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Gut biogeography of the bacterial microbiota

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PREFACE

Animals assemble and maintain a diverse, yet host-specific gut microbial community. In addition to characteristic microbial compositions along the longitudinal axis of the intestines, discrete bacterial communities form in microhabitats, such as the gut lumen, colon mucus layers and colon crypts. In this Review, we examine how spatial distribution of symbiotic bacteria among physical niches in the gut impacts the development and maintenance of a resilient microbial ecosystem. We consider novel hypotheses for how nutrient selection, immune activation and other mechanisms control the biogeography of bacteria in the gut and discuss the relevance of this spatial heterogeneity to health and disease.

Humans and other mammals harbor a complex gastrointestinal ***microbiota***, which includes all three domains of life (Archaea, Bacteria and Eukaryota). This extraordinary symbiosis, formed via a series of exposures to environmental factors, is initiated upon contact with the vaginal microbiota during birth[1]. Abrupt changes during the first year of life follow a pattern that corresponds to gestational age in both mice[2] and humans[3], which suggests that strong deterministic processes shape the composition of the microbiota during development. These population shifts may be explained by influences from diet, the developing immune system, chemical exposures, and potentially founder effects of initial colonizers. Founder effects are not well understood in the mammalian gut, but the profound changes in host gene expression that occur in response to microorganisms, and the great potential for ***syntrophic*** interactions **between bacteria** suggest that early colonizers may have long-term effects on the establishment of the microbiota. The immune system imposes selective pressure on the microbiota through both innate and adaptive mechanisms such as antimicrobial peptides[4], secreted immunoglobulin A (IgA)[5], and other contributing factors[6] (see below). However, current research suggests that diet may have the greatest impact on microbiota assembly.

Prior to weaning, breast milk plays a crucial part in shaping the microbial community composition via transmission of the milk microbiota to the infant gut[7], protection from harmful species by secreted maternal antibodies[8], and selection for certain species by milk oligosaccharides, which can be used by microorganisms as carbon sources[9]. For example, in *in vitro* competitive growth experiments, *Bifidobacterium longum* benefits from its ability to use fucosylated oligosaccharides that are present in human milk to outgrow other bacteria that are usually present in the gut microbiota, such as *Escherichia coli* and *Clostridium*

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perfringens[10]. Several species of *Bacteroides* can also utilize fucosylated oligosaccharides as carbon sources[11], suggesting that their colonization may be aided by ***prebiotic*** properties of milk. Accordingly, children of mothers with nonfunctional fucosyltransferase 2, an enzyme required for fucosylation of milk oligosaccharides, display lower levels of fecal Bifidobacteria and *Bacteroides* species[12]. The importance of diet in determining the composition of the microbial community in the gut is also highlighted by the observation that transition to solid foods coincides with establishment of an adult-like microbiota.

The adult intestinal microbiota consists of hundreds to thousands of species, dominated by the Bacteroidetes and Firmicutes phyla[13]. This ecosystem is distinct from that of any other microbial habitats that have been surveyed[14], and includes many species that exist nowhere else in nature, indicating that coevolution of the host with its gut microbial ***symbionts*** (including ***commensals*** and ***mutualists***) has generated powerful selective mechanisms. A recent study of how different microbial communities colonize ***gnotobiotic*** animals showed that deterministic mechanisms (presumably host-microorganism interactions) led to reproducible shaping of the microbiota regardless of the source of the input community[15].

The adult intestinal microbiota is also partially stable, as a core of ~40 bacterial species (accounting for 75% of the gut microbiota in terms of abundance) persists for at least a year in individuals[16]. A more extensive longitudinal study found that 60% of all bacterial strains within an individual persisted for five years[17]. During severe perturbations such as antibiotic treatment, the fecal community is depleted to a low-diversity consortium, but after a recovery period membership and relative abundance largely resemble the pretreatment state[18]. Some species that are depleted to undetectable levels in stool are later recovered[18], suggesting that there may be reservoirs of bacterial cells that can re-seed the intestinal lumen.

The mucus layer, crypts of the colon and appendix are examples of privileged anatomical sites, protected from the fecal stream and accessible only to certain microorganisms. In this Review, we highlight relevant features of spatial heterogeneity of bacterial species and communities in the gut microbiota, and discuss the impact of microbial localization in engendering specific and stable colonization with profound implications for health and disease.

MICROBIAL COMPOSITION OF THE GUT

The mammalian lower gastrointestinal tract contains a variety of distinct microbial habitats along the small intestine, cecum, and large intestine (colon). Physiological variation along the lengths of the small intestine and colon include chemical and nutrient gradients, as well as compartmentalized host immune activity, which are known to influence bacterial community composition. For example, the small intestine is more acidic, and has higher levels of oxygen and antimicrobials than the colon (Figure 1A). Therefore, the small intestine microbial community is dominated by fast-growing facultative anaerobes that tolerate the combined effects of bile acids and antimicrobials, while still effectively competing for simple carbohydrates that are available in this region of the gastrointestinal tract. Bile acids, secreted through the bile duct at the proximal end of the small intestine, are

bactericidal to certain species due to their surfactant properties and are known to broadly shape the composition of the microbiota, especially in the small intestine. For example, feeding mice excess bile acids generally stimulates the growth of Firmicutes and inhibits Bacteroidetes[19]. Additionally, the shorter transit time in the small intestine compared to colon (an order of magnitude shorter, despite the increased length of the small intestine) is thought to make bacterial adherence to tissue or mucus an important factor for persistent colonization of the small intestine.

In ileostomy samples from humans, the small intestine was found to exhibit lower bacterial diversity than the colon, and was highly enriched in certain Proteobacteria and *Clostridium* species[20]. Furthermore, a metatranscriptomic analysis revealed that the expression of genes involved in central metabolism and in pathways responsible for import of simple sugars by facultative anaerobes was greatly enriched in ileal samples, compared to fecal samples[20]. In mice, Lactobacillaceae and Proteobacteria (especially Enterobacteriaceae) are enriched in the small intestine[21] (Figure 1A). Although bacteria in the small intestine are potentially competing with the host for nutrients, host-derived bile acids and antimicrobial peptides limit bacterial growth to low densities in proximal regions. Only at the distal end of the small intestine (in the terminal ileum) do bacterial densities reach saturating levels similar to those found in the large intestine (Figure 1A).

The cecum and colon cultivate the most dense and diverse communities of all body habitats. Mice, like most herbivorous mammals, have a large cecum between the small and large intestine where plant fibers are slowly digested by the microbiota. Humans have a small pouch-like cecum with an attached appendix, a thin tube-like extension (Figure 1A). In the cecum and colon, microorganisms are responsible for the breakdown of otherwise ‘resistant’ polysaccharides that are not metabolized during transit through the small intestine. Lower concentrations of antimicrobials, slower transit time, and a lack of available simple carbon sources facilitate the growth of fermentative polysaccharide-degrading anaerobes, notably those of the high-abundance families Bacteroidaceae and Clostridiaceae. In the mouse, the cecum is enriched in Ruminococcaceae and Lachnospiraceae, while the colon is enriched in Bacteroidaceae and Prevotellaceae[21]. Rikenellaceae are prominent in both the cecum and colon[21]. Various host factors drive community differences over the cross-sectional axis of the gut. The entire wall of the colon folds over itself, creating compartments between folds (inter-fold regions) that are distinct from the central lumenal compartment (Figure 1B). In mouse studies that used laser capture microdissection to profile the composition of the microbial communities in discrete regions, significant differences were observed between the central lumen compartment and the inter-fold region[22,23]. Specifically, the Firmicute families Lachnospiraceae and Ruminococcaceae were enriched between folds while the Bacteroidetes families Prevotellaceae, Bacteroidaceae, and Rikenellaceae were enriched in the digesta[22]. **Relative to the digesta, the inter-fold regions are likely to contain greater amounts of mucus, which can serve as a nutrient source for certain bacteria.**

Gut microhabitats: mucus and colon crypts

Throughout the human small intestine and colon, specialized epithelial cells called ***goblet cells*** secrete a mucus layer of varying thickness that partially or fully covers the epithelium

depending on the region, creating a boundary between the gut lumen and host tissue (Figure 2A and 2B). The small intestine harbors a single, tightly-attached mucus layer (Figure 2A), whereas in the colon, mucus is organized into two distinct layers: an outer, loose layer, and an inner, denser layer that is firmly attached to the epithelium (Figure 2B). As mentioned above, bacterial densities are much higher in the colon, compared to the small intestine, and examination of the colon by fluorescence *in situ* hybridization (FISH) has shown that the inner mucus layer appears essentially sterile next to the densely populated outer layer[24]. In addition to mucus density itself serving as a physical obstacle for microorganisms, antimicrobial molecules and oxygen secreted from the epithelium accumulate higher local concentrations within the mucosa, especially in the small intestine, greatly restricting potential microbial inhabitants.

