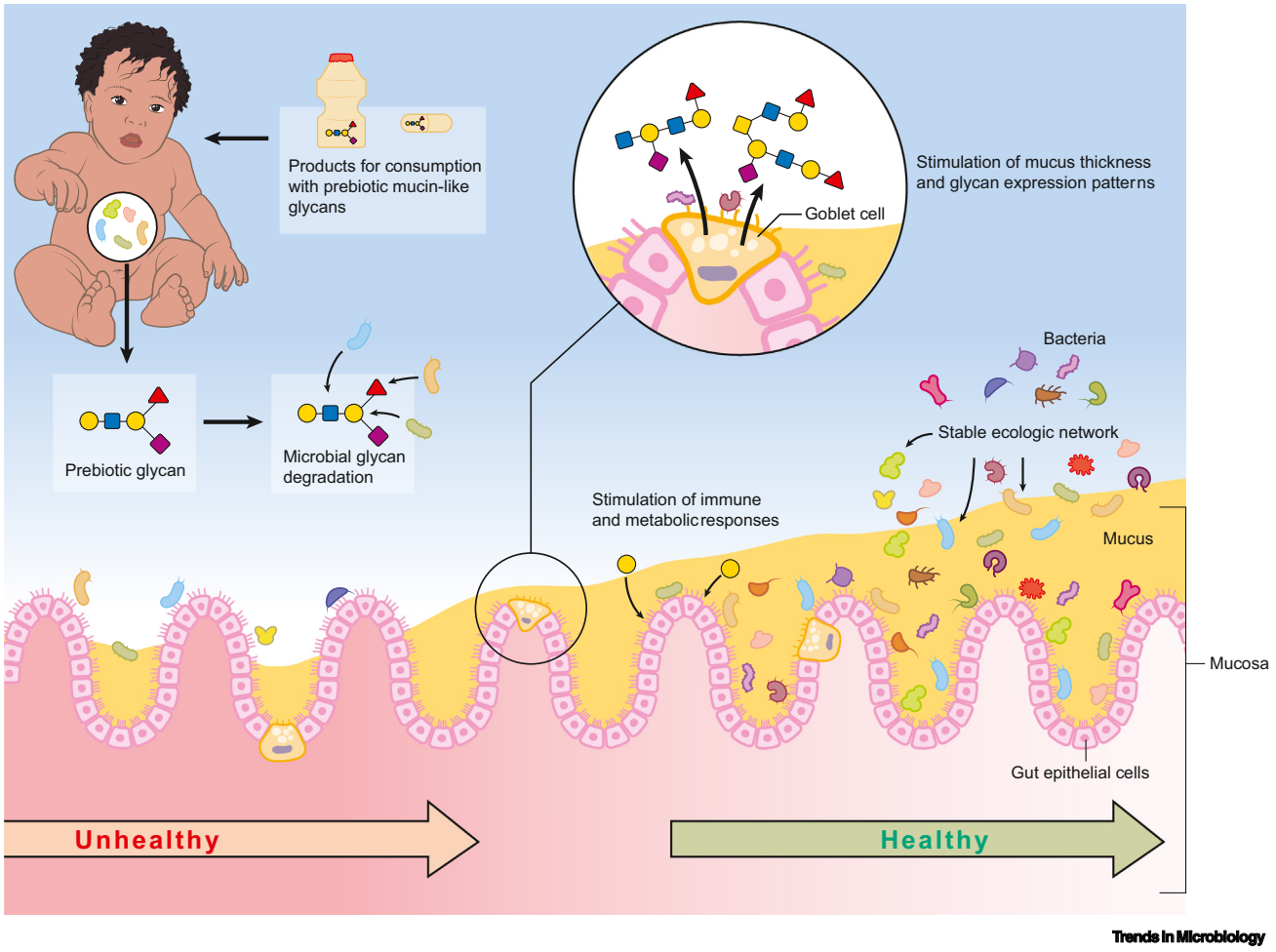

Figure 1. A healthy outer mucosal layer in the colon is colonized by a diverse set of microbes that feed on mucin glycans to form a stable ecological network. The mucosal microbiome stimulates host cells towards a healthy immune and metabolic response. Host goblet cells respond to microbial stimuli by modifying glycan expression patterns, and this leads to an increase in mucus thickness and a modified mucin sugar composition. In an unhealthy state, in which the mucosal layer is thin and compromised, microbes can reach the gut epithelial cells and evoke an inflammatory response. Restoration of the mucosal imbalance could be established through the addition of prebiotic mucin-like glycans that are catabolized by microbes so that these microbes can boost the host to restore mucus thickness and stimulate a beneficial immune and metabolic response.

degradation results in the generation of metabolites that help to modulate mucosal health, making these glycan-degrading microbes good markers of a healthy gut during later life. In addition, the benefits of mucus-colonizing commensals have also been described for mucosal, immune, and metabolic health throughout life. Glycans added to infant formula will therefore promote the growth of beneficial glycan-degrading microbes that can colonize the infant gut. I envision that their presence and activity will promote a healthy microbial network that can benefit mucosal health in later life.

