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The Human Intestinal Microbiome: A New Frontier of Human Biology

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Abstract

To analyze the vast number and variety of microorganisms inhabiting the human intestine, emerging metagenomic technologies are extremely powerful. The intestinal microbes are taxonomically complex and constitute an ecologically dynamic community (microbiota) that has long been believed to possess a strong impact on human physiology. Furthermore, they are heavily involved in the maturation and proliferation of human intestinal cells, helping to maintain their homeostasis and can be causative of various diseases, such as inflammatory bowel disease and obesity. A simplified animal model system has provided the mechanistic basis for the molecular interactions that occur at the interface between such microbes and host intestinal epithelia. Through metagenomic analysis, it is now possible to comprehensively explore the genetic nature of the intestinal microbiome, the mutually interacting system comprising the host cells and the residing microbial community. The human microbiome project was recently launched as an international collaborative research effort to further promote this newly developing field and to pave the way to a new frontier of human biology, which will provide new strategies for the maintenance of human health.

Key words: microbiome; microbiota; gut; metagenomics

1. Introduction

An enormous number of microorganisms, the vast majority of which are bacterial species, are known to colonize and form complex communities, or microbiota, at various sites within the human body. It is estimated that the human microbiota is composed of $\sim 10^{14}$ bacterial cells, which is 10 times more than the total number of human cells. The largest and most complex is the one comprised by intestinal bacteria that includes as many as 10^{12} cells per 1 g of feces in the average human individual.[1] Thus, within each human body, intestinal and other microbiota, along with the 'host' human cells, form a complex ecosystem that, as a whole, interactively performs various biological processes.[2] Therefore, perhaps we should regard ourselves as 'superorganisms' together with the indigenous microbes[3] and that the composite genome should be referred to as the human 'metagenome'.

Studies on human intestinal microbiota should include microbial ecology and analysis of the complex metabolism of the microbial community, as well as various host–microbial interactions occurring at the interface between microbes and host intestinal epithelia. Such studies are expected to lead to understanding of the impact of the microbiota on human health and disease.[4–6] Along these lines, it should be noted that an international collaborative project, 'the human microbiome project (HMP)', was launched[7] in 2007 with the aim of collecting and integrating the

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genomic information from many diverse human microbiomes (the word 'microbiome' was first introduced in 2001 to define the collective genomes of microbiota[8]). This article is intended as an overview of the recent findings in relevant research fields.

2. Microbial diversity of the human gut microbiota

Over the past decade, the bacterial 16S ribosomal RNA gene (16S) sequence (∼1.5 kb) has been a useful landmark for analyzing the microbial diversity of human intestinal microbiota. A large-scale 16S phylotype analysis (grouping only by 16S rRNA sequence similarity) was carried out for three human adult microbiota.[9] The analysis of 13 335 bacterial 16S sequences identified 395 phylotypes at the strain level using a threshold of 99% sequence identity (% ID) and a single archaeal phylotype (*Methanobrevibacter smithii*) within the three samples. Members of the genera *Bacteroides*, *Eubacterium*, *Clostridium* and *Ruminococcus* were the major species found in the adult microbiota. Of the 395 phylotypes, ∼80% represented sequences from species yet to be cultivated. This analysis also indicated high interindividual variations in microbial composition among the three samples. Another large-scale 16S analysis estimated 4074 phylotypes at the species level (≥97% ID) in 18 348 sequences obtained from 14 subjects including 12 obese adults monitored for over 1 year.[10] This and a study using obese mice together revealed the association of the intestinal microbiota with obesity.[11] More recently, 16S analysis was performed for 15 172 sequences from 190 samples including subjects with inflammatory bowel disease (IBD) and healthy adults.[12] The etiology of IBD is known to largely correlate with the intestinal microbiota or certain microbial members.[13–15] These and other studies demonstrated that the intestinal microbiota of IBD patients have reduced microbial diversity compared with those of healthy controls.[12,16] The 16S analysis of other disease-afflicted subjects has also been performed in epidemiologic studies involving allergy[17–19] and cancer.[20,21]

The analysis of more than 45 000 bacterial 16S data combined with the three large-scale surveys described above estimated at least 1800 genera (≥90% ID), ∼16 000 phylotypes at the species level (≥97% ID) and ∼36 000 phylotypes at the strain level (≥99% ID) in the human intestinal microbiota, predicting even greater diversity at the species level.[12] This analysis also revealed that the majority (98% of all species) belongs to only four bacterial divisions: *Firmicutes* (64%), *Bacteroidetes* (23%), *Proteobacteria* (8%) and *Actinobacteria* (3%), whereas other minor taxonomic divisions are quite diverse.

