



Exploring the impact of intestinal ion transport on the gut microbiota

Amy C. Engevik^{a,*}, Melinda A. Engevik^b

^a Department of Surgery, Vanderbilt University Medical Center, Nashville, TN, USA

^b Department of Regenerative Medicine & Cell Biology, Medical University of South Carolina, SC 29455, USA



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ABSTRACT

The gut microbiota and the host are intimately connected. The host physiology dictates the intestinal environment through regulation of pH, ion concentration, mucus production, *etc.*, all of which exerts a selective pressure on the gut microbiota. Since different regions of the gastrointestinal tract are characterized by their own physicochemical conditions, distinct microbial communities are present in these locations. While it is widely accepted that the intestinal microbiome influences the host (tight junctions, cytokine/immune responses, diarrhea, *etc.*), the reciprocal interaction of the host on the microbiome is under-explored. This review aims to address these gaps in knowledge by focusing on how the host intestinal ion transport influences the luminal environment and thereby modulates the gut microbiota composition.

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Abbreviations: GI, gastrointestinal; NHE3, sodium-hydrogen exchanger isoform 3; NHE2, sodium-hydrogen exchanger isoform 2; CFTR, cystic fibrosis transmembrane regulator; Cl⁻, chloride channel; DRA, down-regulated in adenoma; GLUT2, glucose transporter 2; SGLT1, sodium glucose co-transporter 1; NKCC1, Na⁺-K⁺-2Cl⁻ co-transporter; ENaC, epithelial Na⁺ channel; OTUs, operational taxonomic units.

* Corresponding author.

E-mail address: engevika@musc.edu (A.C. Engevik).

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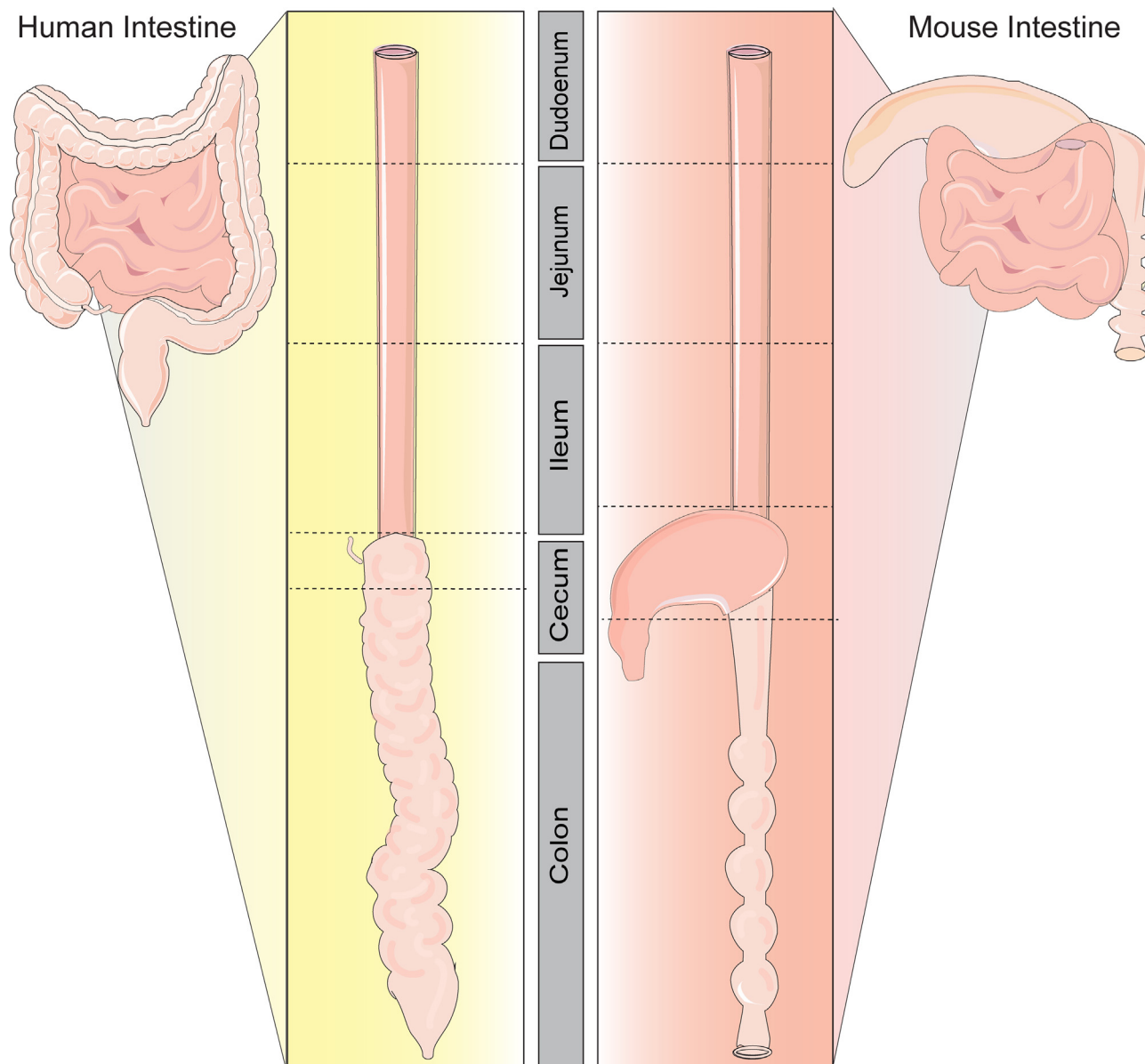


Fig. 1. Graphical representation of the intestinal tract of humans and mice. Both human and mouse intestine can be separated into small and large intestine (also known as colon). Humans and mice have 3 segments of the small intestine: duodenum, jejunum and ileum. The cecum of humans is a small pouch at the beginning of the colon and is connected to the appendix. The cecum of mice is a large fermentation region that is distinct from both small and large intestine. In both humans and mice, the colon is segmented into different regions with distinct structures and function.

