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The Microbes of the Intestine: An Introduction to Their Metabolic and Signaling Capabilities

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Microbiologists estimate that 10^{14} bacteria live in and on each of us. This collection of microbes, known as the human microbiota, contains 10 times more cells than the whole human body. Their collective genomes, the human microbiome, are estimated to contain 100 times more genes than the human genome itself.[1] Because of the gene-centric nature of most of the studies (discussed later), where the identities of the microbes are inferred from the genes observed, the terms, *microbiome* (nucleic acids) and *microbiota* (organisms), are used interchangeably throughout this review.

Recent research on obesity, in mice and humans, has demonstrated that microbes of the intestine can have an important influence on host energy balance. These and other studies are leading to the recognition that the communities of microbes in the gut function as an "organ" with many previously unappreciated metabolic, immunologic, and endocrine-like actions that influence human health.[2] The true nature of this organ is rapidly being charted. What previously was considered a minor player in the sideshow is now approaching status as a star in the center ring.

The goal of this article is to introduce the microbial community of the intestine to endocrinologists and others interested in metabolism, especially with regard to its possible roles in obesity. This article reviews current studies on the composition and functions of gut microbiota in humans and model organisms. Then, current knowledge of surface receptors in the host intestine that facilitate the selection of certain microbes to live in the gut and new knowledge about bacterial-bacterial and bacterial-host communications are reviewed. Although the nature of these interactions is just emerging, it is clear that the cross-talk

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between host, microbes, and environment is complex and involves multiple mechanisms (discussed later). Completeness is impossible for the authors and devastating for the beginner. Instead, this article endeavors to give readers (1) some intellectual tools to explore further, (2) an appreciation of the breadth of possibilities, and (3) an appetite for more.

Composition of Human Gut Microbiota

The gut serves two major functions: nutrition and defense. It digests food, absorbs nutrients, and assists with waste excretion. It also protects the host against invasion by pathogenic bacteria. This is mediated through a layer of intestinal epithelial cells, which, along with other cell types, form a largely impermeable barrier. At the same time, the intestines house an enormous population of microbes (approximately 1 kg found mainly in the ileum and colon), which aid in digestion and guard against pathogenic microbes. The human gut hosts at least 500 different “species” of microbes, according to Eckburg and colleagues'[3] analysis (the species designation is derived from sequence similarities of ribosomal RNA [rRNA] [discussed later]). The majority of these species belong to the superkingdom Bacteria, which is best known, but there also are some from the superkingdom Archaea (single-cell prokaryotes that originally were identified in extreme environments but now are identified in diverse ecologic niches) and unicellular eukaryotes (eg, yeast and protozoa). This diversity encompasses (1) the indigenous gastrointestinal microbiota (the autochthonous microbes) that colonize the gut under normal conditions and (2) transient species (the allochthonous microbes), which may colonize or invade gut tissues only under abnormal conditions.[4] Distinguishing the autochthonous species from the allochthonous species is one of the challenges met by a host via multiple interactions between host and microbes.

Adult humans are consistently colonized by microbes from approximately nine divisions (deep evolutionary lineages) of Bacteria and at least one division of Archaea. This represents only a tiny fraction of the 70 or more bacterial divisions and 13 archaeal divisions detected in the biosphere, indicating that only certain divisions have evolved close associations with human gut.[3,5] Within the confines of those generalizations, there are wide interpersonal variations in gut microbiota as a result of host genetic and environmental factors (eg, mother's microbiota, birthplace, diet, and living conditions). Gut microbiota in many other vertebrate species have been studied and shown to be unique, but each population, in general, shares many of the same features seen in humans—that is, the presence of a few hundred species from a narrow range of divisions.