Besides these 'snapshot' analyses of the intestinal microbiota composition, other long-term surveys have been performed to follow both the overall composition and that of limited members over periods ranging from several months up to 2 years.[10,22–24] These longitudinal studies suggested that the composition of intestinal microbiota do not drastically change in adults within the periods examined.

16S analyzes of infant intestinal microbiota have also been carried out.[25–27] One analysis revealed a dramatic progression in microbial composition until at least 1 year after birth with higher interindividual variations, but less complex than those between adults, converging toward a profile characteristic to the adult type at the end of the first year of life.[25] As might be expected, fraternal twins tend to show a significantly high similarity in their temporal microbial composition profiles.[25,28] No clear correlation was found in overall microbial composition due to the mode of delivery (Cesarean section or vaginal birth) and feeding with breast milk or formulated milk. Therefore, the source of these early colonizers is not clear, whereas some specific species are known to be transmitted from mother to baby.[26,29,30] Infant intestinal microbiota is mostly composed of bacteria such as *Staphylococcus*, *Streptococcus*, *Bifidobacterium* and *Enterobacteria*. Both adult and infant intestinal microbiota members are restricted to a small subset of species as described above, implying that the intestinal microbiota have evolved to shape overall microbial diversity under strong selective pressures.[4,5]

3. Sequence-based metagenomics of the human gut microbiome

The 16S analysis disclosed the existence of numbers of unculturable bacteria in the human intestinal microbiota, with only up to 20% of the 16S data able to be assigned to known species that have been successfully cultured in the laboratory during the past four decades.[9] Purely culturable bacteria are definitely necessary for comprehensive characterization of their biological and genetic natures. On the other hand, it is obvious that the 16S data do not provide any information on functional features of the microbial community. Thus, both culture-based and 16S-based approaches have crucial limitations for further studies, particularly for functional analysis. Metagenomics, the third and newest approach, has made it possible to comprehensively explore the biological nature of these complex communities.[31,32] This culture-independent approach includes shotgun

sequencing of microbial DNA prepared from the microbiota containing unculturable species isolated directly from the environments, followed by intensive computational analysis of obtained genomic sequences. Metagenomics is a quite effective and powerful approach for collecting and analyzing the information of genes present in the microbial community, because the proportion of the protein-coding regions in bacterial genomes can be as high as 80%, so that most of the metagenomic sequences obtained contain at least partial gene regions directly related to functions.[33] Biased data can be minimized in the metagenomic analysis, but a small fraction of genes may be relatively under-represented in the entire data set mainly due to toxicity of the gene products or sequence regions to the host *Escherichia coli* during cloning of the microbial DNA.[34] In addition, the degree of gene coverage is largely dependent on sequencing depth and complexity of the communities. These problems can be overcome by employing next-generation DNA sequencers based on massively parallel sequencing technologies,[35] by which the cloning step is eliminated and sequence quantity is increased by orders-of-magnitude compared with that of conventional Sanger sequencers.

To date, metagenomic data of human and mouse intestinal microbiomes have been published from three separate groups.[11,36,37] Gill et al. obtained ~78 megabases (Mb) unique metagenomic sequence data from the intestinal microbiome of two healthy human adults. Comparison of gene sets annotated in the intestinal microbiomes with human genes identified significant numbers of bacterial genes that are not encoded in the human genome. The function of these gene products contributes largely to the metabolism of glycans, amino acids and xenobiotics, and biosynthesis of vitamins and isoprenoids, which are necessary processes in human biology. These findings indicate the symbiotic relationship with humans and support the concept that we are superorganisms, the union of humans with their microbiota.[3] Kurokawa et al.[37] analyzed 13 human intestinal microbiomes including adults, children and unweaned infants and obtained 479 Mb unique metagenomic sequence data. Unexpectedly, more than half (up to 90%) of total shotgun reads were assembled to form unique contigs in each sample, which is in sharp contrast to soil microbiota in which <1% of total reads were assembled. These results suggest that the amount of sequence data produced in the two metagenomic studies (around 50 Mb of Sanger sequence data for each sample) could substantially uncover the major species with the highest orders of magnitude in quantity in each microbiota, and that these species may be comprised of a very limited number of strain-level species, perhaps accounting for <20 species in each microbiome. The constitution of major species by strain-level species, not by high species-level diversity, could be inferred from base-inconsistency in the generated contigs, with most of those ≥5 kb displaying high sequence similarity of >99.5% ID between assembled reads. In order to uncover the vast numbers of other species present at lower orders of magnitude, it would be necessary to produce sequence data of several more orders of magnitude.