1. Intestinal architecture and function

The gastrointestinal tract is essentially a series of hollow interconnected organs lined by epithelial cells which performs multiple functions including digestion, absorption of water, uptake of nutrients, immune tolerance/homeostasis, regulation of the intestinal environment, and maintenance of the gut microbiota [2]. The architecture of the gastrointestinal tract facilitates these numerous functions. The intestine is divided length wise based on location (Fig. 1). In mammals, the small intestine consists of the duodenum, jejunum and ileum, while the large intestine consists of the colon. In humans the small intestine is approximately (~) 6 m long. In mice the small intestine is approximately ~350 mm [3]. In terms of structure, the small intestine consists of a single layer of columnar epithelium with crypts and villi. The crypts of Lieberkuhn harbor the proliferating stem cells, and finger-like villi contain the majority of differentiated absorptive cells [4,5]. The adult small

intestinal epithelium is composed of different cell lineages: absorptive enterocytes (~90% of cells), mucus-secreting goblet cells (~5%), antimicrobial secreting Paneth cells (~3%), hormone secreting enteroendocrine cells (~1%), chemo-sensing tuft cells (<1%) and proliferative stem cells (~1%) [5–10]. Enterocytes, goblet cells and enteroendocrine cells are located in the intestinal villi, while Paneth and stem cells are located in the crypts [6,7,11,12]. Proliferation of the stems cells results in renewal of the epithelium every 3–6 days in humans [9] and ~2–3 days in mice [13].

The different regions of the intestine have unique functions. The duodenum begins at the base of the stomach's pyloric sphincter. A major function of the duodenum region is to neutralize the acidic chyme, or partially processed food material, from the stomach and enzymatically breakdown food. Enzymes from the pancreas and duodenum aid in digesting proteins and starches as well as emulsifying fats. The duodenum is also the site of amino acid, fatty acid, monoglycerides, phosphorus, and mono and/or disaccharides,

iron, calcium, vitamin A, vitamin B12, and water absorption [5]. The jejunum is adjacent to the duodenum and is a site for the absorption of amino acids, fatty acids, oligosaccharides, minerals, electrolytes, vitamins, and water [5]. The final section of the small intestine is the ileum, which is responsible for absorption of bile salts and fats, as well as vitamin B12 and water.

The large intestine, also known as the colon, is largely responsible for reclaiming electrolytes and water. The colon in humans is ~1.5 m in length [5], while the colon in mice is ~110 mm [3]. Similar to the small intestine, the colon can be further subdivided based on anatomic divisions. In humans, the colon is divided into the ascending colon, transverse colon, descending colon, sigmoid colon, rectum, and anus [5]. In mice, the colon is commonly divided into proximal, mid and distal colon [14]. In contrast to the small intestine, the large intestine consists of crypts without villi [5,9]. These crypts are rapidly renewed by stem cells at the crypt base [15]. Colonic epithelial cells include absorptive enterocytes at the top of the crypts and mucus producing goblet cells that line the colonic crypts. Mucus is critical in the colon as it provides lubrication for the feces and protects the underlying epithelium and immune cells from interacting with luminal antigens.

Although considerable anatomical and physiological features of the intestinal tract are shared between humans and mice, a key

distinction is the cecum. In humans, the cecum is 6 cm and of minor importance for intestinal homeostasis [16]. In mice, the cecum is large, being 3–4 cm in length, and functions as a microbial fermentation vessel. It has been speculated that in mice, the cecum functions to restore the colonic microbiome after insult. In terms of size, the human cecum per kg of body weight roughly calculates to 0.09 cm per kg, while in mice, the cecum is 175 cm per kg body weight [16]. These calculations illustrate that, relative to body weight, the cecum is a much larger organ in mice than in humans. Since the cecum serves very different functions in mice versus humans, acting as a bioreactor for microbes in mice and serving as a reservoir for luminal contents passing from the small to large intestine in humans, this review will only focus on similar anatomical and functional segments (small and large intestine) in relationship to the microbiome in mice and humans.

2. Intestinal ion transport

The combination of digestion, nutrient absorption, water absorption, ion absorption and/or secretion determines the pH and ion composition of intestinal fluid and sets the environmental conditions for the growth of the intestinal microbiota [14,17–21].

