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Regional Diversity of the Gastrointestinal Microbiome

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Abstract

The role of gut microbes in health and disease has often been surmised from stool, which is easily sampled and rich in microbial diversity, density, and abundance. Microbial analyses of stool have been accepted as measures to determine the relationship of gut microbiomes with host health and disease, based on the belief it represents all microbial populations throughout the gut. However, functional heterogeneity of each gastrointestinal tract (GIT) tract segment gives rise to regional differences in gut microbial populations. Herein, we summarize the literature regarding the microbial landscape along the rostral to caudal, i.e. horizontal mouth to anus, axis of the GIT. We aim to identify gaps in the literature, particularly regarding small intestinal microbiota abundance and diversity, highlight the importance of regional microbiota on host health and disease, as well as discuss opportunities to advance this line of research.

In Brief

Microbes inhabit the length of the gastrointestinal tract but differ in type and abundance in a regional fashion (mouth to colon). In this review, Martinez-Guryn, Leone, et al. examine host and environmental pressures that drive regional heterogeneity and how the regional gut microbiota influences physiological host processes and disease development.

Exploring new microbial territories

The gastrointestinal tract (GIT) is a multi-organ system with great regional diversity, housing extensive gut microbes and providing diverse functions. Each region is geographically specialized in gene expression and function, regulating complex and diverse digestive, immunological, metabolic, and endocrinological processes. Whether it is the acidity of gastric juices, the entry point of bile acids (BAs) and pancreatic secretions, the

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Declaration of Interests

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digestive and absorptive surfaces of the small intestine (SI), or water extraction and stool formation in the colon, these properties create critical environmental conditions that determine microbial community assemblage, fitness, and functions that mutually benefit host and microbes (Figure 1). Acquisition of regional microbiota is not random but arises from careful selection customized to the specific host needs through dynamic mutually reinforcing interactions.

This review examines the extensive heterogeneity of gut microbes along the rostral to caudal axis of the GIT. We bring together current knowledge of regional gut microbiota composition and function, outlining how disturbances created by the host, environment, or diet can have detrimental consequences. Many challenges currently hinder studies of regional gut microbiota, especially in humans. This being said, emerging technologies and model systems will enable major advances in the future.

Exploring and mapping the rostral to caudal axis of GIT microbiomes

The abundance and diversity of microbes along the entire GIT generally increases from the proximal to distal intestine, influenced by host features as well as microbial community dynamics (Tropini et al., 2017). Regional oxygen level, nutrient bioavailability, pH, BAs, GI transit time, mucus, and immune factors are all important determinants of microbial selection (Figure 1, Friedman et al., 2018). A deep understanding of the microbial side has been evasive, in that most studies are limited to description of regional membership via 16S rRNA sequencing, with few functional insights.

Mouth

Digestion begins in the mouth with mastication of food and enzymatic digestion of macronutrients facilitated by coordinated action between teeth, tongue and salivary secretions. Multiple factors influence oral cavity microbial assemblage, including differences in surface structure at the micron scale, function and shedding of those surfaces, as well as nutrient flow and oxygen level. Despite ecological differences relative to the distal GIT, the oral microbial community is complex and diverse, containing nearly 20 billion microbes representing over 700 identified bacterial species (Dewhirst et al., 2010).

The microbiota of oral tissues such as the teeth, gingiva, tongue, hard and soft palatal mucosa, and tonsils are distinct and highly structured. For example, the microbiota of supragingival plaques are spatially organized with a “hedgehog” arrangement of *Corynebacterium* setting the foundation of plaque-forming filaments that extend outward, while other taxa take on distinct niches around and within this structure. This confers a strong structural base that resists physical disruption, i.e. teeth brushing (Mark Welch et al., 2016). Non-shedding surfaces such as the teeth, dentures, or implants also serve as a foundation for biofilm formation in the mouth (Lynge Pedersen and Belstrøm, 2019).

Functional features of the mouth include breathing, sensory receptor expression, secretion of saliva for mastication and enzymatic digestion of food, as well as serving as an immunological barrier between the host and environment. These features determine assemblage and elicit bidirectional relationships with resident microbes. For example, direct

exposure to environmental oxygen favors aerobes and facultative anaerobes. Sensory perception of fat is affected by gut microbes, as germ-free (GF) mice display decreased expression of the long chain fatty acid translocase CD36, a lipid sensor on the tongue (Duca et al., 2012). Salivary production impacts microbial composition in various ways such as 1) flushing food and bacteria from the mouth, 2) liberating nutrients via digestive enzymes for bacterial metabolism, 3) releasing mucins that promote bacterial aggregation, and 4) delivering various immune factors, including antimicrobial compounds. Notably, decreased salivary flow can alter pH to affect the oral microbiome (Lynge Pedersen and Belstrøm, 2019).

Commensal bacteria in the oral cavity enhance immune function and confer protection from pathogenic microbes involved in forming supragingival and dental biofilms that promote dental caries. Commensal microbes such as *Veillonella*, *Streptococcus*, and *Granulicatella gingiva* increase anti-microbial peptide (AMP) production and inflammatory cytokine secretion, leading to increased epithelial barrier function and mucosal thickness in 3D-reconstructed human gingiva (Figure 1, Shang et al., 2018).