Mucus is continuously secreted and the outer layers are sloughed off, generating ‘islands’ of mucus that are carried into the fecal stream[25]. In mice, a viscosity gradient of the gel-forming mucus increases from the proximal colon (which includes the cecum and the ascending and transverse colon) to distal colonic sites (which includes the descending colon and the sigmoid colon that connects to the rectum). Accordingly, there are more mucus-associated bacteria in the proximal region[26]. Mucosal ***biofilm*** formation in the proximal colon is conserved from mammals to amphibians[27], suggesting an ancient, evolutionarily conserved origin of this region for interactions with bacteria. Therefore, the mucus layers of the gastrointestinal tract create environments that are distinct, protected habitats for specific bacterial ecosystems that thrive in proximity to host tissue.

Divergence between the mucosal and ***digesta-***associated colonic communities has been observed in several mammals including humans[28], macaques[29], mice[30], cows[31], and flying squirrels[32]. More specifically, human colon biopsy and swab samples have revealed a distinct mucosal community enriched in Actinobacteria and Proteobacteria compared to the lumen community[33]. Certain species are highly enriched in colon mucus, such as the mucin-degraders *Bacteroides acidifaciens* in mice[34], *Bacteroides fragilis* in macaques[29], and *Akkermansia muciniphila* in mice and humans[34,35] (Figure 2B). Human mucosal communities in biopsy[36–38] and lavage[39] samples of the colon contain significant variability between sample locations less than one centimeter apart, suggestive of the existence of mucosal microbial populations in patches. Interestingly, an imaging study using approaches that carefully preserve the structure of feces also identified discrete patches; individual groups of bacteria were found to spatially vary in abundance from undetectable to saturating levels[25]. This spatial niche partitioning in feces may be reflective of aggregates of interacting microorganisms, heterogeneity of nutrient availability in plant fibers, or microenvironments in mucosally-associated communities that imprint the digesta as it transits through the gut. Therefore, microbial profiling of fecal samples, which is the most common strategy employed in microbiome studies, represents an incomplete and skewed view of even the colon, which has distinct mucosal communities and spatial heterogeneity that is lost upon sample homogenization.

Some bacteria completely penetrate the mucus and are able to associate directly with the epithelium, within the crypts of the colon. Crypt-associated microorganisms were first described using electron microscopy[40,41]. Many subsequent imaging studies likely failed to

observe or underestimated the number of tissue-associated bacteria because common washing and fixing methods can remove mucosal biofilms[42]. This led to the hypothesis that the mucosal surface is largely devoid of microbial colonization in healthy individuals. However, imaging studies using Carnoy's fixative, which is known to preserve the mucosal layer, found that there are bacteria in a significant fraction of colonic crypts in healthy mice[43] and humans[44]. More recent work using laser microdissection and sequencing to profile mouse crypt-associated communities revealed that the community is especially dominated by *Acinetobacter* spp. and is generally enriched for Proteobacteria capable of aerobic metabolism[23] (Figure 2B). Evasion of immune responses and particular metabolic activities are likely required for crypt occupancy by microorganisms specialized to reside in close proximity to the host. A well-characterized example of this adaptation is the ability of the human symbiont *B. fragilis* to enter crypts of the proximal colon of mice via a process requiring both modulation of the immune system[45] and utilization of specific host-derived nutrients[46] (see below). While dogma has emerged that microorganisms contact mucosal surfaces exclusively in disease states, it appears that life-long physical associations between specific members of the microbiota and their hosts represent symbioses forged over millennia of co-evolution.

MECHANISMS RESPONSIBLE FOR GUT BIOGEOGRAPHY

Several factors influence the biogeography of bacteria within the gut, including diet, antimicrobials, mucus and adherence, and the host immune system.

Diet and nutrients

Bacterial metabolism in the gut likely contributes to the localization of particular groups of microorganisms. Because fatty acids and simple carbohydrates from food are absorbed and depleted during transit through the small intestine, sustainability of the colonic bacterial ecosystem requires growth by fermentation of complex polysaccharides, the principal carbon sources that reach the colon. Best studied in this regard are *Bacteroides* species, which are able to catabolize polysaccharides derived from the diet and from the host[47]. Compared to other gut bacteria, *Bacteroides* have the largest number and diversity of genes involved in polysaccharide degradation[48]. This extensive array of polysaccharide utilization systems is dominated by those resembling the starch utilization system (Sus), originally described in *Bacteroides thetaiotaomicron* [49]. Sus systems consist of lipid-anchored enzymes either secreted or displayed on the bacterial cell surface that can catabolize particular complex glycans into smaller oligosaccharides, which are then imported through a dedicated outer membrane transporter (Figure 3A). In the gut, *Bacteroides* species use Sus-like systems to break down dietary polysaccharides and host-derived mucin glycans[50]. The genome of *B. thetaiotaomicron* encodes 88 Sus-like systems presumably with different glycan specificities, providing remarkable metabolic flexibility[51]. Based on these findings, *Bacteroides* species, and *B. thetaiotaomicron* in particular, are sometimes referred to as "generalists," capable of occupying a variety of metabolic niches depending on the availability of diverse polysaccharide nutrients.

Diet-derived polysaccharides control microbial community composition in the lumen of the colon. Unsurprisingly, the influence of diet is readily apparent in studies that profile the fecal community. A study of humans that completely switched between plant and animal-based diets showed that the microbiome abruptly shifts with diet[52]. Over small time scales this effect is reversible, suggesting that these changes represent transient ecosystem adaptations via blooms of particular species in the lumen while the mucosal reservoir remains unchanged. Many studies of *Bacteroides* glycan metabolism have shown that restricting the polysaccharide content of the mouse diet allows selection for species (or strains) that are capable of metabolizing the complex glycans present, such as fructans[53], human milk oligosaccharides[11], fucosylated mucin glycans[54], and mannan[55]. Presumably, the variety of Sus-like systems present in the genomes of *Bacteroides* provides the metabolic plasticity to persist in the gut despite short and long-term changes in nutrient availability. However, even in terms of monosaccharide and disaccharide utilization, there is a hierarchy of bacteria that are more efficient consumers, which helps explain how diet can dramatically and rapidly change the composition of the fecal community. Importantly, the nutrient environment of the gut lumen may be in a dynamic state of flux due to potential meal-to-meal variability, especially in omnivorous mammals.

In contrast to the variable conditions in the gut lumen, mammals likely maintain a more consistent nutrient balance in the mucosa, which serves as a stable positive selection factor for certain species of bacteria. Mucus degradation and metabolism by gut microorganisms provides access to privileged spatial niches and therefore a competitive advantage over other species, both ***indigenous*** and invasive. For example, several studies have shown that the ability to grow in an *in vitro* mucus culture is generally predictive of the ability of a bacterial species to colonize the mouse gut[56,57]. ***MUC2*** alone is coated with over 100 different O-linked glycan structures in humans[58]. These glycans differ between mice and humans[59], and differences in complex glycan “preference” by various bacterial species is a suggested mechanism of host-specific selection of a characteristic microbiome profile. In agreement, computational models have shown that positive selection at the epithelium via the ability to metabolize specific nutrients can be a more powerful mechanism for shaping host-associated microbial communities than negative selection driven by antimicrobials[60].