The analysis by Kurokawa et al. also found 647 novel gene families specific to these intestinal microbiomes when compared with genes present in the metagenomic data of other microbial communities such as sea-surface, deep-sea and soil, and significantly enriched genes in these microbiomes when compared with gene sets in known microbes, except for the gut inhabitants. These gut microbiome-enriched (gut-enriched) genes were assigned to 237 and 136 clusters of orthologous groups (COGs) for the adult/child and the infant microbiomes, respectively, sharing 58 COGs between them for a total of 315 COGs in all. In the 315 enriched COGs, the function related to carbohydrate metabolism was remarkable in both types, but the functional repertories clearly differed between the adult and infant types. The adult type was rich in polysaccharide-degrading enzymes and the infant type was rich in sugar transporters. These data indicate that the functionality of healthy intestinal microbiota relies largely on available nutrients in the diet.[38,39]

4. Further analysis of metagenomic genes identified in 13 Japanese samples

Genome analysis of several *Bacteroides* strains dominant in adult intestinal microbiota indicated the richness of genes involved in polysaccharide metabolism,[40–42] exemplifying the functional adaptation of intestinal microbes to gut habitats rich in polysaccharides, which are metabolized by bacteria to generate short-chain fatty acids such as butyrate, the major energy source for the host.[38] It is also valuable to examine and compare the content of genes belonging to the 315 gut-enriched COGs in each genome of bacteria isolated from various environments. Our group recently performed a similarity search analysis of the enriched genes for 371 bacteria whose genomic sequences are already available. The 371 bacteria were classified into seven groups according to their origin of isolation. The ratio of adult gut-enriched genes in each genome is shown in Fig. 1. The average percentage ratios were 3.9% for all 371 microbes, 9.2% for 46 commensal bacteria, 4.0% for 94 pathogens and 2.7% for 231 bacteria from the

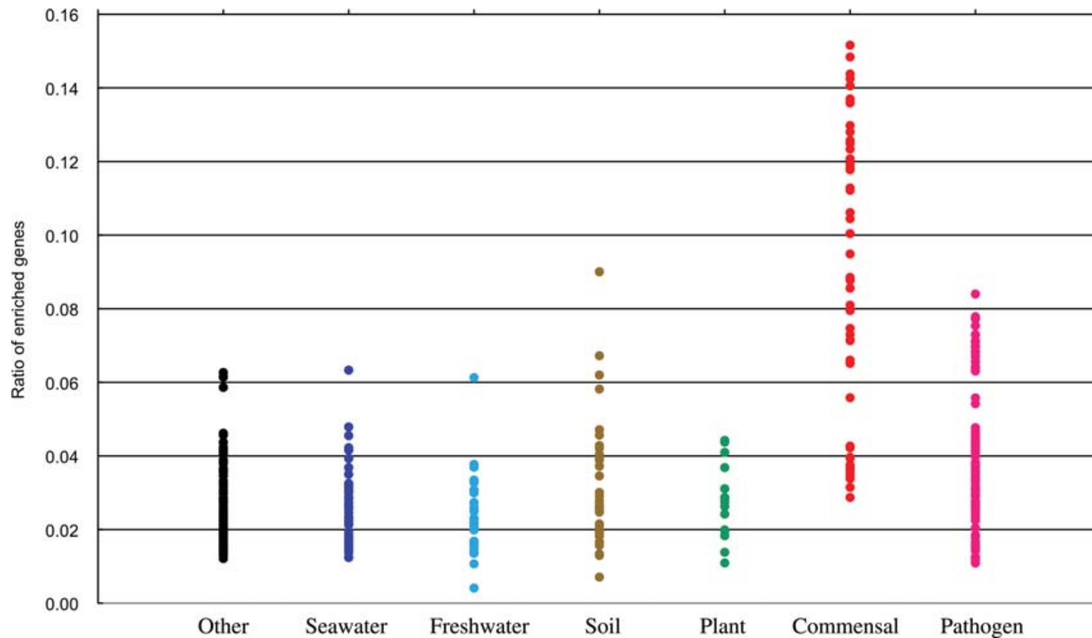

Figure 1. Ratio of gut microbiome-enriched genes (adult) in sequenced genomes of bacteria isolated from various environments. The similarity search was performed with a threshold value of $\leq 1e-8$ for 371 bacteria whose genomic sequences were available from public databases. The 371 bacteria were classified into seven groups according to their origin of isolation; commensals, pathogens, plant-related, soil-born, freshwater-born, seawater-born and others, and shown in red, pink, green, brown, light blue, dark blue and black dots, respectively.