Host Specificities of Gut Microbiota

At birth, a newborn's sterile gut immediately receives inoculations of microbes from the mother, the health care practitioners, and the surrounding environment.[6] The composition and temporal dynamics of the microbial communities vary greatly in infants. By year 1, however, although differences are still detectable, there is a convergence toward a microbiome profile more consistent with that observed in adults.[7] In adult humans, two bacterial divisions, the Firmicutes (predominantly Clostridia class and some Bacilli class) and the Bacteroidetes (including *Bacteroides fragilis* and *B. thetaiotaomicron*) dominate gut microbiota. Proteobacteria (eg, *Sutterella wadsworthensis* and *Escherichia coli*),

Actinobacteria (eg, *Actinomyces* and *Bifidobacterium*), Fusobacteria, Cyanobacteria, and Verrucomicrobia phyla also are present as minor players. The mouse gut microbiota share a majority (six out of nine) of these divisions, suggesting that some divisions may have co-evolved with mammals for millions of years. Alternatively, in zebrafish gut, Proteobacteria dominate.[8] When mouse gut microbiota were inoculated into germ-free zebrafish (animals born and raised without any resident microorganisms) to create chimera animals (animals harboring foreign microbiota) and vice versa, the microbiome profiles of these so-called chimera shift towards the profiles of the conventionally raised members of their respective species.[8] This experiment demonstrated that there are species-specific adaptations of microbes to their hosts.

Functions of The Human Gut Microbiota

Human gut microbes perform many metabolic functions that our own bodies cannot carry out, creating a symbiotic relationship. For example, we consume plant polysaccharides that are rich in xylan-, pectin- and arabinose-containing carbohydrate structures, which we are unable to digest. Encoded in the genomes of gut microbiota, however, are a large number of glycoside hydrolases, which break down these plant products and convert them into usable energy sources.[5] At the same time, gut bacteria derive their own energy from fermentation of these glycans. In addition, microbiota are able to synthesize vitamins and amino acids, degrade dietary oxalates, metabolize host-produced mucosal glycans, and biotransform bile acids (for review see Hooper and colleagues).[1]

Germ-Free Mice

This symbiotic relationship allows host and microbes to use energy sources that they separately cannot use easily.[9] The symbiotic relationship is most striking when comparing germ-free mice to their conventionally raised littermates. Germ-free mice require significantly more (30%) calorie intake to maintain body weight than conventionally raised animals. The phenotype is reversible; when natural mouse gut microbiota are introduced into germ-free mice, calorie balance is normalized, and these mice start to gain weight.[10]

Obese Mice

Microbes affect host energy harvest, and the host's body habitus correlates with the composition of the microbiota. Ley and colleagues[11] compared the microbiome of lean (ob/+ or +/+) mice with that of their obese (ob/ob) siblings, which are homozygous for a mutation in the leptin gene that results in severe obesity. Firmicutes and Bacteroidetes are the dominant divisions of bacteria found in intestines of mice. In ob/ob mice, however, the Firmicutes were more abundant, and the Bacteroidetes population was depressed as compared with lean controls.[11] Moreover, the microbiome associated with obese animals seems more efficient at energy harvesting; the amount of energy remaining in the feces of obese mice is significantly lower than that in lean control animals.[12] A similar shift in microbiome composition has since been observed in several studies of obese versus lean human individuals.[13,14] As discussed later, this shift in microbiome is not a simple microbial response to ecologic changes (ie, host physiology associated with weight gain). Rather,

recent studies suggested that the balance may be a result of intricate cross-talk among microbes and between microbes and host.

Conservation of Host Responses To Microbes in Different Animal Models

Studies using laboratory animals to investigate host responses to gut microbiota showed that there are certain conserved physiologic responses. In mice and zebra-fish, germ-free animals exhibit certain phenotypes that resemble those seen in fasting animals, despite consumption of more food than their conventionally raised counterparts.[15] Presumably, this is the result of a reduced efficiency in the ability to extract nutrients from their diets. Moreover, germ-free animals also have more restricted lipid metabolism and gut epithelial proliferation.[15] The introduction of gut microbiota into germ-free animals is associated with increased hepatic production of triglycerides and fat storage.[10] The molecular mechanism for the increased adiposity is at least partially the result of a microbial signal that suppresses the fasting-induced adipose factor (FIAF) protein of the host, which in turn causes liver to increase production and storage of triglycerides.[10] Gut microbiota can contribute further to the adiposity by providing the building blocks of triglycerides (eg, short-chain fatty acids) through fermentation.[16] Because zebrafish, mice, and humans have distinct microbiota, it is worth noting that despite the differences, there are well-conserved host responses.