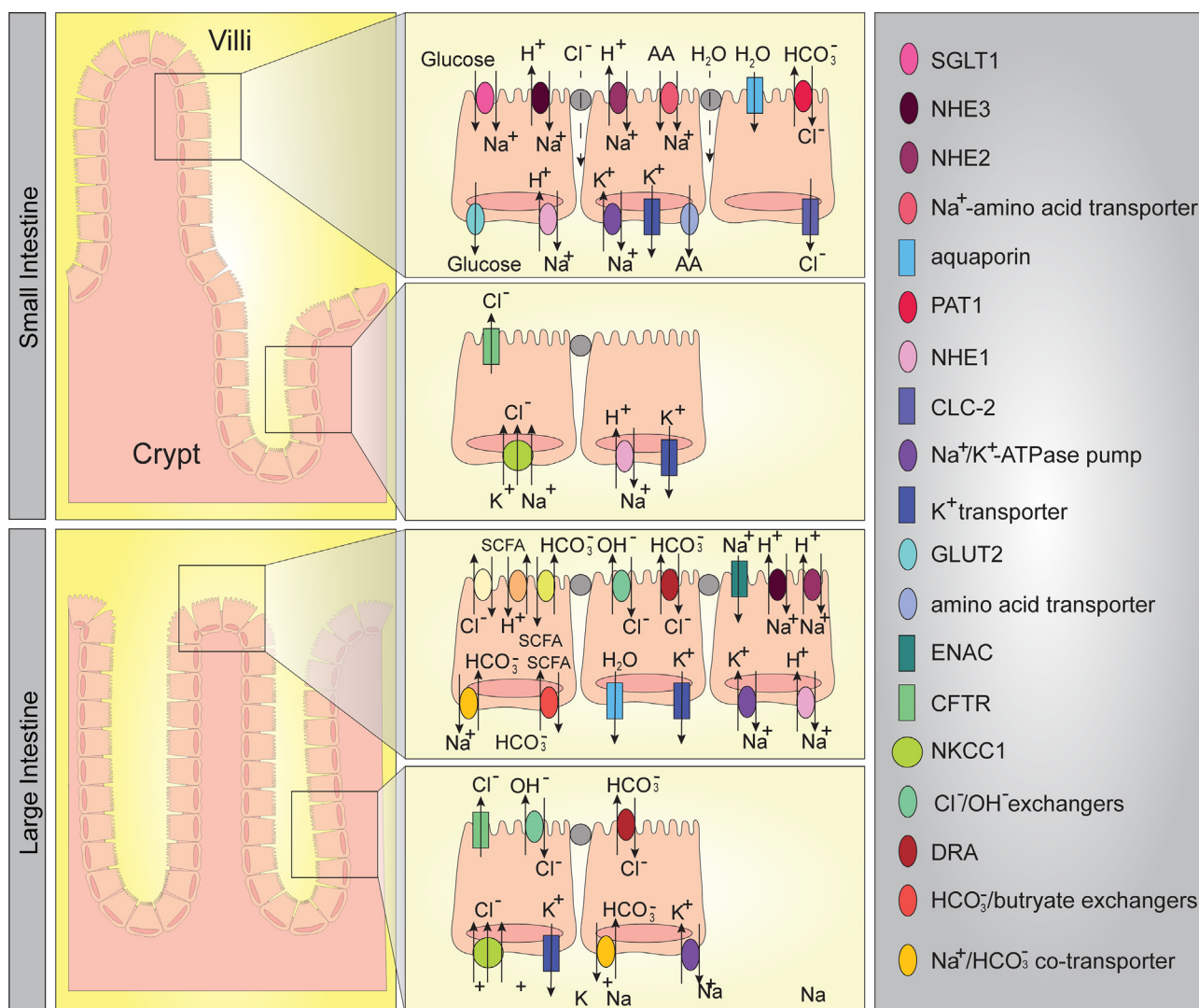


Fig. 2. Graphical representation of intestinal ion transport. Transport mechanisms in the small intestine and colon are depicted. As shown, ion transporters are differentially distributed, creating unique microenvironments. These transporters are present in mouse and human.

Overall, intestinal ion transport is characterized by net absorption of NaCl, nutrients and water and net secretion of bicarbonate (HCO_3^-) and KCl [22,23]. The opposing functions of absorption and secretion are accomplished by transporters located in cells in both villus and crypt regions (Fig. 2). In general, cells in the villus facilitate absorption, while cells in the crypts promote secretion [22–26]. Transporters are found at both the apical (facing the lumen) and basal (facing the blood) membranes, allowing for transport in both directions [22].

Small intestinal fluid absorption predominantly occurs via the net movement of Na^+ . Na^+ absorption is accomplished by either direct absorption of Na^+ by Na^+/H^+ Exchangers (NHEs) or through coordination of Na^+ absorption with other components such as glucose, transported by Na^+ -glucose co-transporter 1 (SGLT1) [17–19,23]. In the intestine, there are three major NHEs: NHE1, NHE2 and NHE3. Apical NHE3 plays a major role in Na^+ absorption, pH and cell volume regulation [23,27,28], while NHE2 appears to contribute to pH regulation and only minimally in Na^+ absorption [17,19,21,29]. Basolateral NHE1 also participates in volume and pH regulation. In addition to Na^+ and water, nutrients such as glucose and amino acids are primarily absorbed in the small intestine. Glucose enters the enterocyte via apical SGLT1 and exits enterocytes via the basolateral glucose transporter 2 (GLUT-2) [23]. Amino acids enter the enterocyte via apical Na^+ -coupled and/or H^+ /dipeptide co-transporters and exit via various basal amino acid transporters.

In contrast to absorption, secretion in the small intestine involves the basal Na^+/K^+ pump, which drives the uphill entry of Cl^- by the basal $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ co-transporter (NKCC1) [30]. Secretion is also supported by basal K^+ channels, which repolarize the cell and maintain the driving force for Cl^- exit. At the apical surface, Cl^- can exit the enterocyte by Cystic Fibrosis Transmembrane Regulator (CFTR, family Cl^- channels (CIC)), and/or the Ca^{2+} -activated Cl^- channels (CICA) [22,31–33]. In addition to Cl^- secretion, enterocytes also actively secrete bicarbonate, which plays a critical role in defining the intestinal pH. In the duodenum in particular, bicarbonate secretion is crucial for luminal alkalization. Bicarbonate secretion is accomplished by the anion exchanger down-regulated in adenoma protein (DRA) [34] and the putative anion transporter PAT1 [35]. Bicarbonate secretion via DRA and Cl^- secretion via CFTR are also essential for mucus excretion from goblet cells [36,37].

Compared to the small intestine, there are far fewer nutrient transporters in the colon (Fig. 2). In contrast to the small intestine where Na^+ and glucose drive water absorption, in the colon water absorption is only driven by Na^+ absorption [23]. The proximal segments of the colon express NHE2 and NHE3, which contribute to the absorption of residual water and maintenance of colonic pH. The more distal segments of colon can also express NHE2, NHE3, but their expression is lower than the proximal colon. Additionally, the distal colon expresses the epithelial Na^+ channel (ENaC) [22,33]. The colon also harbors secretory transporters. The bicarbonate transporter DRA is predominantly expressed in the distal colon [14,23]. In mice, NHE3 is highly expressed in the proximal colon with decreasing expression in the distal colon, while DRA is highly expressed at the most distal end of the colon with decreasing expression toward the proximal colon [14]. The specificity of these ion transporters likely reflects specific intestinal environment regulation and dictates potential environmental changes. The colon also secretes and absorbs K^+ by apical K^+ transporters and the H^+/K^+ -ATPase [23]. Collectively, the balance of absorption and secretion work in concert for the proper absorption of nutrients and water, as well as maintaining the proper intestinal ion composition and pH. This absorption/secretion balance produces feces low in salt, nutrients and water content (<2%). When this absorption/secretion balance is disrupted, diarrhea ensues

[23]. Together these ion transporters regulate the intestinal environment of the small and large intestine.