other five groups. Those of infant gut-enriched genes were 2.8% for all microbes, 6.0% for the commensal bacteria, 3.5% for the pathogens and 1.9% for the other bacteria. These data clearly show that most of the commensal bacteria contain gut-enriched genes at a higher ratio than the other environmental bacteria. Interestingly, many of the commensal bacteria with lower ratios overlap those of pathogens, implying that intestinal microbes with a lower ratio of gut-enriched genes might be primary reservoirs for the corresponding pathogens. Table 1 lists the top 15 species with a higher ratio of adult and infant gut-enriched genes, respectively, which differ considerably from one another, except for the two species, *Ruminococcus* and *Dorea*. For the adult gut-enriched genes, members aggregated to three species, *Bacteroides*, *Eubacterium* and *Ruminococcus*, all of which are known dominant species in adult intestinal microbiota. For the infant gut-enriched genes, members are relatively diverse with *Clostridium*, *Bifidobacterium* and *Lactobacillus* species being characteristic. Some of them (e.g. *Bifidobacterium* and *Lactobacillus*) are prominent probiotics, living microorganisms having beneficial effects on host health.[43] The species with the higher ratios tend to be dominant in intestinal microbiota compared with the species with the lower ratios. For instance, *E. coli* K12 had a ratio of 3.4% for adult gut-enriched genes, much lower than the 15 species listed, which is consistent with the minority of *E. coli* in the adult intestinal microbiota. It is also possible that the metabolic mutualism between intestinal members may allow the species with lower ratios, e.g. the human intestinal archaeon (3.6 and 1.2% for the adult and the infant gut-enriched genes, respectively), to stably colonize the gut.[44]

In summary, the abundance of specific gene sets (i.e. gut-enriched genes) in commensal bacteria suggests that their genomes have evolved to accumulate functions advantageous for competitive survival and colonization in the gut habitat, a possible consequence of the functional adaptation to the gut ecosystem.[5,42] This distinct feature also suggests the difficulty for these intestinal microbes to survive in other environments where available nutrients and surrounding conditions are different in quantity and quality from those in the gut environment. Therefore, intestinal microbes may have additional properties tolerant to transient, but harsh conditions encountered in the mouth and stomach, through which they must travel to reach the gut. Genes encoding these yet unidentified functions may be included in the 315 gut-enriched COGs that contain many conserved but function-unknown genes, accounting for nearly 30% of all gut-enriched COGs, and in the 647 novel gene families identified.[37]

About 75% of the genes annotated in the metagenomic sequences of the 13 human intestinal microbiomes showed sequence similarity ranging from 40 to 100% ID at the amino acid level with known

Table 1. Top 15 species with the high ratio of adult and infant gut-enriched genes

Species having higher ratio of adult gut-enriched genes		Species having higher ratio of infant gut-enriched genes	
Ratio	Species	Ratio	Species
0.152	*Bacteroides ovatus*	0.102	*Bifidobacterium longum* NCC2705
0.148	*Bacteroides* WH2	0.098	*Clostridium ramosum* JCM1298
0.144	*Bacteroides* sp. A01	0.093	*Bifidobacterium catenulatum* JCM1194
0.143	*Bacteroides vulgatus*	0.092	*Clostridium clostridioforme* JCM1291
0.141	*Bacteroides thetaiotaomicron* 3731	0.087	*Collinsella aerofaciens*
0.137	*Bacteroides thetaiotaomicron* VPI-5482	0.082	*Lactobacillus johnsonii* NCC 533
0.136	*Bacteroides thetaiotaomicron* 7330	0.081	*Ruminococcus gnavus*
0.130	*Bacteroides uniformis*	0.081	*Enterococcus faecalis* V583
0.128	*Bacteroides caccae*	0.081	*Lactobacillus acidophilus* NCFM
0.126	*Eubacterium ventriosum*	0.079	*Dorea longicatena*
0.125	*Ruminococcus gnavus*	0.077	*Listeria monocytogenes* EGD-e
0.123	*Dorea longicatena*	0.076	*Lactobacillus plantarum* WCFS1
0.121	*Bacteroides* sp. A03	0.073	*Streptococcus agalactiae* A909
0.121	*Ruminococcus torques*	0.072	*Streptococcus pneumoniae* TIGR4
0.121	*Bacteroides fragilis* NCTC 9343	0.072	*Streptococcus pneumoniae* R6