A. muciniphila, a prominent symbiont in many mammals, is one of the most effective mucin degraders *in vitro*[35] and is consistently found at high abundance in the mucus layer in humans[35] and mice[34]. Consumption of mucus glycans as a carbon and energy source allows *A. muciniphila* and other mucin-degraders to colonize the gut independently of the animal’s diet, providing a clear advantage to the bacteria during conditions of nutrient deprivation. Accordingly, levels of *A. muciniphila* increase in fasting Syrian hamsters[61] and hibernating ground squirrels[62]. Similarly, during intestinal inflammation in mice, the community metatranscriptome indicates increased mucin utilization with a corresponding increase in abundance of the mucin-degrading *B. acidifaciens*[63]. In gnotobiotic mice, restriction of complex polysaccharides in the diet causes the generalist *B. thetaiotaomicron* to shift its metabolism to utilize mucin glycans[50]. Further work has revealed that mutations in Sus-like systems involved in mucin glycan utilization in *B. thetaiotaomicron* cause a defect in competitive colonization and in vertical transmission of bacteria from mother to pup[64].

Therefore, the ability to utilize mucus as a carbon and energy source contributes to the ability of some microorganisms to stably colonize the host and transfer to offspring across generations. Not surprisingly, genetic manipulation of enteric mucus production in mice changes microbial community composition[54,65]. In turn, gut bacteria affect transcription of mucin-encoding genes in mice[66]. Overall, development of a healthy mucosa is a collaborative, bi-directional event between the host and the gut microbiota, creating an environment that allows the specific members to establish persistent colonization via utilization of host-derived glycans.

In some cases, the ability of a bacterium to colonize the gut may be determined by its ability to utilize a specific, yet limiting, nutrient. Bacterial species-specific carbohydrate utilization systems termed commensal colonization factors (CCFs) have been identified in *B. fragilis* and *Bacteroides vulgatus*, and allow these bacteria to colonize saturable nutrient niches[46]. This discovery was made based on the observation that gnotobiotic mice colonized with a specific *Bacteroides* species are resistant to colonization by the same species, but not colonization by closely related species. A genetic screen revealed that a set of genes encoding the CCF system was required for this intra-species ***colonization resistance*** phenotype (Box 1), suggesting that CCFs are responsible for defining the species-specific niche. Accordingly, when the *ccf* genes from *B. fragilis* were expressed in *B. vulgatus*, the resulting hybrid strain gained the ability to colonize an alternate niche. The CCF system was also required for penetration of *B. fragilis* into the crypts of the colon and long-term resilience to intestinal perturbations such as antibiotic treatment and gastroenteritis. Collectively, these data suggest that while metabolic flexibility allows bacterial adaptation in the lumen environment, the occupation of a narrowly-defined, tissue-associated niche is likely very important for stable colonization by some bacteria.

Box 1

Colonization resistance

One of the benefits afforded by the microbiota to the host is colonization resistance to pathogens. Invasive species of bacteria are inhibited from colonizing the gut because they are unable to displace indigenous species that have gained a strong foothold. After years of studying colonization resistance against pathogens in gnotobiotic animals in the 1960's and 70's, Rolf Freter theorized that the ability of a bacterial species to colonize the gut is determined by its ability to utilize a specific, limiting nutrient[135]. This notion has been well supported by studies showing that colonization resistance to pathogens is mediated by the availability of nutrient niches in the cases of *Escherichia coli*[136] and *Clostridium difficile*[137]. But Freter's hypothesis reached even further, suggesting that the relative amounts of limiting nutrients could dictate the abundance of each species in the indigenous community. Correspondingly, the variety of host-derived growth substrates could explain the stable diversity of the gut microbiota if individual species have evolved to specialize in the uptake and metabolism of specific, limiting nutrients, such as in the case of *Bacteroides fragilis*[46]. The concept of spatial niche partitioning being governed by host production of specific and scarce nutrient resources is attractive, and may help

explain both long-term persistence and resilience of the microbiome, as well as colonization resistance to pathogens.

Antimicrobials

Specialized epithelial immune cells called ***Paneth cells*** reside at the base of the crypts of the small intestine, secreting an array of antimicrobials that restrict the growth of bacteria that are found near the mucosal surface[4]. Many of these molecules are cationic antimicrobial peptides that interact with and disrupt negatively charged bacterial membranes (Figure 3B). Modifications to lipid A, a major component of the outer membrane of gram-negative bacteria, are known to confer resistance to cationic antimicrobial peptides in several pathogens[67]. Interestingly, underphosphorylation of this lipid portion of LPS, a modification shared with the ***pathobiont*** *Helicobacter pylori*, was found to be important for resilient colonization by *B. thetaiotaomicron* during inflammation[68] (Figure 3B).

The concentration of a variety of antimicrobials is higher toward the proximal end of the small intestine, creating a gradient that leads to a higher abundance and diversity of bacteria in distal locations (Figure 1A). For example, the lectin RegIIIγ is bactericidal to gram-positive bacteria that dominate the small intestine because it binds to and disrupts their exposed peptidoglycan layer. RegIIIγ is required to prevent massive infiltration of the mucosa and microbial invasion of the tissue[69]. In addition to RegIIIγ, the innate immune system deploys many other antimicrobials (**such as alpha-defensins from Paneth cells and beta-defensins from neutrophils)** with differing specificities to limit access to the epithelium[70], and resistance to these host-derived antimicrobial peptides is a general feature of many indigenous gut species of Firmicutes and Bacteroidetes[68].

In addition to these antimicrobials, gut bacteria, which are largely anaerobic, must contend with reactive oxygen species produced by aerobic host metabolism. Rapid dilution and consumption of oxygen secreted from the host tissue generates a gradient of oxygen that decreases in concentration from tissue to lumen (Figure 2). Accordingly, the mucosal community is enriched in genes required for resistance to reactive oxygen species[33]. Notably, although all *Bacteroides* species are classified as obligate anaerobes, *B. fragilis* can use oxygen as a terminal electron acceptor at nanomolar concentrations[71]. *B. fragilis* and tissue-associated ***microaerophilic*** Lactobacillaceae express catalase, superoxide dismutase, and other enzymes to inactivate reactive oxygen species[72]. Altogether, these mechanisms restrict access to the epithelium to a subset of bacterial species that not only can utilize nutrients found only at the tissue boundary, but can survive host antimicrobial strategies as well.

Mucus and adhesion

To access the epithelium, pathogens and commensals alike must contend with the mucus barrier and the immune system (Figure 4). Secreted MUC2 forms peptide crosslinks to create a viscous gel-like substance[73], serving as a barrier and host defense mechanism[74]. In mice lacking MUC2, the crypts of the colon are filled with bacteria and the tissue is covered in biofilms[24], indicating that the gel-forming mucus is the primary barrier to tissue

association by the microbiota at large. However, certain bacteria are able to penetrate the mucus by swimming or eating their way through.

In the gut, bacterial motility is generally restricted due to the immunogenicity of flagellin, which is a ligand for Toll-like Receptor 5 (TLR5)[75], and the viscosity of mucus limits the effectiveness of swimming (Figure 4). Still, the enteric pathogen *Salmonella enterica* subspecies *enterica serovar* Typhimurium depends on flagella and chemotaxis to penetrate the mucus layer and to reach host tissue[76]. *E. coli* and close relative *Shigella flexneri* opt for an alternative strategy of secreting a mucin-binding serine protease, Pic, which rapidly digests mucus (Figure 4). Interestingly, Pic also causes hypersecretion of mucus, which may interfere with the ability of indigenous bacteria to compete with the pathogen[77]. Similarly, another family of mucus-degrading proteins, M60-like peptidases, are conserved in pathogens and commensal mucosal bacteria from the Proteobacteria, Firmicutes, Bacteroidetes, and other phyla[78]. In enterotoxigenic *E. coli*, an M60-like peptidase was required for association with villi in the mouse small intestine[79].

In addition to the ability to penetrate the mucus layer, bacterial adhesion to the epithelium also influences the microbial composition of the gut, especially in the small intestine (Figure 2A). Species of *Helicobacter* adhere to and colonize the the stomach and small intestine tissue via adherence to epithelial surface glycans[80]. Further downstream in the small intestine, segmented filamentous bacteria (SFB) adhere intimately to the epithelial surface, as first described in imaging studies of mice[81]. Host-specific strains of SFB appear to be present in many mammals, including humans[82]. These bacteria were only recently cultured *in vitro* using tissue-cultured enterocytes as a platform to support their growth, reinforcing the idea that they are obligate symbionts with the mammalian gut tissue[83]. Their mechanism of attachment is still a mystery, though the attachment site is marked by accumulation of actin and leaves a visible indentation on the surface of the epithelial cell following removal of the filaments[83]. By virtue of intimate host association, SFB shape the host immune response[84] and impact autoimmune disease in mouse models[85,86].