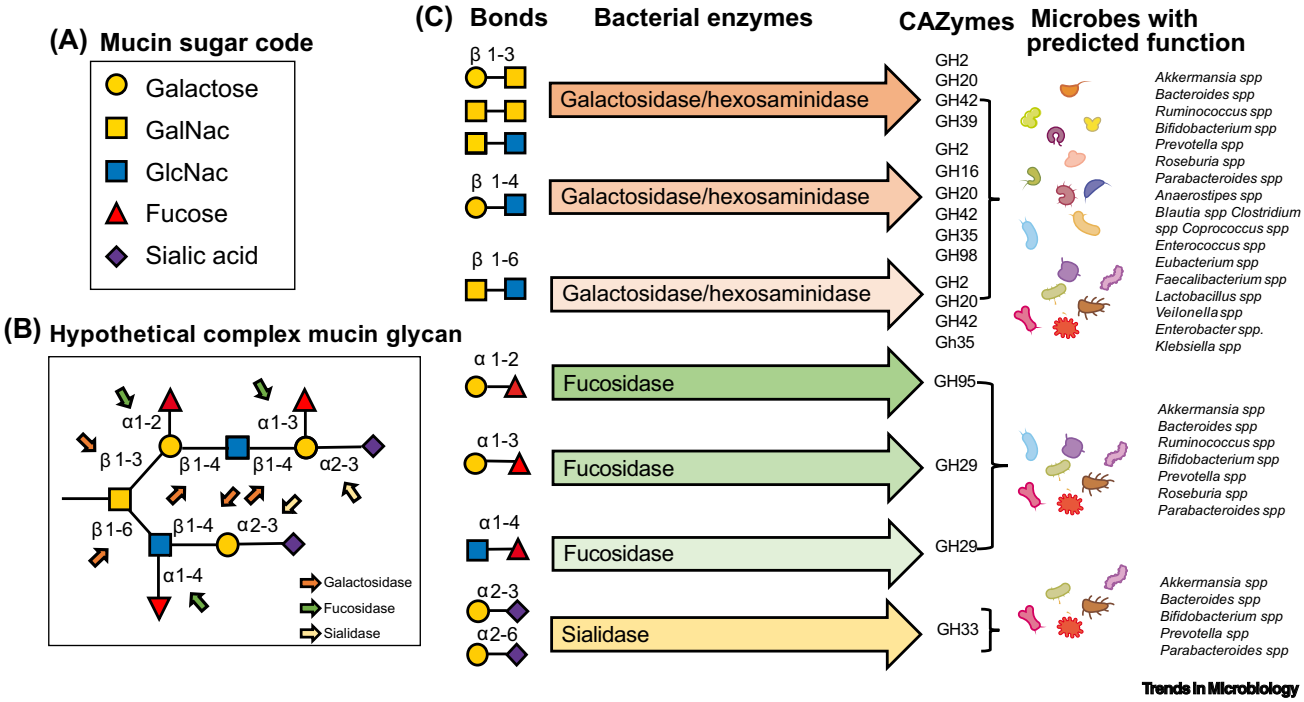

Figure 2. Microbial adaptation to mucin glycans. (A) Sugar monomers present in mucin structures. (B) A hypothetical complex mucin glycan structure with a range of bonds; arrows represent hydrolyzing enzymes encoded in the gut microbiome. (C) Left. Well-characterized mucin glycan structures and their residue bonds. Middle. Microbial carbohydrate-active enzymes (CAZymes) involved in the catabolism of each glycan structure. Right. Representative genera of the major bacterial phyla in the human gut encoding these CAZymes in their genomes.