genes.[37] When, for each gene, the best blastp-hit was used to tentatively assign the gene to a species, the distribution of sequence similarity for each species assigned could be depicted as shown in Fig. 2. Fig. 2 shows two distinct distribution patterns; those that peaked at high similarity of over 80% ID and those with low similarity between 50 and 80% ID. Typical species with the first pattern are *Escherichia*, *Klebsiella*, *Bifidobacterium* and *Bacteroides*, all of which were previously isolated from humans and fully sequenced, so it is reasonable that many of the metagenomic genes showed high sequence similarity with those in these microbes. On the other hand, the metagenomic genes showing low similarity with known genes in tentatively assigned species, such as *Bacillus*, *Clostridium* and *Streptococcus*, probably originate from species that are close to the assigned taxa but have not yet been isolated or sequenced. These data suggest that human intestinal microbes constitute distinct phylogenies from those of other environmental bacteria and have evolved with their own unique histories including co-evolution with the human host and its ancestors.[2,5]

5. Issues to be considered in 16S and metagenomic studies

In both cases of 16S rRNA phylotyping and metagenomic analysis, establishment of a reliable method for microbial DNA isolation from any given microbiota is a critical issue. This is because the intestinal microbiota comprise Gram-positive and Gram-negative bacteria and a small fraction of archaea, some of which may be hard-to-lyse species. So far, methods based on mechanical disruption using zirconium/silica beads[11,22,36,45] and those based on enzymatic lysis[9,25,37,46,47] have been developed and employed for the isolation of microbial DNA from various bacterial sources.[48–52] The DNA extraction method has been assessed with respect to the quality and quantity of obtained DNA as well as fecal sample preservation.[53] Our group recently assessed and compared the published lysis methods using fecal samples. Our preliminary results, including UniFrac[54] analysis of enumerated 16S data, shape and yield of obtained DNA, showed that many of the methods examined gave similar results in quality, but varied by more than 10-fold in quantity and in the degree of DNA fragmentation (data not shown).

The 16S analysis also has an intrinsic problem in quantitative evaluation of the microbial composition because of the existence of multiple heterogeneous copies of the 16S rRNA genes within a genome along with uneven PCR-amplification of the 16S region. The range in copy number of the 16S rRNA gene varies from 1 to as many as 15 in prokaryotic species.[55] In addition, the PCR primer Bact-8F,[56] often used for amplification of the nearly full-length 16S sequence, might not be suitable at least for the quantification of *Bifidobacteria*, of which the 16S sequence has three base mismatches with the primer, underscoring the composition of this species.[25] Recently, an improved primer pool was developed.[57] Next-generation DNA sequencers guarantee the rapid collection of genomic data but provide less read-length than that of conventional Sanger sequencers.[35] The feasibility of pyrosequencing reads of 200–300 bases for the 16S phylotype analysis[58] was evaluated by collecting 141 000 reads from rhesus macaque intestinal microbiota.[59] The results showed high reproducibility of the phylogenetic assignments and similarity of the major types and relative numbers of taxa to those obtained from Sanger sequences.