The molecular mechanisms underlying how microorganisms attach to host tissue have been well-studied in pathogens (reviewed in Ref [87]). Although all of these features were initially discovered and described in pathogens, they are found in many commensal bacteria. Bacteria adhere to mucus and epithelial surfaces by deploying outer membrane proteins, capsules, lectins, adhesins, and fimbriae (attachment pili) (Figure 4). For example, the non-invasive pathogen *Vibrio cholerae* forms a layer of adhered cells on the wall of the small intestine using toxin-coregulated pili (TCP) [88]. *V. cholerae* also binds mucins using an outer membrane N-acetyl-D-glucosamine (GlcNAc)-binding protein, which may also facilitate penetration of the mucus and access to the epithelium[89]. Without attachment, these naturally plankton-associated marine bacteria are unable to colonize the gut, and thus are avirulent. *E. coli* possesses a great number of lectins with diverse sugar specificities allowing it to bind mucins as well as other glycoproteins and extracellular matrix components of epithelial cells[90]. Invasive pathogens also depend on adherence factors as a preceding step to penetration and infection of the tissue. *Listeria monocytogenes* expresses a surface protein, internalin A, which binds epithelial E-cadherin (a host cell adhesion protein) as a first step before exploiting actin to induce phagocytosis[91]. Studies of *S.* Typhimurium also reveal a

critical role of apical surface attachment in inducing neutrophil-mediated inflammation, which appears to paradoxically promote infection[92] by providing a competitive advantage for the pathogen over the resident microbiota[93].

Beneficial microorganisms also adhere to particular regions of the epithelium and can serve to exclude adherent pathogens by occupying limited binding sites, although little is known about the underlying mechanisms or functions of this process (Box 1). Early imaging studies revealed that *Lactobacillus* spp. that form adherent layers on the epithelium in the rat stomach prevent yeast[94] and staphylococcal[95] adherence to the epithelium. Members of the family Lactobacillaceae (such as *Lactobacillus* and *Lactoccocus*) that colonize the small intestine and stomach have become model systems for studying adhesion by commensals, with exopolysaccharides, pili, and cell wall-anchored proteins found to be involved in interacting with mucus, extracellular matrix proteins, and other molecular targets on the epithelial cell surface[96]. Notably, cell wall-anchored mucus-binding proteins (MUBs) unique to lactobacilli are known to be involved in both adherence and aggregation[97]. Strain-specific diversity in adherence and aggregation factors underlies the host specificity of *Lactobacillis reuteri*, indicating that tissue-associated biofilm formation is fundamental to colonization by this species[98]. Other means of attachment involve mechanisms conserved with pathogens, such as adhesive pili in *Lactobacillus rhamnosus* that bind mucus[99]. Analogous mechanisms can be found in unrelated species such as *Bifidobacterium bifidum*, which uses pili to bind extracellular matrix proteins, contributing to bacterial aggregation[100].

Collectively, these studies suggest that interactions with mucus and adherence to intestinal epithelial cells appear to be adaptations used by pathogens during infection, as well as strategies employed by commensals during persistent colonization (Figure 4).

Immunomodulation

In order to persist in the gut, non-pathogenic bacteria that intimately associate with host tissue must be tolerated by the immune system. The mucosa is inundated with large amounts of ***secreted Immunoglobulin A*** (sIgA) to monitor the microbiota. Many bacteria in the gut are coated in sIgA, and this subpopulation broadly resembles the mucosal population[101]. Certain adherent species such as *Helicobacter* spp. and SFB are especially highly coated in sIgA[102]. Binding of sIgA to bacteria may contribute to mucosal biofilm formation, which serves as a barrier to pathogen adherence[103]. Gnotobiotic studies with *Rag1* knockout mice (which effectively have no adaptive immune system) showed that experimental coating of *B. thetaiotaomicron* with sIgA reduces microbial fitness but also leads to reduced inflammatory signaling and changes to bacterial gene expression[5,104]. Through these mechanisms, sIgA mediates homeostasis between the host and the microbiota, as well as potential pathogens at mucosal surfaces. Furthermore, natural antibodies have evolved to recognize bacterial capsular polysaccharides; while largely studied in the context of infectious agents, such antibodies may also represent an evolutionarily conserved strategy used by the host to sense indigenous bacterial species. However, examples on how the immune system can dependably distinguish between harmful and beneficial microorganisms remain limited.

An alternate view is that the immune system is not “hard-wired” to discriminate between various classes of microorganisms, but rather that specific species have adapted to promote

their own immunologic tolerance. A few examples of active, species-specific immunomodulation by beneficial microorganisms suggest that some bacteria display signals that ensure their own tolerance by the immune system (Figure 5). *B. fragilis* is one of the best-understood gut bacteria in terms of immunomodulation. A component of its capsule, polysaccharide A (PSA), signals through an antigen-presenting cell intermediary to stimulate production of IL-10 by an anti-inflammatory subset of immune cells, regulatory T cells[105], contributing to the ability of *B. fragilis* to enter the mucus layer of the colon[45] (Figure 5). Surface fucosylation of the bacterial capsule also contributes to *B. fragilis* fitness in the gut, perhaps by mimicking the host cell surface to elicit a tolerogenic immune response[106]. Through these specific molecular signals, *B. fragilis* induces an anti-inflammatory immune profile that facilitates its own colonization. Similarly, exopolysaccharides of *Bifidobacterium breve* promote immune tolerance by decreasing the production of pro-inflammatory cytokines and preventing a B-cell response[107] (Figure 5). Through a less well-defined mechanism, *B. breve* also induces IL-10 production by regulatory T cells[108]. Notably, both *B. fragilis* and species of *Bifidobacterium* are known to closely associate with the host, which may necessitate immunomodulation to prevent an inflammatory reaction against these bacteria. Similarly, adherent SFB stimulate the development of a subset of T helper cells, Th17 cells, which are required for normal SFB colonization and also confer resistance to the pathogen *Citrobacter rodentium*[84] (Figure 5; Box 1). Clostridia are able to induce regulatory T cells, but a population of many species is much more effective than single isolates or combinations of a few, suggesting this is a combined effect of production of different metabolites, such as short chain fatty acids, by different species (see below)[109] (Figure 5). Similarly, a defined community of eight mouse gut bacterial species (including several members of families Clostridiaceae and Lactobacillaceae) referred to as the altered Schaedler's flora[110], was also shown to modulate immune responses mediated by regulatory T cells. Therefore, it is likely that many other beneficial microorganisms have co-evolved with the immune system to facilitate stable long-term colonization.

Several non-specific signals in the gut also promote tolerance towards beneficial microorganisms. Short chain fatty acids such as butyrate, propionate, and acetate are the end-products of anaerobic fermentation of sugars, which is the dominant metabolism in the colon. The development of regulatory T cells is stimulated by these molecules[111,112], which could be a more general way for the immune system to recognize beneficial bacteria or to assess the total fermentative productivity of the community. Mucus is another non-specific anti-inflammatory signal. When MUC2 is taken up by dendritic cells in mice, it inhibits the expression of pro-inflammatory signals[113], raising the possibility that indigenous mucin-degraders may induce host tolerance by being co-presented with mucus. Pathogens also have an arsenal of anti-inflammatory mechanisms to suppress the immune system to promote infection[114]. Particularly perplexing is the fact that features traditionally regarded as virulence factors in pathogens, such as capsular polysaccharides and pili, are also colonization factors in beneficial bacteria. Our notion of the defining characteristics of pathogens is likely clouded by a historical under-appreciation of similar colonization strategies used by beneficial species (Figure 4). It is not surprising that similar mechanisms of host association (mucus penetration, adherence, immune modulation) are used by

pathogenic and commensal bacteria alike; however, a key distinguishing feature is that commensals have either not evolved traits resembling traditional virulence factors, or have evolved additional features or modifications to offset the host-response to such factors. This perspective suggests that commensal bacteria may have reached an immunologic and metabolic 'truce' with their host, enabling persistent establishment of defined microbial habitats and elaborate microbial biogeographies.