Structural redundancy of mucin glycans and microbial enzyme versatility

Complete dismantling of mucin glycans and mucin proteins demands a series of enzymes responsible for the transportation of glycans and the hydrolysis of their residue bonds. Cross-feeding is an essential component of microbial community development [30,42,43]. Microorganisms are not simply divided into mucin degraders and mucin nondegraders. Most gut microbes encode and express different combinations of CAZymes, the set of enzymes that determine the mucin-degrading properties and which are thus strain- or subspecies-dependent [16] (Figure 2). Together, the highly diverse gut microbiota encode several enzymes with a homologous function for mucus degradation among different microbial species, enabling microbes to hydrolyze a mucin glycan structure using a similar or even a different enzyme. Examples include the diversity of sialidases and fucosidases within the human gut microbiome [13] (Figure 2). Several studies have attempted to pinpoint those genes involved in mucin glycan degradation, and this has led to the identification of several characterized GH enzymes involved in mucin degradation from a range of gut microbial species [19]. Furthermore, the presence of mucin glycan-degrading enzymes within gut **metagenomic** databases showed enrichment in **commensal microbes** compared to pathogens [39].

I would like to propose that the patterns of hydrolytic bonds within mucin glycan structures can be used to deduce a synergy between mucus structure and certain mucin-degrading microbes. The difficulty lies in the fact that the presence and activity of microbes also induces regulation of host mucus glycan expression and composition, increasing diversification of mucin structures [5,31,44–48]. It has been shown that host–microbe interactions can change fucosylation and sialidation patterns [49]. The high diversity of mucin glycans could be an essential component

in stimulating a diverse microbial network that can, in turn, stimulate mucosal expression and, together, form the basis for the host microbial symbiosis. However, given the recurring patterns within mucin glycan structures, and the functional homologies of microbial glycan-degrading enzymes in the gut, I hypothesize the existence of a theoretical optimum in which a minimal structural redundancy of mucin glycans can support a maximal microbial diversity (Figure 3). This hypothesis can be used to design microbial synthetic communities that can express all the enzymes to degrade a set of synthetic glycans to reach an optimal pool of host mucosal modulatory components. As such, this can result in functional glycan-based food ingredients with highly advanced **synbiotics** that can improve mucosal health through synthetic glycans and microbial activity.

New nutritional strategies for microbiota modulation

New microbiota based therapeutic strategies hold great potential for the treatment of infectious, immune and metabolic diseases and disorders. An approach would be to use synthetic prebiotic glycans as microbiome modulators to stimulate mucus barrier, immune and metabolic properties. Current examples of glycans added to nutrition already show their potential to resolve mucus-layer defects associated with inflammatory bowel diseases and metabolic disorders [50–54]. The best instances of the effectiveness of adding prebiotic glycans to nutrition come from infant feeding. Infant formula containing glycan-based prebiotics – such as disialyllacto-*N*-tetraose, sialylated galacto-oligosaccharides, disialyllacto-*N*-tetraose, and 2′-fucosyllactose – have been shown to alleviate diseases such as necrotizing enterocolitis (NEC) [39,55–58]. Apart from prebiotic glycans expressed by a microorganism or derived from plant materials, the use of mucin glycans has also become a field of interest to modulate the microbiota and human health. Animal studies have shown that mice that received orally administered porcine mucin glycan exhibit a reduction in *C. difficile*, a delay in the onset of diet-induced obesity, and an increase in the relative abundance of *A. muciniphila* [39]. Animal studies have also revealed how commensal microbiota members that target mucosal sialidases can protect against *C. difficile* infections [18]. Scientists are now exploring how engineered mucus can prevent pathogen infections [59]. I think that the protective role of the

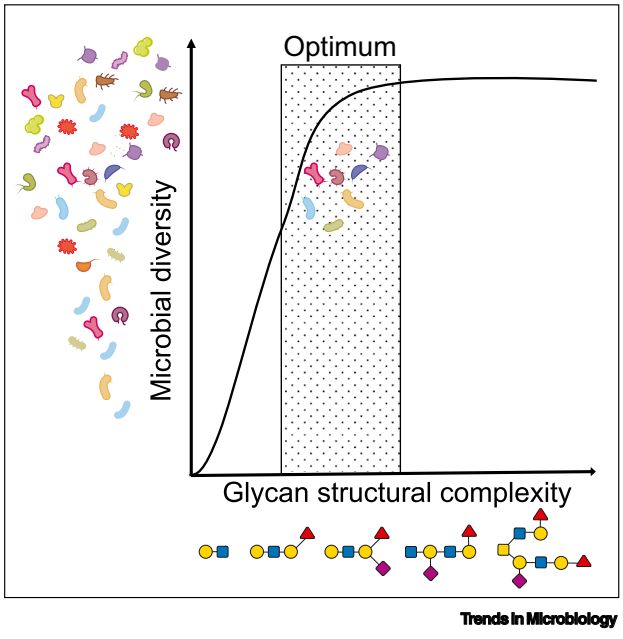

Figure 3. The interaction between microbial enzyme diversity and mucus glycan complexity. As the complexity of glycans and the number of bonds increase (arrowed axis), I hypothesize that there is a redundancy in bond diversity. This redundancy in hydrolytic bonds within glycan structures can be used to define an optimum between mucus glycan diversity and microbes with glycan-degrading enzymes (dotted box).

gut microbiota in the modulation of gut mucosal health can be extended to a range of mucosal-related disorders. I postulate that synthetic prebiotic glycans can, and will, be used in nutrition to target beneficial microbes in the gut and exert their role in modulating mucosal health through being anti-infection, evoking a beneficial immune and metabolic host response, and regulating improved mucus expression and barrier function.