Gnotobiotic Animals as Models of Gut Microbial Interaction

The highly complex and mixed microbial population in the gut presents a challenge to understanding how different members of this population contribute to the ecosystem in the gut. Gnotobiotic animal models provide a means to reduce the complexity. In these studies, animals are born and raised in a germ-free environment and then subsequently receive an inoculum of microbes of known composition. In a study by Bäckhed and colleagues,[10] gnotobiotic mice colonized by a single bacterial strain, *B. thetaiotaomicron* VPI-5482, a representative component of microbiota of the gut, showed an increased weight gain and fat deposition compared to germ-free mice, but this increase was less dramatic than the weight gained by formerly germ-free mice (also known as conventionalized mice) that had received the unfractionated mouse gut microbiota. The result demonstrated that even a single microbial species can have a significant impact on host metabolism. Moreover, the weight gain in the conventionalized mice is attributable to increased host metabolic activity and fat storage.[10]

When a second microbe, *Methanobrevibacter smithii*, was introduced with *B. thetaiotaomicron* to the previously germ-free mice, these two species acted synergistically to further enhance fat storage in the host. Part of this symbiosis involves *M. smithii*'s ability to use *B. thetaiotaomicron*'s fermentation byproducts for the production of methane, thereby making the fermentation reaction more favorable ther-modynamically.[17] In another study using gnotobiotic zebrafish colonized with two different bacteria from indigenous zebrafish microbiota, quantitative analysis of gene expression of selected markers also pointed to differential host responses to each bacterium.[15] These results provide further evidence of host-microbe and microbe-microbe interactions. Although the pathways are not yet well understood, recent studies have demonstrated the importance of such interactions.

Genomes of Two Specific Members of the Human Gut Microbiome

B. thetaiotaomicron and *M. smithii* genomes have been fully sequenced,[18,19] providing a more complete catalog of what these organisms might be capable of and revealing some clues as to how they have adapted to the human gut. The genome of *B. thetaiotaomicron*, one of the prominent members of the human gut microbiota, has an expanded ability to take up and degrade dietary polysaccharides.[18] Moreover, these enzymes seem tightly coupled to many environment-sensing regulators.[20] *B. thetaiotaomicron*, therefore, seems to have the ability to react swiftly and use ingested polysaccharide. In addition to digesting dietary polysaccharides, *B. thetaiotaomicron* has evolved the ability to stimulate gut epithelial cells to produce mucosal glycans and can use these glycans as energy sources and attachment matrices.[21] The details of the regulatory responses of *B. thetaiotaomicron* to environmental changes in the gut remain to be worked out.

M. smithii is the main archaeal species found in human gut.[3] Its genome also possesses many features reflecting its adaptation to the gut environment. Compared to typical archaea that are isolated from nature, *M. smithii* has an enrichment of genes involved in surface variation and defense. The ability to produce polysaccharide capsule and to vary the surface antigenic moieties is a feature shared by many microbes of the distal gut in humans.[19] *M. smithii* has a full complement of genes to produce sialic acid, which is rarely found on the surface of other prokaryotic organisms. This allows *M. smithii* to mimic the glycan landscape of its intestinal habitat and potentially evade host immunity.[19] *M. smithii* genome also contains an enrichment of genes involved in the utilization of bacterial fermentation byproducts (eg, CO_2, H_2, and formate) for methanogenesis.[19] Gene expression studies showed that the enzymes that funnel bacterial fermentation byproducts into the central methanogenesis pathway are up-regulated when *M. smithii* is co-colonized with *B. thetaiotaomicron*,[17] an example of coordination between a bacterium and an archaea in human gut environment.

Effects of Diet on The Gut Microbiome

A diet that is rich in fat and simple sugars (eg, typical Western diet) clearly contributes to obesity and weight-gain.[22] Recent studies using mouse models examined the effect of diet-induced obesity on the gut microbiome and vice versa. In these experiments, mice were fed defined diets consisting of high fat or restricted fat. As previously observed with obesity caused by mutation in the leptin gene, the Firmicutes bloomed, with an associated reduction in Bacteroidetes, as mice gained weight from the high-fat diet.[23] Analysis of the gut microbiome revealed an increase in genes involved in the import and fermentation of simple sugars and host glycans in mice fed the high-fat diet. When the mice were switched to a diet restricted in fat or carbohydrate, the microbiota shifted from Firmicutes to Bacteroidetes, as observed previously.[23] To show that high-fat diet-associated microbiota and restricted-fat diet-associated microbiota influence host adiposity differently, germ-free mice were inoculated with microbiota from mice raised on different diets. The mice that received the high-fat diet-microbiota showed increased adiposity. These and other studies raised the possibility that the Firmicutes-enriched community may be actively affecting host metabolism and storage of absorbed calories (see article by Turnbaugh and colleagues[23] for