MICRO-BIOGEOGRAPHY IN HEALTH AND DISEASE

Microhabitats in the gut are likely to contribute to the development and stability of microbial communities because spatially stratified niches facilitate greater diversity. In mouse pups, the fecal microbiota is initially dominated by Proteobacteria, a signature of the small intestine, but switches following weaning to Clostridia and *Bacteroides*, which are characteristic of the adult colon[2]. The sequential development of the microbiota thus may occur from proximal to distal compartments, which makes sense as dispersal in the gut is largely unidirectional along the fecal stream. Because of this restriction on dispersal, depletion of beneficial species, especially in the colon, could be catastrophic without a mechanism to replenish the community. Therefore, protected regions that are less susceptible to variable conditions in the gut may serve as reservoirs of bacterial cells that can seed growth in the lumen, possibly after an environmental insult (Figure 6). In the case of *B. fragilis*, mutants that are unable to colonize the crypts of the colon are less resilient to intestinal perturbations such as antibiotic treatment and enteric infection[46]. This is also a proposed function of the human appendix, which has a mucus and bacteria-filled lumen contiguous with the cecum[115]. The appendix is protected from the fecal stream, yet harbors a diverse microbial community and a contingent of specialized immune cells. The appendix is also phylogenetically widespread and evolved independently at least twice, providing strong evidence that this is not a vestigial structure as once believed[27]. In the rabbit appendix, indigenous bacteria coordinate the education of B and T-lymphocytes, suggesting that these tissue-associated niches are venues for immunomodulation[116]. Microhabitats such as crypts, mucus, and the appendix may be crucial to facilitate immune homeostasis, to protect microbial inhabitants from competitors, and to re-populate the gut following catastrophic perturbations that alter bacterial community structure or deplete certain species from the lumen.

Micro-biogeography alterations during disease

The adverse effects of altered composition of the healthy microbiota, known as ***dysbiosis***, on host health have long been appreciated. Increasing clinical evidence links dysbiosis with various immune, metabolic, and neurological disorders in both intestinal and extra-intestinal sites. For example, inflammatory bowel disease (IBD) is associated with changes in the gut microbiota, characterized by decreased abundance of *Clostridia*[117–119] and overall reduction in bacterial diversity[118–120]. Childhood asthma is correlated with low intestinal microbial diversity during the first month of life[121]. The obesity-associated microbiota is characterized by reduced microbial diversity and, in some studies, an increased *Firmicutes:Bacteroidetes* ratio[122]. In recent years, the role of gut dysbiosis in the pathogenesis of chronic liver diseases [123,124], colorectal cancer (CRC) [125,126], and even neuropsychiatric dysfunctions[127]

has been explored in animal models and humans. For clinical applications, profiling of the fecal microbiota has been widely used as a surrogate for the gastrointestinal bacterial community due to non-invasive and straightforward sample collection; however, fecal populations may be less informative than mucosal biopsies in defining disease-associated dysbiosis[128], a notion that requires additional experimental support. Below, we detail two examples that illustrate the importance of micro-biogeography alterations of the gut microbiota during disease: IBD and hepatic encephalopathy.

IBD is characterized by inflammation of the gastrointestinal tract resulting in pain, vomiting, diarrhea, and other complications including severe weight loss and behavioral changes. Generally IBD is categorized into two syndromes, Crohn disease, which may involve inflammation throughout the gastrointestinal tract (mouth to anus), and ulcerative colitis, where pathology is restricted to the large intestine. For over a decade, studies have attempted to define a pattern of dysbiosis associated with IBD and yielded inconsistent and sometimes contradicting results[129]. Studies that focused on fecal microbiota reported wide inter-individual differences in composition with overall microbial diversity reduced[118] in Crohn disease patients compared to healthy controls. However, a study in which human gut microbiota were assessed for the ability to drive colitis pathology in mice found that bacteria contributing to the disease are highly coated in sIgA[102], suggesting that the mucosal or tissue-associated population is most relevant. Human studies based on biopsy samples elucidated several consistent features in line with this hypothesis: patients had increased concentration of bacteria on the mucosal surface[130]; decreased microbial diversity; [119,120]; decreased abundance of *Clostridium* species [117]; and increased number of *Enterobacteriaceae* (especially adherent, invasive *E. coli*) in ileal mucosa[131]. Most recently, both the lumen- and mucosa-associated microbiota were profiled in a large cohort of new-onset, treatment naïve pediatric patients with Crohn disease and non-IBD controls. Analysis of the mucosal microbiota revealed a significant drop in species richness, an increase in *Enterobacteriaceae,* a decrease in *Clostridiales*, and significant changes in several other previously unidentified taxa. Importantly, these dysbiotic signatures were lost when stool samples were examined[128]. Intriguingly, a laser-capture microdissection study of colon crypt mucus in patients with ulcerative colitis found that they had lower levels of crypt-associated bacteria[132]. Overall, these studies highlight that distinguishing between fecal and mucosal microbial communities is particularly important for finding a reproducible microbial signature of IBD. Moving from correlations to a potential causal etiology of the microbiota for IBD and other disorders will require further study of mucosal communities, focusing on the interactions between the host and microbiota.

Biogeographical changes in the gut microbiota may also influence liver function. Hepatic encephalopathy is a neuropsychiatric complication of cirrhosis and direct sequelae of gut dysbiosis. As a result of impaired liver function and the presence of porto-systemic shunts (bypass of the liver by the circulatory system), toxic metabolites produced by the gut microbiota evade liver catabolism and cross the blood-brain barrier, leading to cerebral toxicity[123]. Interestingly, a comparison of the fecal microbiota of cirrhotic patients with and without hepatic encephalopathy showed minimal differences, whereas analyzing the microbiota composition of the colonic mucosa revealed significant changes, including lower *Roseburia* and higher *Enterococcus*, *Veillonella*, *Megasphaera*, *Burkholderia*, and

Bifidobacterium in cirrhotic patients with hepatic encephalopathy[133]. The bacterial genera over-represented in the mucosa of patients with hepatic encephalopathy (*Megaspheara*, *Veillonella*, *Burkholderia*, and *Bifidobacterium*) were also correlated with poor cognition, higher inflammation, and higher clinical severity score. In summary, mucosal dysbiosis in the gut, but not in the composition of the fecal community, significantly correlates with the severity of chronic liver disease phenotypes, including hepatic encephalopathy.

CONCLUSION

We have highlighted evidence that the microbiota is biogeographically stratified within the gut on different spatial scales and axes. Progress towards a functional understanding of the microbiota necessitates increased attention to microhabitats within the gut ecosystem, and to spatial relationships between microorganisms and between microorganisms and the host. Fecal community profiling enabled by next-generation sequencing provides a valuable picture of the diversity, specificity, stability, and developmental dynamics of the gut microbiota, but focusing on measurements of abundance in feces neglects the importance of mucus and tissue-associated organisms and cannot account for spatial distributions. Similarly, studies in gnotobiotic animals allow a reductionist approach to studying host-microorganism interactions akin to methods traditionally employed by microbiologists studying pathogens, but this simplified methodology is likely to miss important contributions from interspecies interactions. The functional study of gut microbial ecology using “meta-omics” techniques enables one to account for the behaviors of the community as a whole, but attributing functions to particular microbial members remains a challenge in community-level ecology. Therefore, testing unifying hypotheses using both reductionist and ecological approaches will be essential to our understanding of the microbiota and its biological functions.