In normal conditions, oral microbes are continuously transmitted to the distal GI in numbers greater than expected by ingestion alone (Schmidt et al., 2019). However, transmission of certain oral microbes can potentially promote disease in the distal gut in genetically susceptible hosts. Orally-derived *Klebsiella* was found to colonize the colon, which promoted mucosal inflammation through TH1 cell activation and inflammation (Atarashi et al. 2017). Similarly, *Klebsiella* strains isolated from Crohn's Disease (CD) and ulcerative colitis (UC) patients induced a Th1 immune response in GF mice compared to several other isolates of different genera. Notably, these *Klebsiella* strains were antibiotic resistant, making them well-suited to establish a colonic niche and mount an inflammatory response in genetically susceptible hosts, i.e., IL-10$^{-/-}$ mice.

Esophagus

As a conduit for partially digested food into the stomach, the esophagus houses a complex environment of microorganisms and host immune factors. The esophageal microbiota composition is similar to the oral microbiome with major phyla including Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Fusobacteria, and Saccharibacteria and genera *Prevotella*, *Veillonella*, and *Streptococcus* (Figure 1, reviewed in May and Abrams, 2018).

Much like other GIT regions, the esophageal microbiota is highly influenced by diet. For example, an obesogenic diet alters esophageal microbiota composition with increased representation of the genus *Clostridium sensu strico* (Kaakoush et al., 2017). Others found that increased dietary fiber intake and low fat diet (LFD) promote greater abundance of Firmicutes and reduced abundance of Proteobacteria and gram-negative bacteria, whereas low fiber intake was associated with greater abundance of Prevotella, Neisseria, and Eikenella. Whether these changes are associated with esophageal disease was not investigated (Nobel et al., 2018).

Stomach

The stomach continues digestion through peristaltic action and breakdown of food components within its acidic environment, playing an important role in regulating food intake through its endocrine function. It contains fewer total microbes than in distal regions of the GIT, ~10^1 to 10^3 CFU/ml (O'Hara and Shanahan, 2006). Despite gastric pH, mucosal thickness, and peristalsis that limit growth of microbes, the gastric microbiota is diverse, including major phyla such as Firmicutes, Bacteroidetes, Fusobacteria, Actinobacteria, and Proteobacteria, and at the genus level *Prevotella*, *Streptococcus*, *Veillonella*, *Rothia* and *Haemophilus* (Figure 1, Nardone and Compare, 2015).

The presence of gut microbes may contribute important host functions of the stomach, and may even contribute to systemic diseases. For example, GF mice have significantly elevated levels of the GI hormone ghrelin, which stimulates appetite, food intake, gastric motility and adiposity (Slade et al., 2018) as compared to conventionally-raised mice, suggesting a microbial role in its regulation (Martinez-Guryn et al., 2018). As G cells in the stomach produce ghrelin, gastric microbes may affect host endocrine signaling and metabolism.

Small intestine (SI)

The single layer of polarized intestinal epithelial cells (IECs) of the SI includes diverse cell types, such as absorptive, goblet, Paneth, Tuft, enteroendocrine, and other cell types that are differentially distributed along the length of the SI and impart region-specific assembly rules that determine microbial abundance, diversity, and function. One possible reason why the microbial load is relatively low in the SI compared to more distal regions is that it is purposefully designed for macro- and micronutrient absorption and immune regulation. Its motility pattern, for instance, is primarily determined by a gradient of non-propulsive annular contractions that maximizes mixing of digestive juices and content and produces transit times ranging 3 to 5 hrs (Szarka and Camilleri, 2012). In comparison, the colonic transit time is much longer (>30 hrs). This discrepancy may account for lower microbiota diversity and abundance merely due to less time to colonize and establish stability in the SI. Consistent with this notion, when SI stasis occurs, the luminal microbiota converts to a colonic-like microbiota that exhibit greater diversity, richness, and abundance (Ward et al., 2016).

1. **Duodenum—**Despite its shorter length and rapid transit of gastric chyme, the duodenum is a major site for digestion and absorption where biliary and pancreatic secretions facilitate macronutrient breakdown. Primary bile acids (BAs) made by the liver from cholesterol are conjugated with glycine (humans), taurine (mice), or sulfate prior to delivery into the duodenum (Russel and Setchell, 1992). BAs are amphipathic detergents that not only emulsify fat for digestion, but also favor bile-tolerant microbiota, and signal through nuclear receptors to affect host gene expression. The absorption of digested proteins, carbohydrates, and lipids by IECs begins here, facilitated by a thinner overlying mucus barrier in comparison to that seen in distal gut regions (Ermund et al., 2013).

The duodenum contains roughly 10^1 to 10^3 CFU/ml (O'Hara and Shanahan, 2006). Decreased bacterial abundance in the upper SI is attributed to reduced transit time, higher

oxygen levels, antimicrobial compounds such as BAs, elevated pH levels, and presence of digestive enzymes. Oxygen gradients created by swallowed air, delivery from host tissues, and oxygenation through pancreatic and biliary secretions (Friedman et al., 2018) can influence microbial abundance and type in the upper SI, which consist mostly of Actinobacteria and Proteobacteria in mice. In turn, significant shifts in duodenal gut microbiota membership and function may have important implications to the host. Analysis of duodenal metagenomes in obese and lean participants (Angelakis et al., 2015) showed microbial genes involved in carbohydrate metabolism were decreased, whereas an increase in genes related to lipid metabolism were observed among obese individuals. Thus, duodenal microbiota may affect bioavailability of dietary nutrients to the host.