details of these and additional elegant experiments). Germ-free mice are resistant to obesity induced by a high-fat diet, suggesting that microbes of the gut are crucial for energy harvest and host signaling (eg, via host's FIAF [discussed previously]). Correspondingly, germ-free mice with a mutation in their gene encoding FIAF no longer are resistant to diet-induced obesity and gain weight when put on high-fat diet.[16] A recent study in mice of the effect of differences in diet composition revealed a systematic correlation between diet composition and host adipocyte gene expression.[22] Overall, the interactions among host, diet, and microbiota are complex. Some researchers suggest that the genomes of the host and the indigenous microbiota act as a single coordinated entity, called the metagenome.[24,25]

Host Sensing and Selection of Gut Microbes

In effect, the gut microbiome functions as an organ within the host. Host cells also play an active role in this interaction. How does the intestine simultaneously accommodate the indigenous microbiome, maintain an intact mucosal barrier, and distinguish between symbiotic (autochthonous) species and dangerous pathogenic species? The answer lies in the sensing system found in intestinal cells of the host that recognizes microbes. This sensing system uses pathogen recognition receptors (PRRs). The two major classes of PRRs expressed by host cells of the gut and resident intestinal immune cells include Toll-like receptors (TLRs) and nucleotide-binding oligomerization domains (Nods)[26] (discussed later). Both classes of PRRs belong to the innate immune system. Interactions between intestinal cell PRRs and microbial ligands trigger signaling pathways associated with the innate and adaptive immune systems that are required to maintain healthy intestines. The bacterial components recognized by host cells are (1) microbe-associated molecular patterns (MAMPs), expressed by autochthonous microbes and pathogens, and (2) pathogen-associated molecular patterns (PAMPs), molecular signatures associated with pathogens (Fig. 1).[26]

Toll-Like Receptors

Toll receptors originally were discovered in fruit flies when their embryonic development was being investigated. Further studies revealed the function of Toll receptors in protecting insects from infection by bacteria and fungi. Homologous structures with similar functions were found expressed by mammalian cells and consequently called Toll-like receptors (TLRs) (for review, see Murphy and coworkers[26]). There are 11 human TLRs that are constitutively or inducibly expressed by many cell types, including immune cells and intestinal epithelial cells. TLRs are transmembrane proteins found on the surface of cells where they interact with microbes extracellularly (or intracellularly, within endosomal membranes, where they sense intracellular microbes or microbial products). Each TLR recognizes a diverse class of MAMPs or PAMPs expressed by commensal microbiota and pathogenic microbes, respectively (Table 1).

Most PAMP-TLR binding results in the activation of the innate immune system through the nuclear factor κB (NF-κB) pathway to produce inflammatory mediators (eg, tumor necrosis factor, interleukin [IL]-1 β, and IL-6) necessary to eradicate invading pathogens.[27–29] Signaling through PAMP-TLR interactions can activate several other pathways, including activator protein 1 (AP-1), E26-like protein (Elk-1) cyclic AMP response element binding

protein (CREB), and signal transducer and activator of transcription (STATs), with subsequent downstream transcriptional activation of numerous pro- and anti-inflammatory genes (eg, cytokines and chemokines) associated with pathogen elimination. In addition, TLR signaling can lead to the production of antimicrobial peptides and interferons and adaptive immune responses through effects on T cells and dendritic cells. The cells within the intestines express TLRs 1–9. Abnormal intestinal TLR expression (inappropriate levels or location) is associated with disease.[27] For example, enhanced expression of TLR4 by intestinal epithelial cells is associated with inflammatory bowel disease.