Glycans that improve mucosal health through the microbiota

An increasing number of studies have shown that the intestinal mucus acts as a major modulator of human health by increasing intestinal barrier function and decreasing inflammatory responses [59,60]. Maintenance of a healthy gut through normal mucus encapsulation of fecal pellets can even prevent inflammation and hyperplasia [5]. There are also a few examples of a causative relationship between mucin-degrading microbes and host immune and metabolic health, such as the discovery that the outer-membrane **pili** of *A. muciniphila* can evoke a healthy host metabolic response and increase in intestinal barrier function [61–63]. The use of non-living microbial materials (postbiotics), such as the pili proteins of *A. muciniphila,* instead of viable microbial cells (**probiotics**), has many advantages in the upstream production and application possibilities of products that can be added to nutrition. As mentioned previously, mucin glycans added to the food of mice resulted in a higher abundance of *A. muciniphila* and a delay in the onset of diet-induced obesity.

The ratio between simple sugars versus fibers in a diet plays a role in the activity and abundance of microbes with mucin-degrading abilities, with low fiber consumption being correlated with the onset of intestinal inflammation. In mice, the intake of simple sugars enhances pathogenesis via modulation of gut microbiota and predisposes them to colitis [64]. Mice that were administered a low-fiber diet had an altered microbiota that resulted in mucus defects [60,65]. On top of this, the host makes unfavorable changes in its mucus expression and sugar code in response to an inflammatory type of microbiota, creating a positive feedback loop towards an even more inflammatory state [47]. As such, a long-term low-fiber, high-sugar diet can create a microbiota that is active on mucus degradation, leading to changes in mucus composition and gut barrier function and, subsequently, inflammation. In the case of NEC, inflammatory bowel disease (IBD), and colorectal cancer, the role of the mucosal microbiota has been shown to be associated with the (corresponding) diseased state [55]. Promising results are reported for HMOs and blood type antigens as prebiotic glycans, being effective as microbiota modulators to improve gut and immune health [66–69]. Furthermore, apart from currently available glycans, the use of new synthetic glycans has also been suggested, with the possibility of using not only the glycan chains but also the backbone of the mucin structures in combination with glycan chains [59,70]. I think that the next steps in this field of research should be to apply new glycan structures that improve host health and ameliorate the outcome of to a range of disorders through the microbiota.

Concluding remarks and future perspectives

Glycans have profound effects on the assembly and function of the human gut microbiome. I think that glycans have high potential to be applied in truly new therapeutic nutrition for chronic inflammatory diseases as well as enteric infections in which patients suffer from gut microbial imbalance and impaired mucus integrity. Therefore, I argue that the next phase in the field of gut microbiome research should be focused on the design of nutritional strategies that target the microbes which colonize the mucosal layer in order to improve human mucosal health (see also Outstanding questions). Thus far, the interplay between host mucus production and the role of microbes herein is lacking sufficient mechanistic insights. Knowledge about which microbial components play a role in regulating mucus secretion, and how mucus glycans determine microbiome composition, will elucidate how a symbiosis between the host and microbe is

Outstanding questions

How do different microbiota members stimulate mucin production?

How do microbiota members influence mucin composition?

Which sugars and hydrolytic bonds between sugars are sufficient to stimulate diverse microbial action in the gut?

What prebiotic mucin-like glycans stimulate microbes that, in turn, modulate host mucin production?

How could glycan structures be used as next-generation prebiotics?

Can nutrition steer the mucosal colonization of microbes in early life?

reached and how a disbalance could be treated to overcome dysbiosis. Understanding how interactions between the gut microbiome, dietary glycans, and host glycans regulate gut epithelial function, the immune response to pathogens, and transitions to adaptive tolerogenic immunity will offer *windows of opportunity* to shape lifelong gut, immune, and metabolic health and enhance our knowledge of the causality between microbes and human health.

Declaration of interests

There are no interests to declare.

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