2. **Jejunum—**The jejunum is a major site of nutrient absorption, although not easily accessible. Endoscopic samples are the major sources of information, which limits understanding of the healthy upper GI microbiome because these individuals usually have some underlying condition. Thus, the majority of our knowledge of these regions has been gleaned from animal studies, where both mucosal and luminal samples can be readily obtained. Factors that influence the jejunal microbiota include diet, oxygen levels, bile acids, transit time, and a thinner overlying mucus. The jejunal microbiota is estimated to consist of 10^4 to 10^7 CFU/ml, primarily represented by Firmicutes, but also containing Proteobacteria, Actinobacteria, and Bacteroidetes (El Aidy et al., 2013; O'Hara and Shanahan, 2006). Similar taxa are found in the jejunum of human subjects that markedly differs from fecal microbiota (Seekatz et al., 2019).

Fecal microbial transplantation into GF mice causes functional changes in the jejunal transcriptome as early as one day (El Aidy et al., 2013), whereby metabolic genes (e.g. fat metabolism) were significantly altered compared to GF counterparts. This acute transcriptional response was specific to the jejunum and not observed elsewhere in the GIT. However, a limitation of this study was the use of fecal microbiota which differs from SI microbiota. Our studies, for instance, have shown that high fat diets (HFD) affect membership and function of the murine SI microbiota to a much greater extent than that of cecal and stool microbiota, with increased Firmicutes, particularly the family Clostridiaceae (Martinez-Guryn et al., 2018). When HFD-induced jejunal microbiota were transferred into GF mice, lipid absorption was increased compared to GF mice receiving LFD-induced jejunal microbiota. Thus, HFD-induced microbiota differentially primes the naïve GF gut compared to LFD microbiota, enhancing fat absorption.

3. **Ileum—**Digestion and absorption in the ileum is unique from the other regions, and not surprisingly its microbial community membership and functions differ. The ileum has an estimated microbial load of 10^3–10^8 CFU/mL, mostly comprised of facultative and obligate anaerobes. From limited studies in humans, the ileal microbiota appear to consist mainly of Bacteroides, Clostridium, Enterobacteria, *Enterococcus*, *Lactobacillus*, and *Veillonella* (Li et al., 2017; Zoetendal et al., 2012). Collectively, ileal IECs function as the primary absorption site for nutrients such as B vitamins, residual nutrients not absorbed more proximally, as well as BA reuptake and reentry into enterohepatic circulation, the latter influenced greatly by gut microbes.

The distal ileum expresses nuclear receptors involved in BA signaling, such as the farnesoid x receptor (FXR) and the G-protein coupled receptor TGR5, that elicit profound effects on downstream metabolic pathways (Ridlon et al., 2016a). GF mice have elevated levels of total BAs as well as abundance of SI muricholic acid compared to conventionally-raised animals (Sayin et al., 2013). The distal ileum of conventional mice expressed increased levels of basolateral BA transporters as well as *FXR* target genes, specifically *Shp* and *Fgf15,* important mediators of the BA enterohepatic pool. The microbially-mediated upregulation of *Fgf15* decreased hepatic BA synthesis mediated by decreased expression of hepatic *Cyp7a1,* a rate limiting BA synthesis enzyme. Conversely, levels of these genes could be restored to those observed in GF mice by treating conventionally-raised animals with antibiotics (Sayin et al., 2013).

BAs themselves can in turn directly and indirectly impact the regional gut microbiota. Oral administration of certain conjugated BAs inhibits bacterial overgrowth and restores barrier integrity (Hegade et al., 2016). Recent work revealed activation of FXRα-upregulated mucosal immune factors, specifically in the ileum, which protected against bile duct ligation-induced overgrowth of both anaerobic and aerobic bacteria (Inagaki et al., 2006). Thus, BAs and gut microbiota can profoundly affect enterohepatic circulation to impact host metabolism and immunity.

Specific microbial community members can alter the genetic landscape of the ileum in isolation. Monoassociation of adult GF mice with *Bacteroides thetaiotamicron* significantly influenced expression of genes related to micro- and macronutrient uptake (Hooper et al., 2001). These findings partially explain why GF mice consume more calories daily to maintain the same amount of body weight compared to conventionally-raised counterparts (Bäckhed et al., 2007). In addition, *B. thetaiotamicron* dramatically increases *colipase* expression of ileal crypts and many genes associated with intestinal barrier function, immune responses, and host xenobiotic metabolism. IgA-producing B cells and *Polymeric Immunoglobulin Receptor* (*PIgR*) involved in IgA transcytosis were also elevated following colonization, indicating that both innate and adaptive immune function in the ileum are dramatically impacted by gut microbes (Hooper et al., 2001).

The terminal ileum is an important site for mucosal immunity where the mucus is thinner and Paneth cells produce a wide array of innate immune factors such as AMPs, including cathelicidins, C-type lectins, and defensins. Various microbe-associated molecular patterns (MAMPs) including LPS, peptidoglycan, flagella, bacterial DNA/RNA, fungal cell walls, and Lipid A can induce AMP expression and other mucosal adaptive immune components, such as IgA. Conventionalization of GF mice increases transcriptional and translational levels of AMPs (Natividad et al., 2013), particularly REGIIIγ, a C-type lectin made by both Paneth cells and absorptive enterocytes that exhibits bacteriocidal activity against Gram-positive organisms (Hooper and Macpherson, 2010). Commensal gut microbiota increase REGIIIγ expression (Cash et al., 2006), while HFD-induced microbial communities in conventionally-raised mice decreases expression of AMPs, including *RegIIIγ,* in the ileum (Everard et al., 2014), suggesting an important mechanism of dietary modulation of regional gut immune function.