Commensal microbiota also can interact with TLRs via MAMPs. MAMP-TLR interactions within the intestines, however, usually promote gut homeostasis, attenuate host inflammatory responses, and maintain the integrity of the mucosal barrier. Precisely how TLR signaling is triggered to initiate pro-inflammatory responses by pathogenic microbes and dampened by commensal bacteria is not well understood. There are several proposed mechanisms to explain how commensal microbiota suppress pro-inflammatory responses within the intestines: (1) exposure of intestinal epithelial cells to commensal microbiota prior to invasion by pathogenic bacteria may cause the cells to become refractory to their pro-inflammatory stimuli; (2) commensal bacteria may induce differential downstream TLR signals compared to pathogenic microbes (eg, MAMP signaling promotes the nuclear export of NF-κB, thereby subverting the pro-inflammatory response typical of pathogenic bacteria); and (3) molecular changes in ligands of commensal bacteria may prevent recognition by TLRs.[26,28–30] Dysfunctional interactions between commensal microbiota and TLRs are believed to facilitate gut inflammation and loss of intestinal integrity associated with inflammatory diseases of the bowel.

Nucleotide-Binding Oligomerization Domains

The family of nucleotide-binding oligomerization domains (Nods) is the other major class of intestinal PRRs that interacts with commensal microbiota and microbial pathogens.[26] Currently, there are more than 20 different mammalian Nods. Nod1 and Nod2 are two family members found within the intestines that recognize major components of bacterial cell walls (Table 2). Like TLRs, Nods act as sensors for bacteria within the intestine to maintain health (ie, intestinal homeostasis) and to regulate pro-inflammatory signaling after interactions with pathogenic microbial products.[27,28,31] In addition, Nod2 expressed by intestinal epithelial cells exerts antibacterial activity. Nods are constitutively or inducibly expressed by intestinal cells and signal through several transcription factors, including NF-κB. Similar to TLRs, genetic mutations and abnormal expression of Nods (specifically Nod2) are associated with inflammatory diseases of the bowel (eg, some forms of Crohn's disease).[28] Unlike TLRs, which are transmembrane proteins exposed to extracellular and intracellular environments, Nods are located exclusively within the cytosol. Therefore, intestinal Nods only recognize bacterial components that are within host cells.

Intercellular Communication Between Microbes

As discussed previously, microbes can act coordinately as a multicellular organism. This coordination, achieved through chemical signaling and shared metabolites, is only beginning

to be understood (Fig. 2).[32] Current understanding of how microbes use intercellular signaling is summarized briefly.

Early Studies of Intercellular Communication between Microbes

Pheromones, molecules that signal among organisms of the same species, are widely recognized. Unicellular eukaryotes are known to regulate reproduction and feeding with ligands and receptors that are like mammalian counterparts (eg, small peptides that bind to seven transmembrane domain G-protein linked receptors and also steroid-related ligands that bind to intracellular receptors that act as nuclear binding proteins that regulate gene transcription).[33,34] In pioneering work with prokaryotes, Myxobacteria, a bacterium that can form a multicellular fruiting body with some cell specialization, was shown to use chemical signals to respond to changes in its environment.[35]

Quorum Sensing

Over the past decade or so, molecules that carry out intercellular signaling have been described extensively across many different types of bacteria. The process of intercellular communication, called quorum sensing, allows the bacteria to monitor the environment for other bacteria of the same or different species and to alter their behavior in response to, for example, changes in the cell density. Multiple forms of behavior, gene expression, secretion of virulence factors, biofilm formation, reproductive processes, and sporulation are among the processes regulated via quorum sensing in a community of bacteria.[36] For example, virulence factors that promote attachment and invasion of enteric pathogenic *E coli*, a major gut pathogen that causes neonatal diarrhea in developing countries, may be activated by quorum sensing at body temperature (37°C).[37]

Most processes undertaken by bacteria under the control of quorum sensing are more effective when carried out simultaneously by a large proportion of the bacterial community. This communication is not limited to members of the same species; there are now multiple examples where these ligands also can affect organisms of another species. The targets may be other bacteria or unicellular or multicellular eukaryotes. This newly understood concept narrows the distinction between unicellular and multicellular organisms; with quorum sensing, bacteria can approach the behaviors of a multicellular organism. Eavesdropping, deception, antagonism, cooperation, and disease modification are among the consequences of interspecies communication.[38–41]

Quorum sensing among microbes of the gut is beginning to be explored. Recent studies show that a molecule produced by *B fragilis* in the gut promotes maturation of the host's intestine-based immune system and also blunts the deleterious effects of *Helicobacter hepaticus*.[42]

Definitions

When a molecular signal is sent and received among members of the same species it is designated a pheromone. When the species of the sender and recipient of the signal are different, the signal molecule is considered to be an allomone or kairomone, when it is

beneficial to the sender or to the recipient, respectively. As the biology is better understood, it is likely that the nomenclature will be refined.