3. Mouse and human gut microbiomes

In both humans and mice, the gut microbiota is dominated by two major phyla: Firmicutes and Bacteroidetes [38–42]. Although mice and humans appear to harbor similar bacterial communities at the high-taxonomic levels (phyla, class, order), they differ at the lower-taxonomic levels (genera, species, subspecies) [43–47]. A comparison of fecal 16S rDNA data from four public datasets from healthy adults [48,49] and five murine studies [50–54] by Nguyen *et al.* revealed that mice and humans harbor 79 shared genera [55]. *Clostridium* (Firmicutes), *Bacteroides* (Bacteroidetes) and *Blautia* (Firmicutes) were found in both humans and mice at similar relative abundance. However, in human samples, *Prevotella* (Bacteroidetes), *Faecalibacterium* (Firmicutes) and *Ruminococcus* (Firmicutes) were found in high abundance, while *Lactobacillus* (Firmicutes), *Alistipes* (Bacteroidetes) and *Turicibacter* (Firmicutes) were more abundant in mice [55]. Other studies have identified differences between *Mucispirillum schaedleri* (Deferribacteres) [56] and segmented filamentous bacteria (Firmicutes) [57–61], which both appear higher in mice than humans. Despite lower-taxonomic differences, the microbiota of both humans and mice share similar metagenomic core functions [44,45,48,62–64]. In mouse and human gut microbiome cores, 25 genera have been identified as shared [63]. Moreover, almost 80% of annotated functions were found in common between the mouse and human microbiome, indicating significant functional overlap in microbiome function.

A confounding factor in studying the microbiome of mice and humans is the techniques employed by each individual study [65,66]. Common techniques include 16S rRNA sequencing and shot-gun metagenomic approaches. 16S rRNA gene sequencing employs PCR to target and amplify portions of the hypervariable regions (V1–V9) of the bacterial 16S rRNA gene. Amplicons are then given molecular barcodes, pooled together, and sequenced. Raw sequencing data undergoes trimming, error correction, and comparison to a 16S reference database which assigns phylogenetic rank to reads. In contrast with 16S sequencing, which only targets 16S rRNA genes, shotgun metagenomic sequencing sequences all the genomic DNA. The workflow for library preparation is similar to regular whole genome sequencing. Similar to 16S sequencing, shotgun metagenomic sequencing also includes quality trimming and comparison to a reference database comprising whole genomes or marker genes to generate a taxonomy profile. Since shotgun metagenomic sequencing covers all genetic information, the data can be used for additional analyses, e.g. metabolic function, antibiotic resistance, metabolite prediction, *etc.* A major difference between 16S and shotgun metagenomics is the taxonomic resolution: 16S is limited to the genus level, while shotgun can obtain high resolution at the species and strain level. For both 16S sequencing and shotgun metagenomic sequencing, multiple reference databases exist, as well as different versions of each database, and these databases may generate different taxonomy assignment; leading to potential differences in microbiome output. Another consideration is sequencing depth. Jovel *et al.* randomly sampled libraries at depths of 500, 1000, 5000, 10,000, 50,000, and 100,000 to investigate the minimal sequencing depth sufficient for accurately profiling bacterial community composition in stool samples [66]. The authors found that sequencing depths of 1000 and 50,000 were remarkably consistent, but the assignments of some bacteria required increasing sequencing depth to augment artifacts. A recent study comparing 16S rRNA gene-based analyses to shotgun metagenomic sequencing identified that many aspects

of bacterial community characterization were consistent across methods [67]. In this study, Rausch *et al.* found that single-step amplification of the V3-V4 region yield more comparable results to shotgun metagenomics than multi-step amplification and use of the V1-V2 region of the 16S rRNA gene [67]. Based on these studies, it is evident that the microbiome composition of both mouse and human studies rely in part on the sequencing methodologies and thus information should be interpreted with caution. Despite these variables, it has been speculated that mouse microbiome studies can yield valuable information on the potential function of the microbiome in the setting of disease.

4. The gut microbiome composition along the length of the intestine

The gastrointestinal tract harbors diverse and dynamic microbial communities [68–71]. In general, the gut microbiota is dominated by the phyla Firmicutes and Bacteroidetes, with Proteobacteria, Actinobacteria, Fusobacteria and Verruomicrobia present in lower abundance [39,44,72–76]. The composition of these microbial communities differs based on intestinal locations (duodenum, jejunum, ileum, colon) as well as proximity to the host (luminal vs mucosa-associated) [14,19,21,45,77–84]. The differences in microbiota composition reflect the differences in the local environments: the microbiota is exposed to varying pH conditions (acidic stomach and alkaline intestine), various ion concentrations, intestinal motility, redox potential, nutrient supplies, and host secretions (e.g. hydrochloric acid, digestive enzymes, bile juice, pancreatic secretion and mucus) [1,85–87].

The microbiota increases in number and diversity from the stomach to the colon, with the colon being the most densely populated region [86,88]. The Human Microbiome Project (HMP) and the Metagenomics of the Human Intestinal Tract (MetaHIT) initiatives have helped define the healthy human microbiome and identify region specific microbial ecosystems [89,90]. Based on these initiatives and current literature, we know that the small intestine commonly harbors *Streptococcus* (Firmicutes), *Lactobacillus* (Firmicutes), *Clostridium* (Firmicutes), and *Prevotella* (Bacteroidetes) [91–93]. Analysis from luminal contents along the length of the small intestine in two separate studies has revealed a dominance of *Streptococcaceae* (Firmicutes, ~55% of sequences) in the duodenum and jejunum followed by *Veilonellaceae* (Firmicutes, ~35%), and *Lactobacillaceae* (Firmicutes, ~5%), with lower abundance of *Lachnospiraceae* (Firmicutes), *Clostridiaceae* (Firmicutes), *Erysipelotrichaceae* (Firmicutes), *Pasteurellaceae* (Proteobacteria) and *Prevotella* (Bacteroidetes) [94,95] (see graphical representation in Fig. 3, Table 1). In contrast, the feces of these healthy individuals were dominated by *Ruminococcaceae* (Firmicutes), *Blautia* (Firmicutes), *Bifidobacteria* (Actinobacteria), and *Lachnospiraceae* (Firmicutes) (~35%). Analysis of ileal luminal contents from a separate study has shown that the ileum harbored high levels of *Bacilli* (which include *Streptococcus* and *Lactobacilli*, Firmicutes, ~60%), followed by *Bacteroides* (Bacteroidetes, ~10%) and *Clostridium* (Firmicutes, ~25%) [96]. These findings are consistent with ileal swabs which also confirmed the dominance of *Streptococcus* (Firmicutes, ~60%), followed by *Lactobacillus* (Firmicutes) and other microbes [97]. In one study, the pH of the luminal contents was correlated with microbial operational taxonomic units (OTUs) [95]. In this study, Seekatz *et al.* demonstrated that 15 OTUs significantly correlated with pH changes. Six OTUs classified as Bacteroidetes, mainly