Microbes are essential to drive both innate and adaptive mucosal immunity of the ileum as well as systemic immune function. For example, CD4+ RORγt+ T helper 17 (Th17) cells, which produce Interleukin (IL)-17A/IL-17F, IL-21, and IL-22, are dependent upon the presence of Segmented Filamentous Bacteria (SFB) (Ivanov et al., 2009). Indeed, GF animals, as well as newborns exhibit very few, if any, Th17 cells within the lamina propria. However, SFB adheres tightly to enterocytes and Peyer's patches in the terminal ileum, a requirement for its immune-mediated stimulation of Th17 cells (Atarashi et al., 2015). Moreover, IL17a expressing RORγt+ T cells were found to be localized to the ileum in response to SFB (Sano et al., 2015). SFB can deter enteric pathogens like *Citrobacter rodentium*, *Salmonella Typhimurium*, or the nematode Nippostrongylus brasiliensis by enhancing Th17-mediate immunity (Stockinger and Omenetti, 2017). The presence of SFB may also mitigate the development of diabetes in the non-obese diabetic mouse model (Kriegel et al., 2011), although it does not appear to affect obesity outcomes following exposure to HF diet (Harley and Karp, 2012).

Circadian rhythmicity aids in maintaining metabolic function and immunity, that can be disrupted based on meal timing or type of diet. Several studies recently revealed a link between the host circadian system and gut microbiota. Gut microbiota, for example, drive rhythmic signaling events downstream of innate immune toll-like receptors (TLRs) within the ileal IEC compartment to regulate molecular circadian clock function (Mukherji et al., 2013). Gut microbiota also exhibit diurnal patterns in community membership and function that provide important inputs into host circadian rhythms and downstream metabolic functions (Leone et al., 2015). Both dietary and host factors are major determinants of microbial membership and their oscillatory properties. The distal gut microbiota (cecum, colon, and feces) also exhibit the diurnal variation that can be affected by circadian disruption by genetic manipulation, induction of jet lag, as well as to time of food intake (Thaiss et al., 2014; Zarrinpar et al., 2014). In the SI, circadian transcription nuclear factor interleukin 3 (NFIL3 or E4BP4) drives immune and metabolic function in a microbe-dependent manner (Wang et al., 2017). Here, ileal IECs from GF mice exhibit reduced expression of *Nfil3* relative to conventionally-raised counterparts. IEC-specific *Nfil3* knockout mice remain lean relative to WT littermates even on HFD, similar to that observed in GF animals. NFIL3 was ultimately shown to contribute to diet-induced obesity via affecting lipid absorption, as *Nfil3* knockout mice had decreased epithelial lipid levels and increased lipids in the stool, overall demonstrating an important microbiota, host circadian, and metabolic interaction in the ileum.

Colon

The colon harbors much greater microbial abundance and diversity relative to the SI, through its unique functions, storage capacity, and physiological role. Despite its shorter length, the colon has a transit time that is considerably longer (>30hr) than the SI (Szarka and Camilleri, 2012). The thicker inner and outer mucus layers are essential barriers against the estimated ~10^{10} to 10^{12} CFUs/ml of (O'Hara and Shanahan, 2006) bacterial phyla such as Firmicutes and Bacteroidetes and families like Lachnospiraceae, Bacteroidaceae, and Prevotellaceae. The colon has functionally distinct regions, where the cecum and proximal regions of the colon are the major sites of fermentation, and the distal colon primarily

extracts fluid and electrolytes (~ 1.3L/day) (O'Hara and Shanahan, 2006). Regional heterogeneity in metagenomic profiles of colonic mucosa-associated microbiota can also be appreciated when unprepped colonoscopy is performed (Wang et al., 2010). Here, proximal and distal colonic microbiota differed in representation by functional subsystems including short chain fatty acid (SCFA) production, primary to secondary BA conversion, and regulation of GI motility.

Strict colonic anaerobes such as Clostridia, Eubacteria, and Roseburia ferment complex carbohydrates and fiber (Koh et al., 2016), producing SCFAs like acetate, propionate, butyrate, and valerate (Sommer and Backhed, 2016) that are mildly acidic 2 to 5 carbon molecules and primary fuel sources for colonocytes. They are actively and passively transported by epithelial cells, but also activate G-protein coupled receptors (GPCRs) in the colon and peripheral tissues. Both butyrate and propionate modulate histone deacetylases (HDACs), which are implicated in immune function and cancer (Koh et al., 2016). SCFAs also enhance anti-inflammatory properties of adaptive immune cells (Tao et al., 2007) and influence a range of physiological processes (MacFabe et al., 2011; Weitkunat et al., 2016).