Microbial Mimics of Host Hormonal Signals

Multiple laboratories have detected factors native to bacteria that have properties similar to peptide hormones of vertebrates.[43] The functions of these peptides in the bacteria are not known. Likewise, it is not known whether these peptides act on other organisms (eg, microbes or vertebrates).

Pastan and colleagues,[44] using standard techniques of the day, characterized a thyrotropin-like peptide from *Clostridium perfringens* that resembled native pituitary thyrotropin in its ability to enhance glucose oxidation, phospholipid synthesis, and colloid droplet formation in thyroid slices. More impressive, when injected systemically into chicks, it stimulated the thyroid to release iodine into the bloodstream. On gel filtration, the thyrotropin-like peptide eluted in a region typical of a globular protein of 30 kd and it was destroyed by pronase, a broad-spectrum agent of proteolysis. Because proteases and lectins, for example, also are able to generate hormone-like receptor-mediated bioactivities in target cells, further studies are needed to define better the relationship of the clostridial peptide to the pituitary hormone.

Studying bacteria and yeast, other groups have reported material that has biologic and immunologic characteristics resembling human chorionic gonadotropic (hCG) hormone and molecules that have binding properties that resemble those of hCG receptors.[45]

In studies of *E coli* grown in a simple synthetic medium, Roth and colleagues detected peptides that resemble mammalian insulin in extracts of the bacteria and in conditioned (cell-free) medium. The peptides behaved like insulin in a standard radioimmunoassay, in an in vitro adipocyte bioassay, and in several chromatographic systems. On gel filtration, the peptide eluted broadly in a region comparable to a globular peptide of approximately 8 kd. The bioactivity was blocked by anti-insulin antibody and by anti-insulin receptor antibody.[43,46–49]

In similar studies of *E coli* grown in a simple synthetic medium, these researchers isolated and characterized a melanocortin-like material that corresponds in structure to the C terminus of elongation factor G, which shares some structural similarities to alpha-melanocyte-stimulating hormone (alpha-MSH) and to corticotropin.[50,51] A synthetic replicate of the *E coli* peptide mimics alpha-MSH in its interactions with four mammalian melanocortin receptor classes (melanocortin 5 receptor [MC5-R] not tested), therefore named MECO-1 (melanocortin from *E coli*). MECO-1 and alpha-MSH show affinity and biologic potency in vitro and in vivo that are extremely similar, despite the divergences in structure.[51]

The authors have considered the possibility that MSH-like peptides from microbes, acting on MC1-Rs on immune cells in the intestine, bolster the anti-inflammatory forces there. More speculative is the suggestion that MECO-1-like peptides, acting through MC3-R and MC4-R, may modulate feeding. More broadly, the authors have raised the suggestion that

microbes of the gut, in addition to their role as a metabolic organ in the host, also may be a source of hormone-like signals to host cells. In other review articles, the authors have referred to similar peptides in unicellular eukaryotes, such as Neurospora and Candida species, and also catalogued early examples of systems in prokaryotes and eukaryotes that resemble systems in vertebrates.[43]

Shedding Light on The Contents of The Black Box

The microbiome of the gut remained uncharted until recently. It is not entirely clear why the methods of culture and isolation, used so brilliantly by Koch and Pasteur and their descendants, produced such a low yield with organisms of the gut. It is becoming clear, however, that microorganisms, like humans, live in communities.

There are some intriguing examples of microbes living in communities and producing metabolites needed and used by each other.[52] More subtle influences may include the need for certain species to anchor on scaffolding material secreted by other species in order to grow (eg, biofilm).