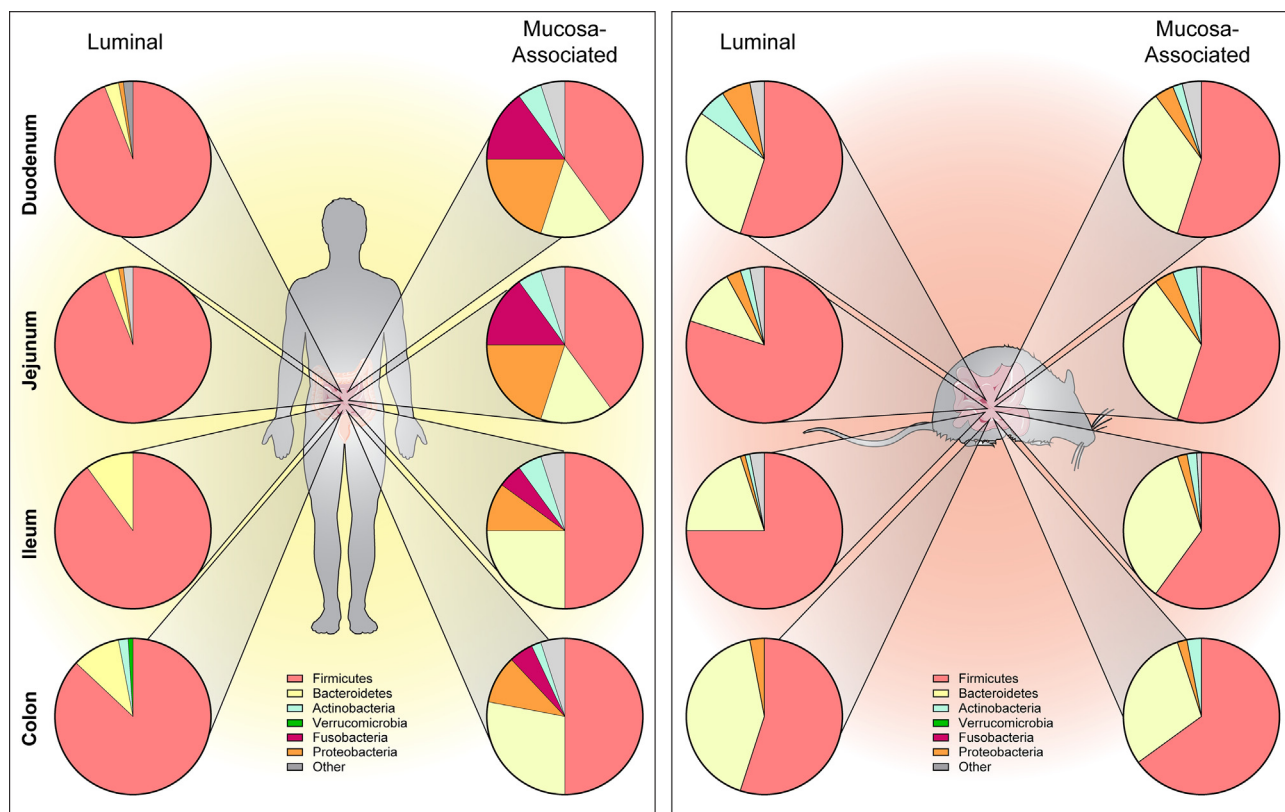


Fig. 3. Graphical representation of the microbiome composition along the length of the intestine. Pie graphs depict the relative abundance of the major phyla (Firmicutes (pink), Bacteroidetes (yellow), Actinobacteria (blue), Verrucomicrobia (green), Fusobacteria (fusia), Proteobacteria (orange) and other (grey)). Composition is noted for both the luminal population as well as the mucosa-associated microbiome for human and mouse. Composition is approximated from published literature. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1

Bacteria that are commonly identified in human luminal and mucosa-associated microbiota from various intestinal segments.

Luminal Microbiota				Mucosa-associated Microbiota		
Small Intestine		Feces		Small Intestine	Ileum	Colon
Duodenum	Jejunum	Ileum	Colon	Duodenum	Ileum	Colon
<i>Streptococcaceae</i>	<i>Streptococcaceae</i>	<i>Streptococcus</i>	<i>Ruminococcaceae</i>	<i>Bacillales</i>	<i>Lachnospiraceae</i>	<i>Lachnospiraceae</i>
<i>Veillonellaceae</i>	<i>Veillonellaceae</i>	<i>Lactobacilli</i>	<i>Blautia</i>	<i>Streptococcaceae</i>	<i>Bacteroidaceae</i>	<i>Bacteroidaceae</i>
<i>Lactobacillaceae</i>	<i>Lactobacillaceae</i>	<i>Bacteroides</i>	<i>Bifidobacteria</i>	<i>Veillonellaceae</i>	<i>Ruminococcaceae</i>	<i>Ruminococcaceae</i>
<i>Lachnospiraceae</i>	<i>Lachnospiraceae</i>	<i>Clostridium</i>	<i>Lachnospiraceae</i>	<i>Pseudomonadaceae</i>	<i>Enterobacteriaceae</i>	<i>Enterobacteriaceae</i>
<i>Clostridiaceae</i>	<i>Clostridiaceae</i>			<i>Fusobacteriaceae</i>	<i>Fusobacteriaceae</i>	<i>Fusobacteriaceae</i>
<i>Erysipelotrichaceae</i>	<i>Erysipelotrichaceae</i>					<i>Akkermansia</i>
<i>Pasteurellaceae</i>	<i>Pasteurellaceae</i>					
<i>Prevotella</i>	<i>Prevotella</i>					

Table 2

Bacteria that are commonly identified in mouse luminal and mucosa-associated microbiota from various intestinal segments.