The cecum and colon are predominant sites for conversion of primary to over 20 types of secondary BAs via a broad range of microbial processes (Ridlon et al., 2016a). While oxidation and epimerization of primary BAs via hydroxysteroid dehydrogenase enzymes are major pathways, conversion of primary glycine or taurine-conjugated BAs to secondary BAs occurs by deconjugation via bile salt hydrolase (BSH). A broad range of bacterial genera harbored in the colon, including *Clostridium, Lactobacillus, Bifidobacterium, Bacteroides*, and enteric pathogens such as *Listeria* encode BSH and are able to perform this function (Gerard, 2014). Depending on the nature of the microbe, BSH activity detoxifies BAs. Colonic BSH deconjugation is followed by 7α/β-dehydroxylation resulting in the formation of deoxycholic (DCA) and lithocholic acids (LCA) predominantly. The BA-inducible gene network that encodes dehydroxylation enzymes were identified in *Clostridium scindens* and *C. hyelomona*e (Kang et al., 2008; Ridlon et al., 2016b). Short-term human consumption of an animal-based diet led to increased DCA abundance in stool that corresponded with increased BSH expression compared to baseline or after plant-based diet consumption (David et al., 2014). Both DCA and LCA have been linked to colon cancer. While DCA can be reconjugated with taurine or glycine in the liver reducing its toxicity, LCA must undergo amino acid conjugation and sulfation, after which it is mainly eliminated from the body in feces (Ridlon et al., 2016b).

Conversion of primary to secondary BAs involves formation of esters via microbially-derived esterification enzymes as well as sulfatase activity. Rodents fed high saturated fat diets derived from milk promoted an expansion of cecal and colonic *Bilophila wadsworthia*, known for its ability to utilize the taurine-derived sulfite as a terminal electron acceptor (Devkota et al., 2012). This expansion corresponded with increased Th1 interferon-gamma producing CD4+ T cells, leading to increased severity of colitis in genetically-susceptible IL-10$^{-/-}$ mice (Devkota et al., 2012). This finding was supported by the aforementioned human study in which consumption of animal-based products increased bile-tolerant *Bilophila, Alistipes*, and *Bacteroides* abundance along with increased gene levels of the bacterial sulfite reductase enzyme (*dsrA*) that reduces sulfite to H_2S (David et al., 2014).

GI motility is impacted by types and functions of microbes harbored in the colon. GF mice have reduced GI motility and increased transit time vs conventional mice. *Ex vivo* stimulation of colonic rings from GF and conventionally-raised counterparts revealed GF colons are more sensitive to carbachol (a cholinergic agonist that stimulates muscle contraction) and KCl, indicating hyper-reactivity (Touw et al., 2017). Constipation induced by Loperamide, which slows intestinal transit, alters gut microbiota composition and functional capacity, which directly affected colonic contraction (Kashyap et al., 2013; Touw et al., 2017). Microbes impact GI motility via SCFA-induced peristalsis, as SCFA treatment of *ex vivo* rat middle to distal colons induced peristalsis via the release of 5-hydroxytryptamine (5-HT, serotonin) (Grider and Piland, 2007). Furthermore, tryptophan production by spore-forming gut microbes associated with a healthy gut can increase 5-HT production by colonic enterochromaffin cells, to stimulate GI motility (Yano et al., 2015). Together, these results highlight the bi-directional communication between host and microbe in a region-specific manner to regulate motility.

Region-specific gut microbiota and disease

The regional tropism of GIT microbiota can be a determinant of specific diseases. For instance, gastric *Helicobacter pylori,* can promote peptic ulcer diseases and gastric cancer in humans, whereas different species like *Helicobacter hepaticus* can cause colitis in susceptible mice (Fox et al., 2011). Similarly, severe perturbations in the colonic microbiota and niche conditions (e.g. increased primary bile acids) caused by broad spectrum antibiotics favor the germination and bloom of *Clostridium difficile,* and development of colitis.

1. Diseases of the Mouth, Esophagus, and Stomach

Dental caries and periodontal disease are influenced by periodontal pathogens including *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Actinomyces israelii* (Shim et al., 2018). Saccharibacteria which is plentiful in dental and supragingival plaque formation may also contribute to periodontal disease (Liu et al., 2012).

Esophageal microbiota were previously overlooked as having a role in the development of esophageal diseases. However, their role in disorders such as Barrett's esophagus, eosinophilic esophagitis, esophageal adenocarcinoma, and esophageal squamous cell carcinoma are now being revisited (reviewed in May and Abrams, 2018). Fecal microbiota from HFD-fed L2-IL1B mice , a mouse model of Barrett's esophagus, increased tumor development when transferred into LFD-fed GF mice, suggesting a causal role for microbes in esophageal tumor development (Münch et al., 2019). However, since fecal microbiota were used in this study, the precise role of esophageal microbes in this disease remains unclear.

Gastric acid creates a unique niche for *H. pylori*, which was one of the most prevalent human indigenous microbes due to its ability to thrive under such conditions. Prior to improved hygiene, many individuals benefited from *H. pylori* through enhanced induction of gastric acid and mucosal immune mechanisms capable of deterring many pathogens. However, as hygiene improved and life span lengthened across societies, the negative

consequences of indigenous *H. pylori* became apparent, e.g. peptic diseases and cancer (Nardone and Compare, 2015). In addition, immunological tolerance to *H. pylori*-induced T cell responses has been shown to increase bacterial levels by ten-fold in murine neonates (Fung et al., 2019), potentially affecting digestion and absorption and susceptibility to other pathogens.