In the 1980s, Pace and coworkers introduced a new culture-independent method to identify microbes in the environment that used molecular sequencing of the small subunit of the bacterium's rRNA gene.[53] The small subunit rRNA gene, or 16S rRNA gene, contains highly conserved sequences that can be used as anchors for the polymerase chain reaction. The nucleotide sequences in the variable regions of the 16S rRNA gene are characteristic of that species and can be used to identify each organism in a mix. A percentage cutoff (typically 97%-99%) then is used to assign an organism to the same genus or species. Applying this technique to gut microbiome using the high throughput sequencing machines (described later), more than 500 “species” have been identified in fecal samples obtained from a few subjects, each individual harboring approximately 200 to 400 “species”. Moreover, it is recognized that the vast majority (80%) of the detected species have not been cultivated.[3]

Technologic Advances

The metagenomic studies were possible because of recent advances in high-throughput nucleic acid sequencing and other large-scale technologies. All of these are supported by new developments in the field of bioinformatics (or computational biology) that allow genomic researchers to computationally process and analyze large amounts of biologic data. Some of the current technologic advances are discussed briefly as a springboard to speculation on future advances.

Since 2005, several next-generation DNA sequencers have become commercially available. Compared to the standard capillary sequencer, which produces approximately 70 kilobases per run, these sequencing platforms can produce between 100 megabases and 2 gigabases of sequences in a single sequencing run, representing a greater than 1000-fold increase in throughput. The vast improvement in sequencing technologies at reduced cost enables researchers to design experiments to interrogate the changes in the gut microbiota under various conditions at a depth of coverage not previously possible. Microbial DNA can be

isolated from fecal samples, swabs, or biopsies and then subjected to sequencing to decipher the composition and the functions of the microbiota. Despite the ability to sequence these community genomes, typically less than 40% of the genes found in a gut microbiome sample have inferred or known functions, leaving many potential genes with unknown functions and organismal sources. Novel experimental designs and computational tools need to be created to elucidate the functions of these novel genes and how they contribute to host-diet-microbiome interactions. For example, it will be necessary to couple the data obtained from metagenomic sequencing to functional studies involving gene expression profiles (the metatranscriptome), large-scale studies of the function and structure of proteins (the metaproteome), or the metabolic products produced by microbial communities (the metabolome) to gain insights into which organisms contribute to which metabolic processes.[14,54] These approaches are especially powerful as ex vivo studies of gut microorganisms often are impossible because of inability to culture these organisms.

Applications to Medicine

Knowledge of the constituents and properties of the microbiota of the intestine has emerged recently. Like other nascent research fields, the list of accomplishments is small but the future is extraordinarily promising. The authors' predictions, all highly theoretic, are offered. The microbes that populate the gut at birth are presented by chance. They come from mothers and other caregivers. As humans grow up, they continue to receive daily inocula from their diets and other environmental sources. Their bodies interact with diverse microbes and accept microbiota belonging to a narrow range of lineages. This selection process, however, is not always precise; also, certain harmful microbes can gain a foothold in the gut by chance. In the future, microbiota will be highly cultivated and selected for a specific purpose.

Until now, experiments to colonize the gut from the outside have been empiric. The effects of probiotics (“live microorganisms which when administered in adequate amounts confer a health benefit on the host”)[55] and prebiotics (“non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the ‘guts’, and thus improve host health”)[56] have been studied on a few microbes rather than the whole microbiota. The new knowledge obtained from metagenomics approach will provide a scientific basis to these experiments. Current data suggest that a “systems approach” to manipulate an entire microbial population is more likely to be successful in terms of stability of the engrafted population and its benefits.

As examples, obese patients might receive a satiety-promoting and calorie-wasting population; people who have cachexia and inanition might benefit from a community that enhances appetite and more efficient calorie absorption. Newborns and the elderly, who are particularly vulnerable to infections from an intestinal source, likely will have prescribed microbial populations selected for safety. The microbial population of the gut influences the course of inflammatory bowel disease. There is a long record of experimental approaches to affect changes in populations of gut microbes in these conditions. The scientific basis of such experiments in the future will be much richer. The specific strains used will have to be

matched to a patient's genetic background, thereby achieving the goals of personalized medicine.

Future Prospects

To facilitate understanding of the impact of human microbiome on health, the National Institutes of Health (NIH) has initiated the Human Microbiome Project[57] to survey the microbiomes of five different body sites, including the gut. Moreover, efforts have been made to sequence hundreds of reference genomes of bacteria found in the gut.[24] One day, how the microbes found in intestines contribute to well-being, metabolically, endocrinologically, and immunologically, may be understood. Furthermore, a more thorough understanding of how the immune system recognizes and interacts with beneficial and pathogenic species encountered in the gut also may be gained. Through studying gut microbiota, one day it may be possible to manipulate the gut microbiota to improve health, such as weight reduction and diabetes prevention, in patients. In summary, many normal states and disease conditions are influenced by the microbes of the gut (and vice versa). The rapidly expanding (but now known to be finite) catalog of organisms and the new understanding of their properties promise a bright future for rational interventions.