More than half a century ago, in “Microorganisms Indigenous to Man,” the microbiologist Theodor Rosebury lamented on the lack of a general theory for influences that control composition of the microbiota, the roles of individual members, and functions that affect the host[134]. With the true complexity of the problem revealed recently by sequencing advances, research is only now in a position to fulfill Rosebury’s call for a general theory. Rolf Freter’s nutrient niche hypothesis[135], which states that limiting nutrients control the population level of species that are particularly adept at utilizing them, provides a metabolic foundation to explain some of the nascent observations in the field. But when Freter proposed his ideas, we were unaware of the role of immunomodulation by non-pathogens, which requires access to the tissue. Based on evidence outlined in this review, we propose that the host presents limiting nutrients as well as attachment sites in privileged locations. Furthermore, the immune system has an active role in allowing only beneficial species to access these locations during homeostasis. Selection for particular species close to the epithelium creates protected, stable reservoirs for microorganisms to persist in the face of rapidly changing conditions in the gut lumen. Through localized, immune-facilitated, and adherence-dependent nutrient selection, the host maintains stability of a diverse community of microbial symbionts.

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Glossary

Symbionts	in ecology, these are organisms that participate in a close relationship with other organisms. The term symbionts encompasses organisms that participate in different types of relationships, including mutualists, commensals and parasites.
Mutualists	in ecology, these are organisms that participate in a symbiotic relationship in which both parties benefit.
Commensals	in ecology, these are organisms that participate in a symbiotic relationship in which one party benefits from the other without affecting it. Historically, commensals is also used as a term for the resident gut bacteria, though many of these may be mutualists
Pathobiont	a symbiont with the potential to promote pathology under conditions that deviate from homeostasis, (such as in immunocompromised or nutrient deprived individuals)
Indigenous	organisms that are native to a particular habitat (also termed autochthonous), as distinct from organisms that are simply passing through a habitat (allochthonous)
Dysbiosis	deviations from a normal microbial community, such as imbalances in abundance, membership or localization of microorganisms.
Gnotobiotic	usually refers to formerly germ-free animals that carry a defined microbiota. The composition of the microbiota in these animals is usually determined experimentally.
Paneth cells	specialized epithelial cells at the base of crypts in the small intestine that secrete antimicrobial peptides.
Goblet cells	specialized epithelial cells throughout the gastroinstestinal tract that secrete gel-forming mucins. Goblet cells can also be present in other mucosal epithelial surfaces throughout the body.
Colonization resistance	the prevention of invasion of an exogenous species into a microbial community. In the gut, colonization resistance may be a result of resource competition, spatial exclusion or direct inhibition by commensal microorganisms or of selection mediated by host factors.

Microaerophilic	obligate aerobic microorganisms that only thrive in environments with relatively low oxygen concentrations, such as at the epithelial surface in the gut.
Microbiota	The microorganisms that inhabit a particular habitat
Syntrophic	A metabolic relationship between difference species in which one feeds another
Prebiotics	Foods that stimulate the growth of commensal or mutualistic gut bacteria
Biofilm	An aggregation of bacteria stuck to each other and to a surface
Digesta	The bulk of dietary fibers that is digested as it transits the gastrointestinal tract
MUC2	The most abundant mucin protein in the human gut (also in mice, where it is referred to as Muc2).
Secreted Immunoglobulin A (sIgA)	By far the most abundant class of antibody found in the gut

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Biographies

Gregory P. Donaldson is a graduate student in the Mazmanian lab at Caltech, studying the microbial genetic and molecular basis of gut colonization in species of *Bacteroides*. He received his B.S. in Microbiology from the University of Maryland, College Park, where he studied biofilm regulation in *Pseudomonas aeruginosa*.

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Sarkis K. Mazmanian is the Louis and Nelly Soux Professor of Microbiology at Caltech. He received his B.S. and Ph.D. in Microbiology, Immunology and Molecular Genetics from UCLA, studying mechanisms by which *Staphylococcus aureus* displays surface protein adhesins during animal infection. As a fellow at Harvard Medical School, he investigated how gut bacteria influence immune system development. Dr. Mazmanian's laboratory focuses on studying mechanisms by which the gut microbiome impacts the immune and the nervous systems.

Online summary

The gut microbiota is spatially stratified along the longitudinal and cross-sectional axes of the gut. Chemical and nutrient gradients, antimicrobial peptides, and physical features of the gut contribute to differences in microbial community composition in different locations.

The mucosal and lumenal microbiota of the gut represent distinct microbial communities. On a smaller scale, patchiness within these communities suggests that they are highly spatially organized.

Diet imparts a large effect on microbial colonization and relative abundance, but some bacteria can thrive independently of dietary changes by living on host-derived nutrients such as mucin glycans. Therefore, the mucus layer can harbor a reservoir of bacteria that are maintained regardless of food intake. The appendix and colon crypts may also be examples of such microbial reservoirs.

Only a subset of gut symbionts are able to access the epithelial surface. Mucus, antimicrobial peptides, and adaptive immune activity limit tissue accessibility. Direct interfacing between the host and microbial symbionts may be important for maintenance of homeostasis.

Immunomodulation by certain symbionts allows the host to tolerate intimate relationships with potentially beneficial microorganisms. This may be a way in which commensals distinguish themselves from pathogens and prevent their elimination by the immune system.

While many diseases have been associated with dysbiosis, understanding the function of the microbiota in health and disease requires accounting for the biogeography of the community. Recent human studies have found differences specific to the mucosal community in cases of inflammatory bowel disease and hepatic encephalopathy.

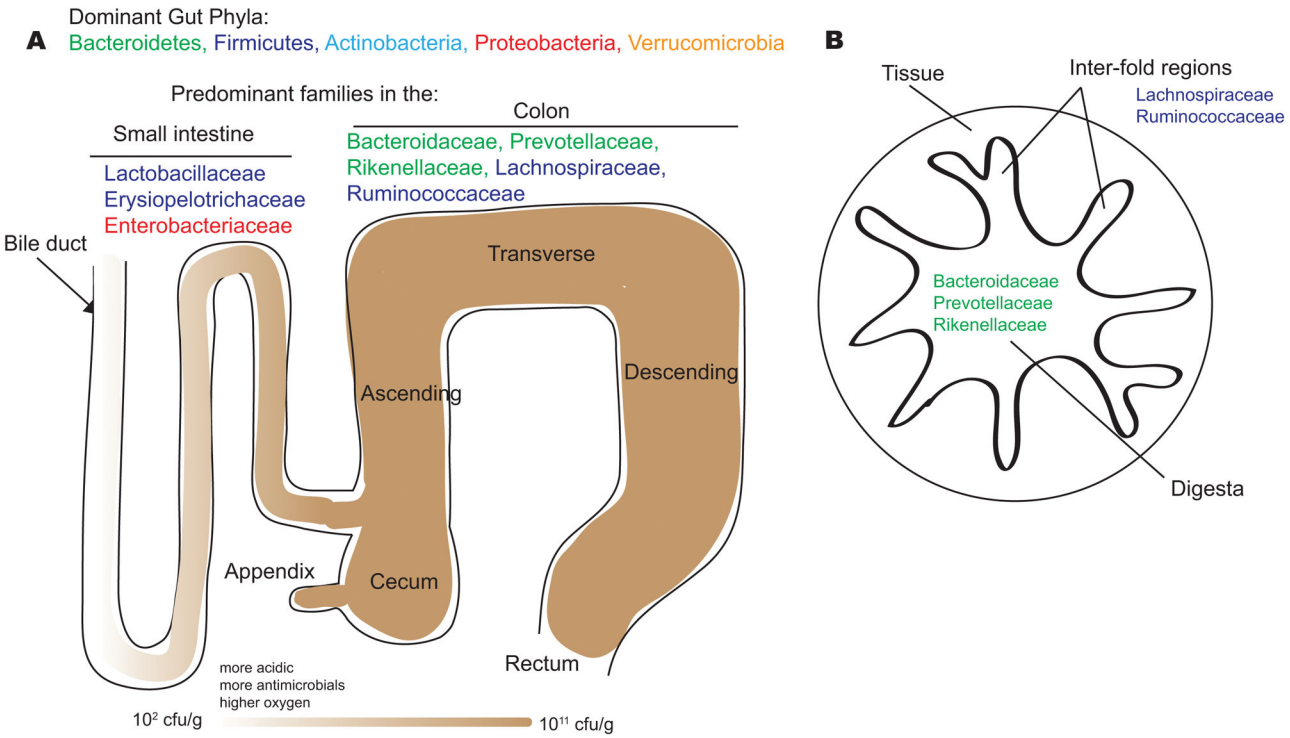

Figure 1. Microbial habitats in the human lower gastrointestinal tract

The dominant bacterial phyla in the gut are the Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria and Verrucomicrobia. The dominant bacterial families of the small intestine and colon reflect physiological differences along the length of the gut. For example, a gradient of oxygen, antimicrobial peptides (including bile acids, secreted by the bile duct), and pH limits the bacterial density in the small intestinal community, whereas the colon carries high bacterial loads. In the small intestine, Lactobacillaceae and Enterobacteriaceae dominate, whereas the colon is characterized by the presence of Bacteroidaceae, Prevotellaceae, Rikenellaceae, Lachnospiraceae and Ruminococcaceae. A cross-section of the colon shows the digesta – which is dominated by Bacteroidaceae, Prevotellaceae and Rikenellaceae – and the inter-fold regions of the lumen – which are dominated by Lachnospiraceae and Ruminococcaceae.