2. The Small Intestine: Small Intestine Bacterial Overgrowth (SIBO), Bariatric Surgery, Undernutrition, and Celiac Disease

SIBO caused by decreased small bowel motility, impaired host immune functions, and reduced gastric acid barrier, is characterized by a bacterial load of 10^5 or 10^6 CFU/ml as opposed to normal levels of 10^3/ml (Dukowicz et al., 2007). In addition to maldigestion and malabsorption, SIBO can contribute to several other conditions including irritable bowel syndrome, celiac disease, Crohn's disease, nonalcoholic steatohepatitis, and obesity. The stasis associated with SIBO creates conditions that favor colonization by microbes may not be indigenous to the region (e.g. colonic-like), potentially resulting in overgrowth of microbes that compete for nutrients, perturb critical commensal microbe-host interactions, and/or promote mucosal inflammation (reviewed in Bohm et al., 2013).

Restrictive and bypass bariatric surgery, e.g. roux-en-y gastric bypass (RYGB), sleeve gastrectomy, gastric banding and biliopancreatic diversion with duodenal switch (BPD/DS) promote weight loss by altering small bowel anatomy and function, but also likely set into play other mechanisms that change the state of host metabolism. For example, these procedures alter small bowel microbiota which can directly prevent diet-induced obesity (Liou et al., 2013). Similarly, dramatic changes in small bowel microbiota were observed following biliary diversion to the jejunum or ileum were associated with prevention of diet-induced obesity in mice (Pierre et al., 2016). These effects are due to the direct impact of bile acids on the host (e.g. through FXR activation) or through bile-mediated alterations in the gut microbiota.

Undernourished children in Bangladesh present with a condition known as environmental enteropathy (Petri et al., 2014) characterized by small intestinal villus blunting and inflammation with an associated immature microbiota. GF mice receiving fecal microbiota transplant (FMT) from growth-stunted vs healthy infants fed a Malawian diet displayed impaired growth, while cohousing with mice given healthy donor microbiota prevented growth impairments, implicating a causal role for microbiota in undernutrition (Blanton et al., 2016). Supplementing with ready-to-use therapeutic food only partially restored metabolic disruptions (Smith et al., 2013). Similarly, microbe accessible carbohydrate (MAC)-deficient diets led to the generational loss of commensal microorganisms. However, replenishment of MAC did not restore the abundance of depleted microorganisms, which were only restored by conventionalization (Sonnenburg et al., 2016). Notably, both groups examined stool microbiota, which begs the question whether "missing" regional microbiota might be better in restoring SI digestive and absorptive functions.

Celiac disease (CD) is a chronic enteropathy of genetically-prone patients expressing the MHC molecules HLA-DQ2 or DQ8, which is triggered by the dietary component gluten found in wheat, rye, and barley (Kim and Jabri, 2015). Chronic mucosal inflammation can

cause long-term damage to the gut epithelium leading to maldigestive and malabsorptive issues. While HLA-DQ haplotype is a requirement, additional factors likely play a role because only 2 to 5% of HLA-DQ2/DQ8 individuals develop CD. Among these, changes in small bowel microbiota, resulting from altered immune states associated with HLA-DQ haplotype, have been implicated. While some studies suggest an expansion of proinflammatory SI microbiota, others have posited that the loss of key microbes important for proper immune development plays a role (reviewed in Verdu et al., 2015). Regional biopsy samples from duodenum and jejunum have revealed changes in the local gut microbiota of patients with active CD. For instance, rod vs. cocciform bacteria were identified adhered to the mucosa in distal duodenum/proximal jejunal biopsies collected from children with untreated CD, treated CD, oral gluten-challenged CD and controls. Mucosally-adhered rod-shaped bacteria increased in untreated and oral gluten-challenged CD patient biopsies relative to controls and treated CD subjects (Forsberg et al., 2004). Several studies have also shown increases in duodenal Proteobacteria in CD subjects relative to controls (Sánchez et al., 2013; Wacklin et al., 2014), while others have shown that SCFAs are dramatically decreased in active CD patients, which can be restored following a year's long adherence to a gluten-free diet (Tjellström et al., 2013).

Interleukin-15 (IL15), a cytokine made by IECs, is often upregulated in CD (Abadie and Jabri, 2014). In mice that overexpress IEC IL15 (IL15tg), gut microbiota are affected throughout the intestine, with the most pronounced differences observed in the ileum (Meisel et al., 2017). Relative abundance of butyrate-producing bacteria and butyrate levels were significantly decreased. While the IL15Tg mice did not develop overt CD, these findings showed that altered IL-15 expression can significantly alter microbial community membership and function, possibly contributing to CD progression.

3. Colonic Diseases: Inflammatory Bowel Diseases and C. difficile-induced colitis

Inflammatory bowel diseases (IBD) are complex immune disorders that arise from convergence of host genetic, gut microbial, and environmental factors. Regionality and topical nature of lesions are hallmarks of the diseases and suggest that local factors (microbe and microbial products) are germane to their development. Ulcerative colitis (UC), one of the clinical IBD phenotypes, only involves the colon, usually starting at the rectum and proceeding proximally in a confluent mucosal inflammatory front, suggestive of propagative processes. In individuals with left-sided UC, a clear line of delineation between diseased and non-diseased regions is often seen. Here the differences in mucosa-associated microbiota are considered an important underlying factor causing or contributing to this phenomenon. Similarly, Crohn's disease, another clinical phenotype of IBD, can involve any part of the GI tract, from mouth to anus. The typical starting lesion, an apthous ulcer, starts at the mucosal surface and as the disease progresses, the lesions widen and deepen, with the latter capable of penetrating the bowel wall. Yet, tissue surrounding the Crohn's mucosal lesion is endoscopically and histologically normal. A few studies (Hirano et al., 2018; Walker et al., 2011) have attempted to map differences in taxonomical profiles of involved and non-involved mucosal regions in IBD patients based on 16S rRNA amplicons. While differences have been reported, it remains unknown whether they are a cause or an effect and what their functional significance might be. Crohn's involving the terminal ileum is often associated

with a perturbed bacteria populations and increased fungal load (Liguori et al., 2016). With regard to the latter, many patients exhibit serum levels of anti-Saccharomyces cerevisiae antibody (ASCA), which is directed against the oligo-mannin component of the cell wall of polymorphic fungi such as *Candida albicans*. Antibody levels to other microorganisms including *Ecsherichia coli* and *Pseduomonas fluorescens* can also be seen (Mitsuyama et al., 2016).