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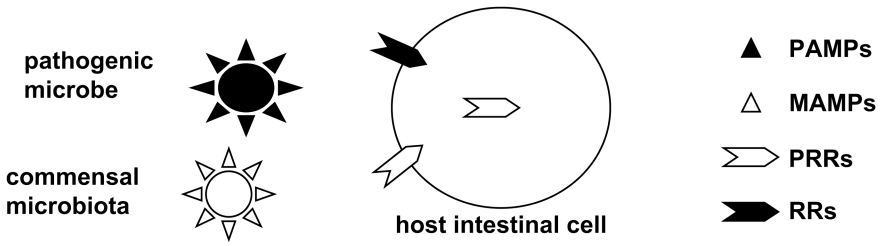

Fig. 1.

Diagram showing interactions of PRRs expressed by host intestinal cells and MAMPs or PAMPs. Commensal and pathogenic bacteria express MAMPs and PAMPs, respectively, which interact with PRRs (TLRs and NODs) found on the cell surface and within host intestinal cells.

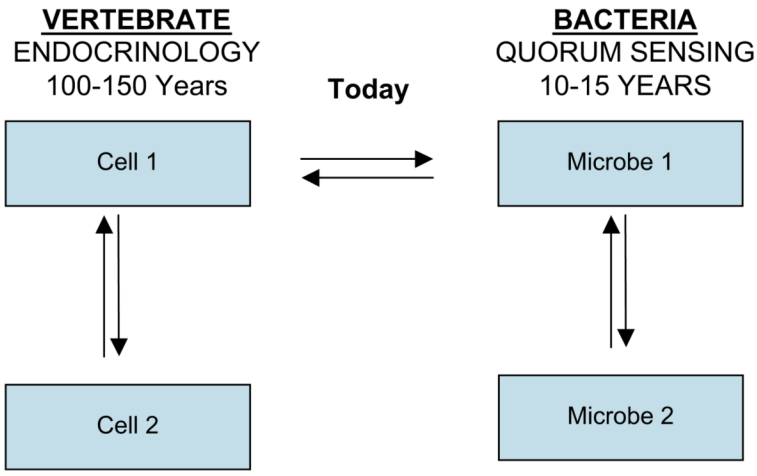

Fig. 2.

Studies of intercellular communication. Endocrinology led the way in the elucidation of communication between cells of vertebrates, starting 100–150 years ago. Similar communication among bacteria (quorum sensing) emerged 10–15 years ago. The exchange of endocrine-like signals between microbes and vertebrate cells is now emerging.

Table 1
Human Toll-like receptors and their ligands

Toll-Like Receptor	Ligand
TLR1:TLR2 heterodimer	Peptidoglycan Lipoproteins Lipoarabinomannan (*mycobacterium*)
TLR2:TLR6 heterodimer	Glycosylphosphotidylinositol (GPI) (*Trypanosoma cruzi*) Zymosan (yeast)
TLR3	Double-stranded RNA (most viruses)
TLR4 dimer (+ MD-2 and CD14)	Lipopolysaccharide (LPS) (gram-negative bacteria) Lipoteichoic acid (gram-positive bacteria)
TLR5	Flagellin
TLR6:TLR2 heterodimer	GPI (*T. cruzi*) Zymosan (yeast)
TLR7	Single-stranded RNA
TLR8	G-rich oligonucleotides
TLR9	Unmethylated CpG DNA
TLR10	No known ligand
TLR11	Uropathogenic ligand

Data from Murphy K, Travers P, Walport M. Janeway's immunobiology. 7th edition. New York: Garland Science; 2008.

Table 2
Well-described human intestinal Nods and their ligands

Nod	Ligand
Noc1	N-acetylglucosamine-N-acetylmuramic acid tripeptide Gamma-D-glutamyl-meso-diaminopimelic acid (iE-DAP) Source: gram-negative bacteria
Nod2	N-acetylmuramic acid-L-Ala-D-isoGln, also known as muramyl dipeptide (MDP) Source: gram-negative and gram-positive bacteria