Luminal Microbiota		Mucosa-associated Microbiota	
Small Intestine	Feces	Small Intestine	Feces
Ileum	Colon	Ileum	Colon
<i>Clostridium</i>	<i>Clostridium</i>	<i>Bacteroides</i>	<i>Bacteroides</i>
<i>Lactobacilli</i>	<i>Bacteroides</i>	<i>Prevotella</i>	<i>Prevotella</i>
<i>Bacteroides</i>	<i>Prevotella</i>	<i>Clostridium</i>	<i>Clostridium</i>
<i>Prevotella</i>	<i>Lactobacillus</i>	<i>Lactobacillus</i>	<i>Lactobacillus</i>
<i>Enterococcus</i>	<i>Lachnospiraceae</i>		<i>Akkermansia</i>

Prevotella, and two OTUs were classified as *Pasteurellaceae* (Proteobacteria) were found to negatively correlate with pH (with decreased abundance at higher pHs). The other OTUs, classified as Firmicutes, mainly *Streptococcus* and *Lactobacillales*, as well as *Actinomyces* (Actinobacteria) were found to positively correlate with pH [95]. These findings highlight the link between intestinal pH and microbe composition.

Analysis of biopsy specimens from healthy humans has revealed that the mucosa-associated microbiota differs significantly from the luminal microbiota. In a study examining mucosal biopsies along the length of the gastrointestinal tract, the authors found that the duodenum mucosa-associated microbiome contained *Bacillales* (Firmicutes, ~25%), *Streptococcaceae* (Firmicutes, ~20%), *Veillonellaceae* (Firmicutes, ~10%), *Pseudomonadaceae* (Proteobacteria, ~10%), and *Fusobacteriaceae* (Fusobacteria, ~5%), with lower levels of other microbes [98]. Interestingly, the ileum mucosa-associated microbiome was found to harbor *Lachnospiraceae* (Firmicutes, ~35%), *Bacteroidaceae* (Bacteroidetes, ~30%) *Ruminococcaceae* (Firmicutes ~5%), *Enterobacteriaceae* (Proteobacteria ~5%) and *Fusobacteriaceae* (Fusobacteria ~3%). This composition greatly differs from the documented members of the luminal microbiome. Similarities were found in the mucosa-associated bacterial populations of the ascending and descending colon mucosa-associated microbiomes to that of the ileum, which also harbored *Lachnospiraceae* (~40%), *Bacteroidaceae* (~30%) *Ruminococcaceae* (~5%), *Enterobacteriaceae* (~5%) and *Fusobacteriaceae* (~2%). These human studies establish relative community structures of the healthy microbiome along the gastrointestinal tract.

Similar to the human microbiome, the mouse microbiome has also been shown to vary along the length of the intestine (Fig. 3, Table 2). Whole segment microbiome analysis has shown that the small intestine (duodenum, jejunum and ileum) contained higher levels of *Lactobacillaceae* (Firmicutes ~30%), *Bacteroidales* (Bacteroidetes ~30%) followed by *Lachnospiraceae* (Firmicutes, ~10%), while the colon contained *Bacteroidales* (Bacteroidetes, ~40%), *Clostridia* (Firmicutes, ~30%), *Lachnospiraceae* (Firmicutes, ~20%), and *Ruminococcus* (~4%) in mice [99]. Analysis of luminal contents has revealed a dominance of *Lactobacilli* (Firmicutes,

~20%), *Clostridium* (Firmicutes, ~20%) and other Firmicutes members (~20%), followed by *Bacteroides* (Bacteroidetes, 5%) and other microbes in the ileum of mice [19,21,100]. In the mouse colon, the lumen was dominated by *Clostridium* (Firmicutes, ~35%) and *Bacteroides* (Bacteroidetes, ~20%), followed by *Prevotella* (Bacteroidetes, ~5%) and other microbes. In contrast, the mucosa-associated population was found to contain higher levels of *Bacteroides*, *Prevotella* and *Mouse Intestinal Bacteroides* (MIB) in the ileum and higher levels of *Clostridium* and MIB in the mucosa of the mouse colon than the luminal population [19,21,100]. These studies set the stage for examining how intestinal ion transport shapes the microbiome.

5. Connecting ion transport with the gut microbiome

The interconnection between the intestinal environment and the microbiome has been elegantly demonstrated using various animal knockout models. Among the best characterized ion transport knockout mice are the NHE3, NHE2, CFTR, DRA and GLUT2 deficient mice (Fig. 4).

5.1. NHE3 and NHE2 knockout mouse microbiome

Given the importance of NHE3 in sodium and water absorption, NHE3^{-/-} mice exhibit chronic diarrhea with an alkaline intestinal fluid high in sodium compared with wild type (WT) littermates [17–19,21]. Two different groups have reported on the microbiome of NHE3^{-/-} mice [19,101,102]. NHE3^{-/-} mice housed at one university exhibited an ileal and colonic luminal microbiome higher in Bacteroidetes compared to WT littermates [19]. Interestingly, the mucosa-associated microbiome exhibited even higher levels of Bacteroidetes in both the ileal and colonic microbial populations in the NHE3^{-/-} mice compared to WT littermates and compared to the luminal contents. At another institution, NHE3^{-/-} mice were likewise found to have increased Bacteroidetes in both the luminal and mucosa-associated colonic microbiomes, as well as expanded Proteobacteria [102]. These microbiome findings are consistent with previous studies that indicate that Bacteroidetes have improved growth at slightly higher pHs [103]. Select *Bacteroides*, like *B. thetaiotaomicron*, which were increased in NHE3^{-/-} mice, were found to have optimal growth in conditions that resemble the NHE3^{-/-} ileal environment *in vitro* [19]. Mice in the second facility exhibited decreased colonic mucus and developed spontaneous colitis, exhibited increased sensitivity to dextran-sodium-sulfate (DSS)-induced colitis, and when crossed with Rag2^{-/-} mice for T-cell transfer experiments, NHE3^{-/-}Rag2^{-/-} mice experienced dramatically accelerated and exacerbated disease in a microbiome dependent manner [101,102,104]. These studies highlight the role of NHE3 in dictating the microbiome, which in turn can promote inflammation in the right setting.