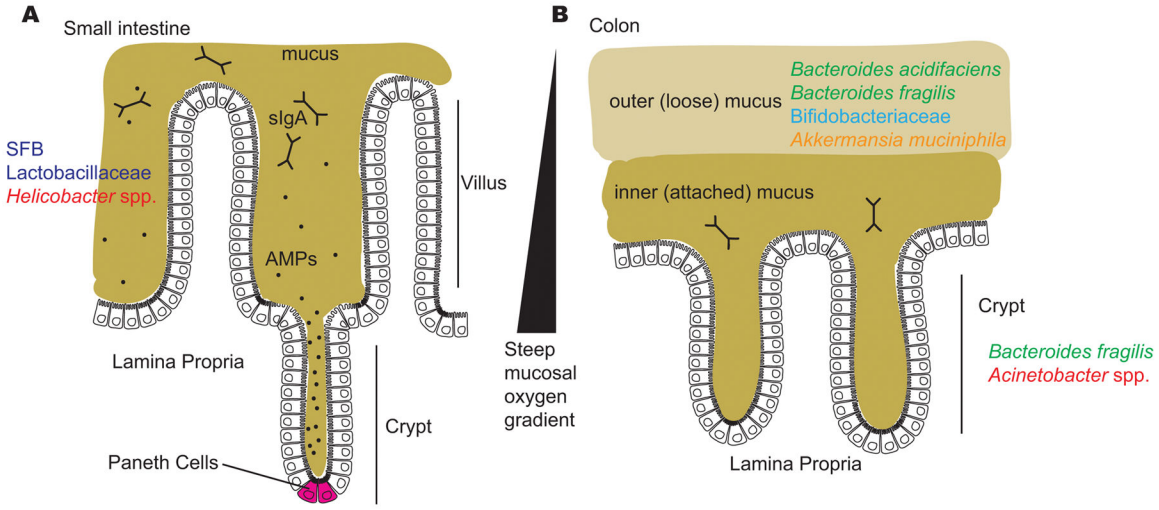

Figure 2. The mucus layers of the small intestine and colon

Several factors limit the ability of gut bacteria to access host cells, including the mucus layers in the small intestine and the colon; antimicrobial peptides in the small intestine, including those produced by Paneth cells at the base of the crypts; secreted immunoglobulin A (sIgA) in both the small intestine and colon; and a steep oxygen gradient that influences which bacteria are capable of surviving close to the epithelial surface. A) The surface of the small intestine is shaped into villi and crypts and is colonized by certain adherent species, including segmented filamentous bacteria (SFB), Lactobacillaceae and *Helicobacter* spp. B) The colon has two distinct mucus structures: the outer layer is colonized by mucin-degrading bacteria and is characterized by the presence of *Bacteroides acidifaciens*, *Bacteroides fragilis,* Bifidobacteriaceae and *Akkermansia muciniphila* and the inner layer and crypts are penetrated at low density by a more restricted community that includes *Bacteroides fragilis* and *Acinetobacter* spp.

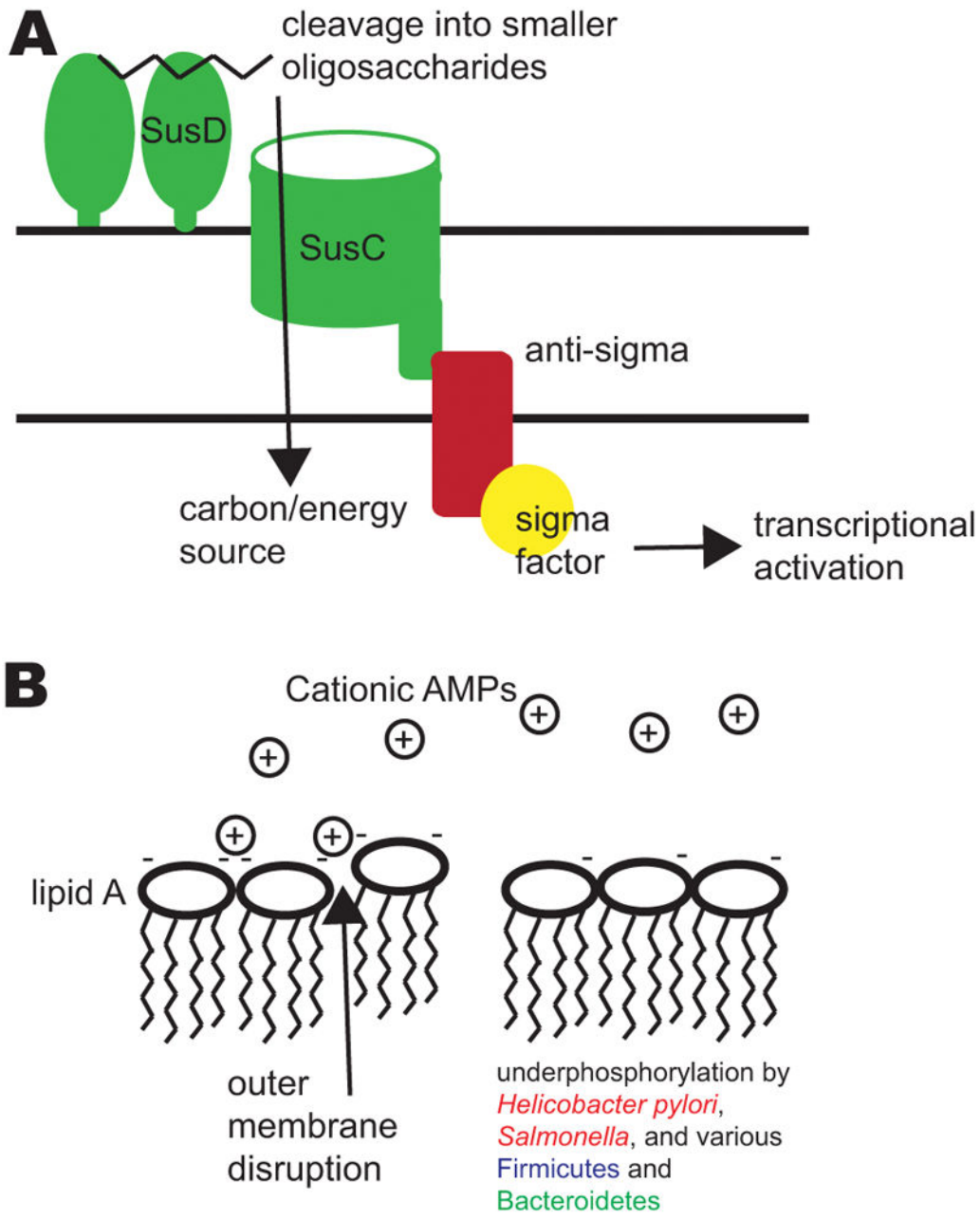

Figure 3. Bacterial colonization determinants

Several factors affect the localization of bacteria within the gastrointestinal tract, including the ability to utilize different glycans and to resist antimicrobial peptides (AMPs). A) Sus-like systems in *Bacteroides* species allow the utilization of complex polysaccharides from the diet or the host. The figure illustrates a generalized schematic of a Sus-like system. Homologues of SusD and other outer membrane lipid-anchored enzymes bind and cleave the glycans (such as starch) into smaller oligosaccharides that are then imported by the SusC-like outer membrane transporter. Interaction with the cognate glycan often leads to transmembrane signaling to activate gene regulatory mechanisms, such as a two-component system or a transmembrane anti-sigma factor which releases and activates a sigma factor.