The obligate spore-forming Gram-positive anaerobe, *Clostridium difficile,* is the major cause of recurrent antibiotic-induced toxin-mediated colitis (reviewed in Libertucci and Young, 2019). While *C. difficile* has been observed at low levels in healthy humans, particularly infants, antibiotic-induced disruption of the indigenous colonic microbiota and resulting increases in colonic primary bile acid concentrations promote its outgrowth, toxin production, and invasion (Seekatz and Young, 2014). The latter is important for germination of *C. difficile* spores (Theriot et al., 2016), while production of specific secondary BAs can inhibit this process (Libertucci and Young, 2019). Commensal microbes, such as *C. scindens* and *C. sordellii*, which have the capacity to perform 7α-dehydroxylation play a role in inhibiting outgrowth of pathogenic *C. difficile* through secretion of antibiotic-like molecules derived from tryptophan metabolism (Kang et al., 2019). The actions of these molecules are further enhanced by the presence of secondary BAs, DCA and LCA. First line treatment of *C. difficile* colitis continues to be with antibiotics such as metronidazole or vancomycin, but FMT from a healthy donor is becoming increasingly used for recurrent *C. difficile* infection. Following therapeutic FMT, increased levels of DCA, LCA and SCFAs, even after 6 months, are observed and attributed to the expansion of families like Lachnospiraceae, Ruminococcaceae, and Clostridiales (Seekatz et al., 2018).

On the horizon: Next Generation technologies and approaches to navigate the unknown

The human gut microbiota from stomach to distal ileum (regions difficult to sample) remain largely unexplored. What can be inferred from animal studies has been useful to gain conceptual insights, but human microbiota in these regions are likely very different in speciation, composition, and function. The technical challenges and confounders associated with human studies of this domain are formidable.

Sampling of the upper GI is invasive and limited to disease settings where endoscopic examination is indicated. Thus, information of “healthy” upper GIT microbiomes is very limited. One possible non-invasive approach is to sample patients with ileostomies, but even then, their underlying disease could be a confounding factor. Non-invasive approaches are being developed such as the esophageal string test, inflatable balloons covered in cotton mesh and attached to a rubber tube, or the Cytosponge, which is a tethered capsule that dissolves in the stomach to release a 3cm mesh screen that is then pulled back through the mouth (described in May and Abrams et al. 2018). The limitation to these methods is potential contamination with oral microbes upon retrieval. Additionally, swallowed bio-sampling capsules programmed or triggered to sample luminal content during passage

through the GIT are under development (Nakamura et al., 2017), although they have currently no capacity to sample the more stable mucosa-associated regional microbiota.

Other challenges to consider are those generic to the study of microbial communities in general. The field has relied heavily on 16S rRNA amplicon sequencing which provide limited, if any, functional information. Metagenomics and metatranscriptomics on regionally-collected samples could provide more information, but are often difficult to perform because of limited biomass and heavy contamination by human DNA and transcripts. Resolution of these data to identify genomes and functions of specific microbial strains or consortia is a major hurdle, although headway is being made through newer metagenome-assemble genome approaches (Eren et al., 2015). Matrix-assisted laser desorption ionization time-of-flight mass-spectrometry (MALDI-TOF) coupled to culturomics and sequencing based approaches has also been successfully used to isolate and identify single strains in human fecal samples in the clinical microbiology setting (Seng et al., 2013; Stevenson et al., 2010). Development of microfluidic cultivation systems (Liu and Walther-Antonio, 2017) has allowed for more rapid isolation and characterization of previously non-cultivable strains of microbes (Ma et al., 2014). Others have since coupled high-throughput microfluidic droplet platforms that allow for single cell assessments to gain information about functional parameters of individual microbial community members (Terekhov et al., 2018). These approaches applied to regional samples may aid to identify and characterize novel strains that are important to critical host-microbe interactions.

There is also the need to understand organizational structure of regional microbial communities of the gut. Confocal microscopy combined with immunofluorescence can provide information that complements more standard approaches using FISH (Tropini et al., 2017). The Sonnenburg group showed that fluorescently-tagged Bacteroides strains were useful to gain insight into the dynamic interactions between mucosally-associated bacteria and the host in different intestinal regions (Whitaker et al., 2017). Engineering bacterial sensors through synthetic biology has allowed for both detection and tracking of specific events within the intestine (Kotula et al., 2014). Similar approaches have been used to engineer bacteria, particularly strains of *E. coli*, that can sense and/or modulate the environment in a potentially therapeutic manner (Kurtz et al., 2019; Tscherner et al., 2019), a few of which have already passed Phase I trials in humans. Refinement of these tools and identification of novel targets could be accomplished using swallowable devices that can be made to detect important host or microbial factors.