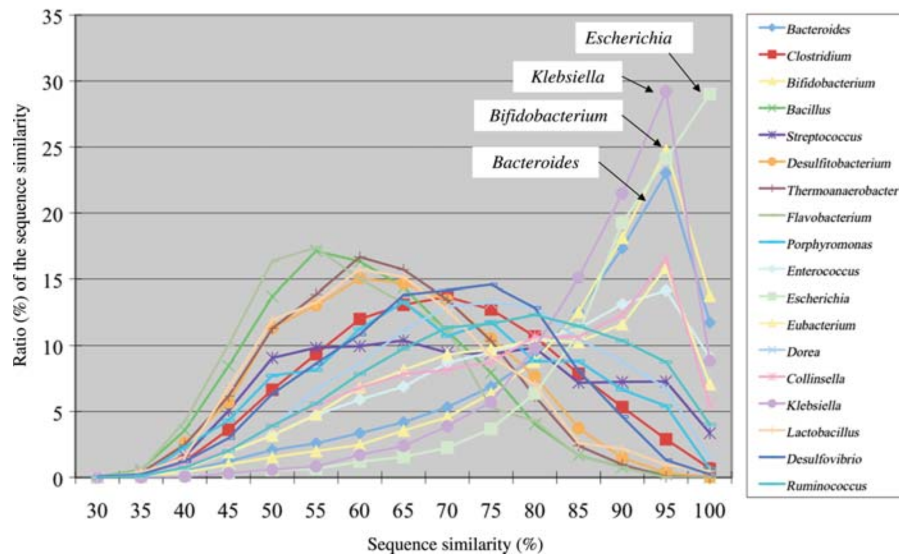

Figure 2. Distribution of sequence similarity of genes identified in the human intestinal microbiome. Only the data for the species that had sufficient numbers of best blastp-hit to known genes were represented. Species names are indicated in the box. Of these, four typical distribution patterns, which peaked at high sequence similarity (≥80% ID), are indicated by name in the figure.

6. Functions of the gut microbiota

Deciphering biological features of a taxonomically complex and ecologically dynamic microbial community is a challenging issue in gut microbiome research. Germ-free and gnotobiotic mice,[60] pig[61] and zebrafish[62] provide simplified model ecosystems that allow detailed evaluation of functions of colonized microbiota or microbes and the corresponding host responses *in vivo*,[63,64] as well as their impact on various host physiologies.[11,65–69] Immunity in germ-free mice is premature, but is restored by colonization of commensal bacteria and even by a single microbe.[70,71] Protection against pathogenic infection is also a characteristic feature of commensal bacteria.[72–74]

Investigations have been made regarding the functions of commensal bacterial genes on their colonization in the mouse intestine. Flexible transcriptional regulation for adaptation to changes in available nutrients, including those during weaning, was found in *Bacteroides thetaiotaomicron*.[39,75] Surface glycans expressed by *Bacteroides fragilis* are essential for its colonization.[76] The analysis of certain *Lactobacillus* strains, which are thought to have health-promoting properties as probiotics, identified genes inducible upon their colonization.[77] Expression profiling of both bacterial and host genes in mono-associated mice colonized by either *Bifidobacterium longum* or *B. thetaiotaomicron* and di-associated mice colonized by both bacteria were examined.[78] The co-existence of *B. longum* expanded diversification in the carbohydrate substrates accessed by *B. thetaiotaomicron* in a host-independent manner. On the other hand, the presence of *B. longum* significantly reduced the expression levels of host genes responsible for antimicrobial activity against Gram-positive bacteria compared with that by *B. thetaiotaomicron* alone, suggesting the involvement of host responses in competitive colonization between these bacteria.

Commensal bacteria share many indistinguishable features with pathogenic bacteria relating to host immune response.[79–81] For example, lipopolysaccharide (LPS), lipoteichoic acid (LTA) and peptidoglycan, major cell wall components of all bacteria, are well known ligands recognized by membrane-bound toll-like receptor 4 (TLR4) and TLR2 (TLR2), that serve as sensors of bacterial infection and lead to the production of pro-inflammatory cytokines such as TNFα and IL-6.[82] Commensal bacteria have the ability to activate pro-inflammatory responses leading to harmful effects on the host via TLR signaling in mice lacking IL-10,[83] an anti-inflammatory cytokine.[84] Commensal bacteria also have the ability to activate anti-inflammatory responses leading to beneficial effects on the host via TLR signaling when the cascade to pro-inflammatory responses is lacking.[85] These results indicate that the impact of intestinal microbiota on the host physiology largely depends on the state of host immunity and that host-commensal bacteria interactions are considered to be placed at the exquisitely equilibrated state between pro-inflammatory and anti-inflammatory responses,[81,86] where the host preserves intestinal microbes while still being able to sense the bacteria that penetrate across intestinal borders.

Mammalian epithelia including Paneth and dendric cells are major sources of endogenous antimicrobial