Downstream transcriptional regulation allows *Bacteroides* species to respond to local availability of glycans. B) Cationic AMPs in the small intestine, which also pass into the colon via the fecal stream, disrupt bacterial outer membranes by interacting with negative charges on their surface. By removing phosphate groups from lipid A of lipopolysaccharide (LPS), pathogens and commensals alike – such as *Helicobacter pylori, Salmonella* spp., and various Firmicutes and Bacteroidetes –reduce the negative charge on their membranes and evade attack by cationic AMPs.

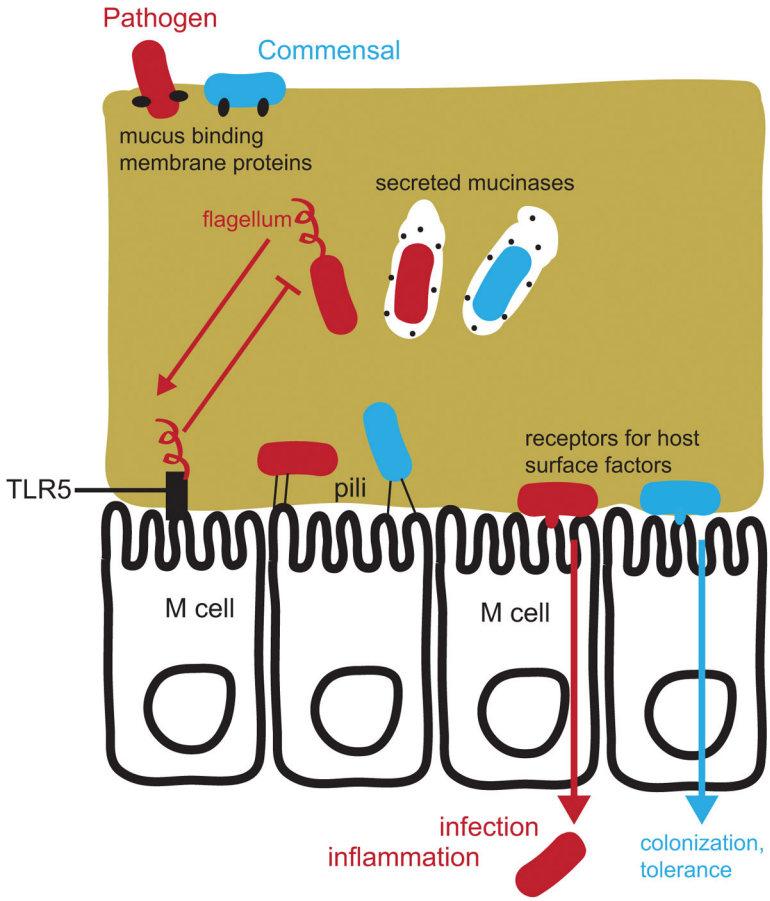

Figure 4. Bacterial access to the epithelium

Both bacterial pathogens (red) and commensals (or mutualists; blue) have the ability to cross the mucus layer and access the gut epithelium. Lectins and other mucus-binding proteins facilitate initial interactions with the mucus layer. Mucinases and proteases are used to degrade mucus for bacteria to "eat" their way through, while some pathogens such as *Salmonella* spp. use flagella to swim through the viscous mucus. TLR5 sensing of flagellin effectively leads to inhibition of flagellar biosynthesis for most bacteria in the gut. Adherence to the tissue is achieved by both commensals and pathogens through pili, lectins, and other outer-membrane proteins that target ligands on the epithelial cell surface. Adherence facilitates gut colonization for both commensals and pathogens, and also allows tissue invasion by pathogenic bacteria. Microfold cells (M cells) are specialized immune sentinel epithelial cells that detect gut bacteria and are also exploited by many pathogens as a means of translocation across the epithelium.

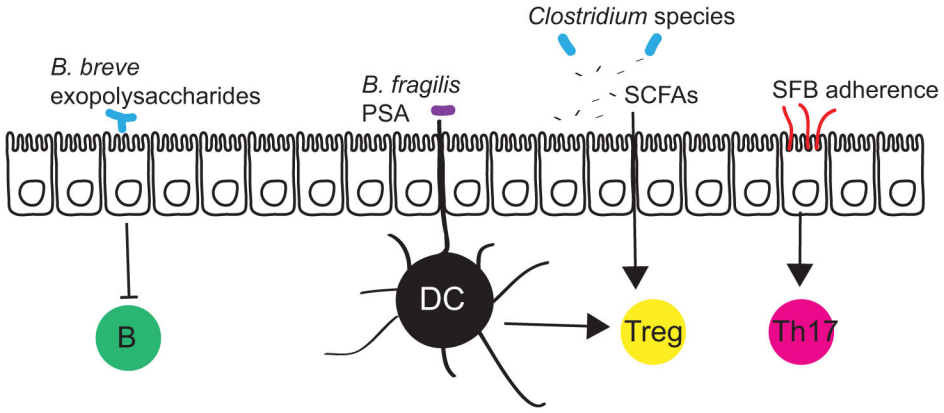

Figure 5. Immunomodulation by commensal gut bacteria

Commensal gut bacteria induce immunomodulation via interaction with epithelial cells, antigen presenting cells (such as dendritic cells (DCs)), and via production of signaling metabolites. The exopolysaccharides of adherent *Bifidobacterium breve* reduce the production of inflammatory cytokines to dampen B cell responses. The capsular polysaccharide PSA of *Bacteroides fragilis* and short-chain fatty acids (SCFAs) produced by many species of *Clostridia* (and other genera) stimulate the production of the anti-inflammatory interleukin-10 (IL-10) by regulatory T cells. Segmented filamentous bacteria (SFB) intercalate between microvilli of epithelial cells and stimulate the development of Th17 cells, which are important for mucosal immunity to extracellular pathogens.

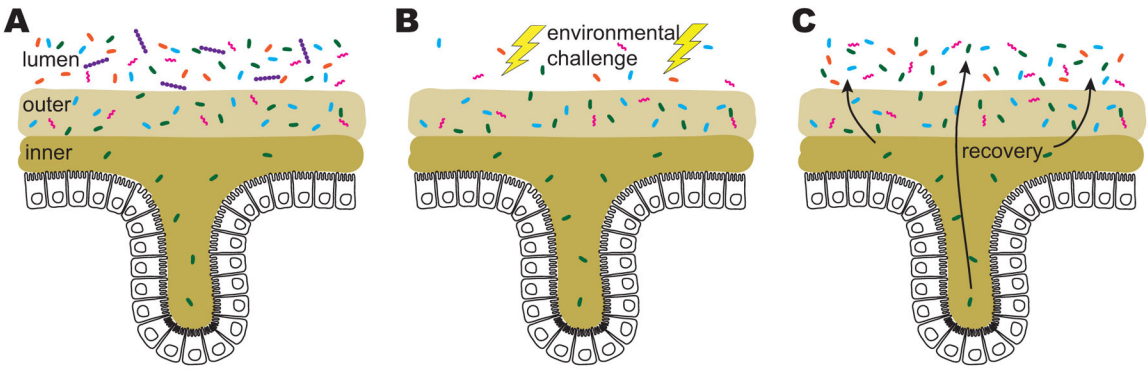

Figure 6. Gut microhabitats as reservoirs of bacterial diversity

Specific niches such as crypts, the inner mucus, and the appendix may be crucial to facilitate immune homeostasis, to protect microbial inhabitants from competitors, and to re-populate the gut following perturbations that alter bacterial community structure or deplete certain species from the lumen. A) A subset of species is able to penetrate the inner mucus layer and enter crypt spaces. B) Environmental challenges such as diet perturbations, abnormalities in gastrointestinal motility, and antibiotic consumption massively alter the lumen community. However, the more stable mucosal environment and crypts protect important bacterial species. C) The crypts and mucosa serve as reservoirs to repopulate the lumen.