To identify and probe mechanisms behind region-specific host-microbe interactions, an expansion on *ex vivo* and *in vitro* model systems is required. For instance, organoids (intestinal epithelial organoids) can be derived from differentiated cells obtained via biopsy (from human) or at the time of tissue harvest (mice) from varying regions of the gut, or they can be made from human intestinal organoids (HIO) (Bartfeld and Clevers, 2017) derived from iPS cells/embryonic stem cells. Both 2- and 3-dimensional organoids can be treated with either conditioned media (Martinez-Guryn et al., 2018; Wang et al., 2017) from individual organisms or micro-injected with live organisms to examine how they impact regional specific epithelial-based pathways involved in macro-nutrient uptake or inflammation (Leslie and Young, 2016). These approaches, combined with additional tools,

such as gut-on-a-chip (Kim and Ingber, 2013), co-culture experiments with immune cell populations procured from regional mucosa, and/or bioreactors could provide novel insight into regional specific host-microbe interactions in health and disease.

The future: Things are only impossible until they are not

Much remains to be discovered, among them how to define healthy versus unhealthy microbiomes of the gut, which is likely to be determined by host, microbial, and environmental factors. An important step towards this goal is to gain a better understanding of the regional organization and functions of gut microbial communities along the GI tract and their relevance to conditions of health and disease. While there are many challenges on the horizon, particularly in studies of human subjects, great promise lies ahead to advance the field through new approaches and next generation technologies002E

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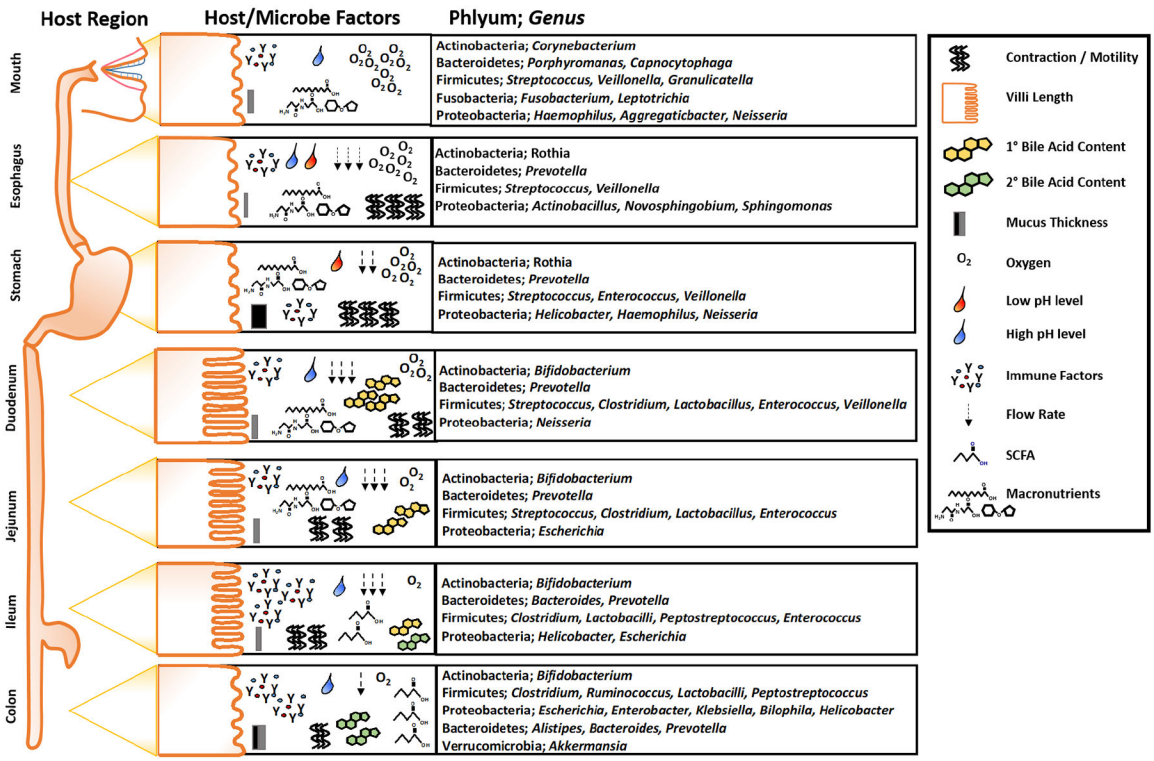

Figure 1. Representative host and microbial factors and community membership along the rostral to caudal (horizontal) axis of the GI tract.

Different regions of the GI tract perform unique functions in regards to macro- and micronutrient digestion and absorption. These unique features along with a number of host derived factors, such as epithelial cell types and surfaces, mucus thickness, motility and contractility, pH, oxygen tension, and flow rate drive diversity and abundance of gut microbes from mouth to anus. Additionally, each area of the intestine secretes unique immune factors that can interact with the intestinal microbiota, shaping the community membership in a region-specific manner. Several host factors secreted into the intestine, such as primary bile acids (BAs), are important for digestion, but can also elicit direct antimicrobial effects i.e., primary vs. secondary BAs. Other regions of the intestine, due to pH and oxygen tension support microbial fermentation of complex fiber sources, resulting in an increasing abundance of short chain fatty acids in the distal portions of the GI tract that not only impact local epithelial cells, but further influence the regional microbial communities and distal tissue sites important for health and disease.