substances, including: lysozymes, phospholipases and various antimicrobial peptides such as α-defensins,[87] angiogenin4[88] and RegIIIγ.[89] Several studies have examined the relationships between these antimicrobial peptides and their effect on various intestinal microbes. RegIIIγ is a C-type lectin, of which the expression is mediated by TLR signaling[90] and down-regulated by a Gram-positive *Bifidobacterium* strain.[78] The expression of mouse cryptdins, a counterpart of human α-defensins, requires Nod2 (nucleotide-binding oligomerization domain containing 2), a cytoplasmic pattern recognition receptor expressed in Paneth cells that senses for bacterial peptidoglycans.[91,92] Mutations in the *Nod2* gene are highly correlated with the etiological risk of a subset of Crohn's disease (CD).[14,93] The expression of α-defensins at the ileal of patients with CD of the ileal was significantly down-regulated, but not at the ileal of patients with CD of the colon.[94] Certain *Enterococcus* strains have been shown to regulate the phosphorylation of peroxisome proliferators-activated receptor γ (PPARγ) to induce the expression of downstream target genes including interleukin-10 (IL-10).[95] PPARγ-deficient mice exhibited dysfunction on the maintenance of gut homeostasis.[96] *Bacteroides thetaiotaomicron* induced PPARγ-mediated cytoplasmic re-distribution of the NF-κB subunit RelA in intestinal cells, selectively attenuating the inflammatory response.[97] These findings indicate that PPARγ is a nuclear factor associated with anti-inflammatory response. The expression of antimicrobial cathelicidin LL-37 is induced by butyrate, the product of polysaccharide fermentation by intestinal microbes.[98] These antimicrobial peptides may suppress microbial overgrowth and excessive contact of bacteria to the epithelia by directly killing them, resulting in minimizing inadequate stimulation of inflammatory responses.[80,99]

Some antimicrobial peptides were shown to dramatically increase in expression during the post-natal period.[88,89,99,100] And, it has been shown that TLR signaling by LPS is activated in vaginally delivered newborn mice immediately after birth but not in newborns delivered by Cesarean section.[101,102] These findings suggest that exposure of maternal-derived commensals to the intestinal epithelia in neonates is involved in initiating the development of intestinal homeostasis. Secreted (or mucosal) IgA produced by gut-associated lymphoid tissues is largely involved in shaping the intestinal microbiota composition, whereas lack of IgA expression can lead to adaptive immune response.[103–105] Intestinal alkaline phosphatase (IAP) was found to dephospholyrate the phosphate moiety in LPS, resulting in detoxification of LPS and prevention of bacterial penetration across the epithelial barrier, suggesting that IAP plays an important role in maintenance of gut homeostasis.[106,107] Recently, a study on gene expression of the TLR4-mediated signaling cascade in LPS-stimulated macrophages identified two classes of genes: those responding only to initial LPS stimulation and those responding to repeated LPS stimulation. The former class of genes includes pro-inflammatory cytokine genes, whereas the latter includes antimicrobial genes such as cathelicidin-related peptides. Histone modifications are involved in this regulation,[108] which could also be linked to disease susceptibility from environmental factors.[109]

In summary, the host has evolved to establish many processes that sustain unresponsiveness toward the commensal bacteria while at the same time maintaining responsiveness toward pathogens (Fig. 3). These processes include the production of IgA, IAP and various antimicrobial peptides and epigenetic control of pro-inflammatory responses, all of which sever routes leading to excessive inflammatory response. On the other hand, pathogens also have evolved to equip various virulence factors, including effectors, that confer additional abilities for evading the host defense system, eventually inducing pro-inflammatory responses[79,110] via change of the microbiota composition in favor of the pathogens.[72] In contrast, commensal bacteria may also have evolved not only to acquire specific functions adaptive to the gut habitat, e.g. carbohydrate metabolism, energy production, cell maturation and proliferation toward intestinal homeostasis,[111] but also to eliminate undesired appendages that could result in sensing for pro-inflammatory responses, e.g. profound depletion of genes for cell motility function in the metagenomic data of human intestinal microbiomes[37] and attenuation of host immune response by loss of flagellar function.[112]

7. HMP and future directions

The HMP aims at a better understanding of the roles of human microbes on human biology including their relationship with health and disease.[7] The project includes metagenomic and 16S analysis of the microbiota inhabiting various body sites such as the oral and nasal cavities, the gastrointestinal and urogenital tracts, and skin in several hundreds of healthy and disease-afflicted subjects, as well as genome sequencing of nearly 1000 human commensal microbes. In addition, various metadata concerning the host are needed, including variations in host genotypes[113] and metabolic phenotypes[114] that largely influence host–microbe interactions. In this regards, one challenging issue is the construction of an integrated metabolic map, of both human and