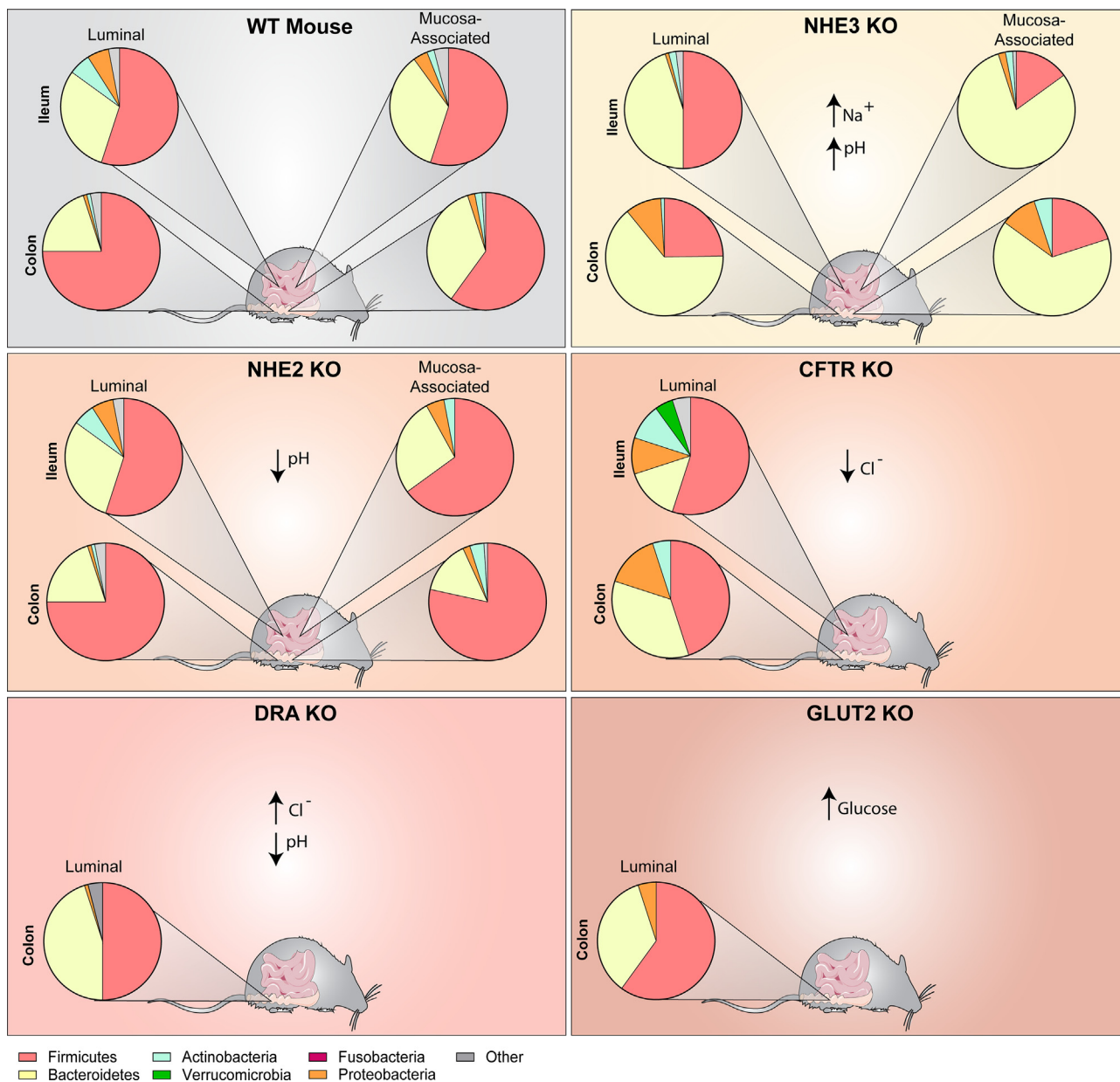


Fig. 4. Graphical representation of the microbiome of WT type mice highlighting the communities in the ileum and colon, for both luminal and mucosa-associated communities. Pie graphs depict the relative abundance of the major phyla (Firmicutes (pink), Bacteroidetes (yellow), Actinobacteria (blue), Verrucomicrobia (green), Fusobacteria (fuscia), Proteobacteria (orange) and other (grey)). In contrast to WT mice, NHE3, NHE2, CFTR, DRA and GLUT2 knockout (KO) mice harbor an altered intestinal environment and bacterial populations. Composition is approximated from published literature. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

In contrast to NHE3^{-/-}, NHE2^{-/-} mice do not exhibit diarrhea, but have an acidic intestinal fluid throughout the gastrointestinal tract [17–19,21]. Interestingly, no differences were observed in the microbial communities of the luminal contents in the NHE2^{-/-} mice compared to WT littermates [21]. However, significant differences were observed in mucosa-associated ileal and colonic microbiomes. In the ileal mucosa-associated microbiome, there were dramatic increases in Actinobacteria and decreases in *Bacteroides* (Bacteroidetes), MIB (Bacteroidetes), and other Firmicutes in NHE2^{-/-} mice compared to WT controls. In the colon, significant increases were observed in *Clostridia* and *Lactobacillus* (Firmicutes) in NHE2^{-/-} mice. Shifts in *Lactobacillus* and *Clostridium/Ruminococcus* correlates with changes in host mucus oligosaccharide composition [21]. These studies were among the first to identify a direct role of intestinal ion transport in shaping the intestinal environment and the microbiome composition.

5.2. CFTR knockout mouse microbiome

The microbiome is also altered in chloride transporter cystic fibrosis transmembrane conductance regulator (CFTR) deficient mice [105,106]. CFTR^{-/-} mice exhibit decreased luminal Cl⁻ and increased mucus secretion with no change in pH [105]. In the terminal ileum of CFTR^{-/-} mice, total bacteria were increased with enrichment of *Enterobacteriaceae* (Proteobacteria), *Mycobacteria* (Actinobacteria) and *Bacteroides* (Bacteroidetes), with an associated reduction in *Lactobacilliales* (Firmicutes) and *Acinetobacter lwoffii* (Proteobacteria) [105]. In a separate study, analysis of small intestinal luminal contents found increased Firmicutes and decreased Verrucomicrobia in CFTR^{-/-} mice compared to WT mice [107]. OTU classification revealed increased abundance of *Lactobacillus* (Firmicutes) and *Porphyromonadaceae* (Bacteroidetes), with decreased abundance of *Akkermansia* (Verrucomicrobia) in CFTR^{-/-}

mice. Interestingly, another study examining small intestinal luminal contents found the reverse. In CFTR^{-/-} mice they observed increased *Akkermanisa* (Verrucomicrobia) and *Erysipelotrichaceae* (Proteobacteria), and decreased Firmicutes (*Lactobacillus*) [108]. These studies highlight the need to identify region specific differences and emphasizes the microbiome variations that can be observed in different animal housing facilities. Fecal analysis of CFTR^{-/-} mice from another study indicated a significant increase in *E. coli* (Proteobacteria) compared to WT controls [109]. To the best of our knowledge, there are no mucosa-associated microbiome studies in CFTR^{-/-} mice. Collectively, these studies confirm the importance of ion transport and particularly the role of Cl⁻ in modulating the microbiome composition and depict the need for more studies on the CFTR microbiome.