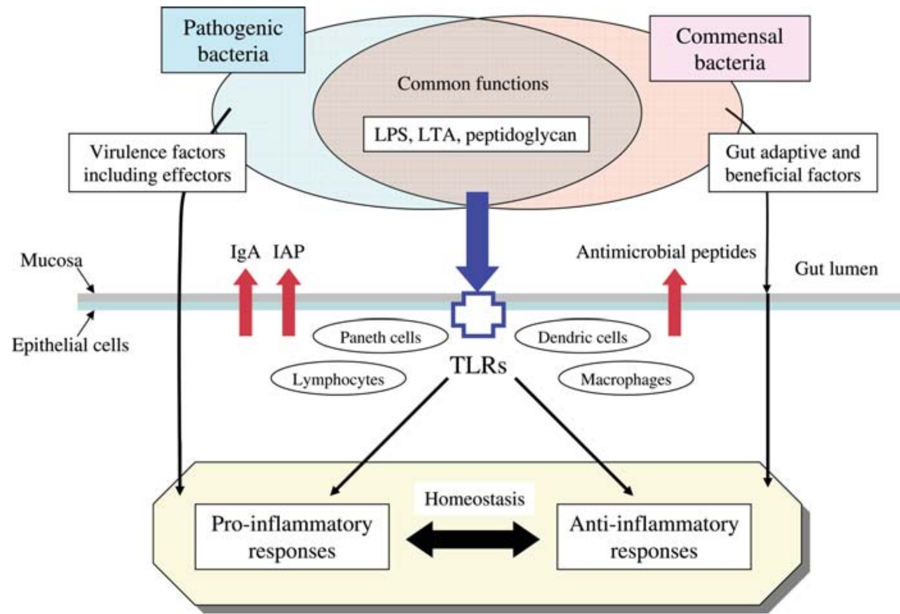

Figure 3. Molecular interactions at the frontline between the host, intestinal commensal bacteria and pathogenic bacteria. Commensal bacteria possess specific functions adaptive to the gut habitat and are beneficial to host cells, including maintenance of intestinal homeostasis, but also include functions such as those of TLRs that signal immune responses to pathogenic infection.[81,86] Host cells produce various antimicrobial substances such as IgA, IAP and antimicrobial peptides at the frontline to suppress excessive immune response to commensal bacteria, while maintaining responsiveness to pathogens equipped with various virulence factors to evade the host defense system.

microbiota,[115] which will become the new frontier for medical purposes such as the development of biomarkers for prediction of disease predisposition of individuals, extensive drug design targeting the intestinal microbiota and personalized drug therapies.[116,117]

A simplified model system using gnotobiotic animals has provided fundamental knowledge of the molecular mechanisms involved in intestinal host–microbe interactions, in which active bacterial components have been identified.[118,119] However, an enormous number and variety of bacterial components and products must participate in these interactions, and most remain unknown. Future studies will include those to explore and identify intestinal bacteria and their gene products (including metabolites) that are involved in host–microbial interactions, to identify human genes that respond to

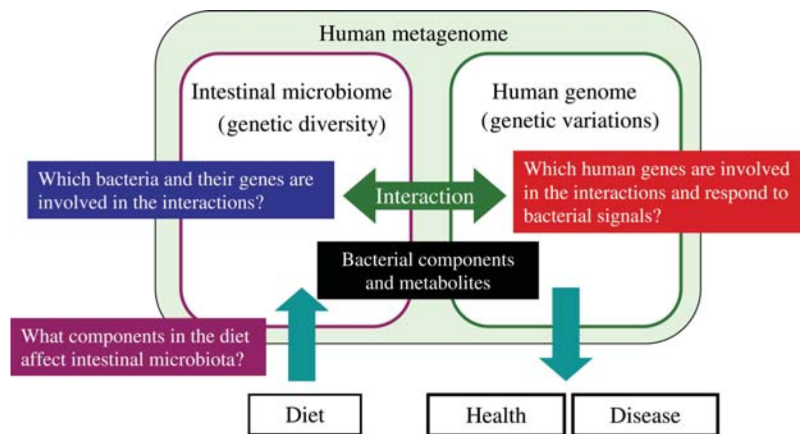

Figure 4. Future direction of human intestinal microbiota research. Human intestinal microbiota function is responsible for both human health and disease in accordance with its own genetic diversity and in association with human genetic variation. The study of human microbes, especially the vastly abundant intestinal microbes, is a new frontier in human biology. Many questions remain to be answered about host–microbe interactions, including: what factors and dietary components shape microbiota diversity, which bacteria and their components interact with host cells, which human genes respond to and how do they react to bacterial signals affecting human physiology.

bacterial signals crucial for human physiology and to identify dietary components that influence and shape the intestinal microbiota composition. These scientific challenges will be achieved by using advanced 'omics' technologies coupled with the vast quantities of genomic data that are already being accumulated by the HMP. Thus, the human intestinal microbiome will pave the way leading to a new frontier in human biology, in which the human genome and the intestinal microbiome are tightly linked together as an integral part of the 'human metagenome' (Fig. 4).

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