5.3. DRA knockout mouse microbiome

Recently, two groups have established the microbiome of DRA^{-/-} mice [110,111]. Mutations in mouse DRA resembles that of congenital chloride-losing diarrhea in humans. Mice exhibit diarrhea with a high chloride, volume depletion, and growth defects [112]. DRA^{-/-} mice also have an acidic colonic pH-microclimate, similar to NHE2^{-/-} mice [111]. At an institution in the United States, DRA^{-/-} mice exhibited an expansion in Bacteroidacea (Bacteroidetes) with significant increases in *Parabacteroides* and *B. ovatus* in the feces [110]. DRA^{-/-} mice also had an expansion in Erysipelotrichaceae (Firmicutes) and Porphyromonadaceae (Bacteroidetes) and a retraction in Actinobacteria and Bacteroidales family S24-7 (Bacteroidetes). In an institution in Germany, DRA^{-/-} mice also exhibited expansion of Bacteroidetes and retraction of Firmicutes in the proximal and distal colon [111]. These DRA^{-/-} knockout mice also had decreased levels of Actinobacteria. DRA^{-/-} mice from both institutions exhibited decreased colonic mucus and inflammation [110,111]. These two studies highlight the importance of DRA in regulating the gut microbiota and intestinal homeostasis.

5.4. GLUT2 knockout mouse microbiome

The transporter GLUT2 facilitates the passage of dietary sugars, glucose, fructose, and galactose in the intestine [113]. Reduced GLUT2 leads to higher sugar content in the distal intestine. Animals deficient in GLUT2 exhibit decreased microvillus length in the jejunum compared to control mice and reduction in absorptive epithelial cells [114]. Sequencing demonstrates that GLUT2^{-/-} mice have increased fecal levels of *Clostridium* cluster IV (Firmicutes) and *Enterococcus* (Firmicutes) compared to control mice. These findings indicate that glucose persistence in the intestinal lumen can impact gut bacterial composition.

6. Conclusions

Although multiple aspects of host physiology can influence the microbiome, including oxygen content [115–118], bile acids [119–122], antibiotic use [123–125], diet [126–129], mucus [130–134], etc., in this review we have focused on the unique link between intestinal ion transport and the gut microbiota. The studies presented herein demonstrate that endogenous ion transport (luminal Na⁺, K⁺, Cl⁻, pH) alters the intestinal microenvironment making it inhabitable for particular bacterial groups. Since multiple factors can influence the gut microbiome, at present we lack a strong understanding of exactly how transported ions impact the gut microbiota. In the future, studies using bioreactors with human and mice feces might shed more insight into how ion composition and pH can

directly modulate gut microbes (independent of the immune system, mucus secretion and subsequent host responses, etc.). These types of studies would provide valuable information of microbial tolerance and niche development and may provide insights into how to shift an altered microbiome back towards a healthy composition.

The highlighted studies emphasize that knowledge of the stool microbiota does not necessarily fully reflect intestinal changes upstream (small intestine) or fully reflect the mucosa-associated bacterial populations, which dramatically change in the setting of altered host ion transport. Since it can be challenging to obtain human small intestinal samples, mouse models provide a valuable scientific tool for these types of analyses. Analyzing the microbiota regionally along the length of the intestine and by population (luminal or mucosa-associated) could be used in the future to determine mechanistic interactions. Since mucosa-associated bacteria live in closer proximity to the intestinal epithelium, it is likely they execute different functions within the GI ecosystem compared with luminal microbiota [1].

Although mouse models can be useful, mice differ from humans in some key aspects. Mouse diet, fur, and behavior (e.g., nocturnal behavior, grooming practices, and coprophagia) clearly differ from humans [44,135]. These differences likely influence the gut microbiota composition and confound analysis. Furthermore the mouse immune system differs from the human immune system [135], which affects the way the host responds to the gut microbiota. As a result, mouse disease does not always reflect human disease. Despite these differences mouse models can still be a useful tool for unraveling mechanisms of host-microbiota interactions. Kostic *et al.* eloquently stated “acknowledging this complexity and the potential pitfalls is not meant to suggest that using mice for host-microbiota studies is a flawed approach; rather, the point is to highlight that studying host-microbiota interactions in mice requires careful experimental design” [44].

Mouse genetic background has been shown to impact the composition, diversity, and richness of the gut microbiota in both WT and knockout mice [136,137]. In a study by Campbell *et al.* eight core inbred strains were examined by 16S rRNA. Effects were shown to exist in the gut microbiota based on litter, co-housing and in some mouse strains, gender [138]. Effects of mouse background can provide an advantage for selecting specific traits and determining how they influence the gut microbiota. However, it can also confound data analysis, and as such mouse background should be considered in experimental design. Animal age is also an important consideration as the microbiome of both rodents and humans shifts overtime.

Despite the potential impact of microbial communities on human health and disease, our understanding of how microbial communities are maintained in the gut remains incomplete. Although much is known about basic host physiology and stool gut microbiota composition, more studies are needed for a better understanding of the interplay between the gut microbiota and host environment set by ion transport. Current work in the field supports the notion that ion transport shapes the microbiota composition and ultimately the microbe-host interactions. Knowledge of how the intestinal environment affects specific bacteria will likely aid in the development of future therapies for diseases with abnormal bacterial composition.

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CRediT authorship contribution statement

Amy C. Engevik: Conceptualization, Writing - original draft, Writing - review & editing, Funding acquisition. **Melinda A. Engevik:** Conceptualization, Writing - review & editing, Visualization, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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