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Fredrik Bäckhed, *et al.*
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Host-Bacterial Mutualism in the Human Intestine

Fredrik Bäckhed,* Ruth E. Ley,* Justin L. Sonnenburg, Daniel A. Peterson, Jeffrey I. Gordon†

The distal human intestine represents an anaerobic bioreactor programmed with an enormous population of bacteria, dominated by relatively few divisions that are highly diverse at the strain/subspecies level. This microbiota and its collective genomes (microbiome) provide us with genetic and metabolic attributes we have not been required to evolve on our own, including the ability to harvest otherwise inaccessible nutrients. New studies are revealing how the gut microbiota has coevolved with us and how it manipulates and complements our biology in ways that are mutually beneficial. We are also starting to understand how certain keystone members of the microbiota operate to maintain the stability and functional adaptability of this microbial organ.

The adult human intestine is home to an almost inconceivable number of microorganisms. The size of the population—up to 100 trillion—far exceeds that of all other microbial communities associated with the body's surfaces and is ~10 times greater than the total number of our somatic and germ cells (*1*). Thus, it seems appropriate to view ourselves as a composite of many species and our genetic landscape as an amalgam of genes embedded in our *Homo sapiens* genome and in the genomes of our affiliated microbial partners (the microbiome).

Our gut microbiota can be pictured as a microbial organ placed within a host organ: It is composed of different cell lineages with a capacity to communicate with one another and the host; it consumes, stores, and redistributes energy; it mediates physiologically important chemical transformations; and it can maintain and repair itself through self-replication. The gut microbiome, which may contain ≥100 times the number of genes in our genome, endows us with functional features that we have not had to evolve ourselves.

Our relationship with components of this microbiota is often described as commensal (one partner benefits and the other is apparently unaffected) as opposed to mutualistic (both partners experience increased fitness). However, use of the term commensal generally reflects our lack of knowledge, or at least an agnostic (noncommittal) attitude about the contributions of most citizens of this microbial society to our own fitness or the fitness of other community members.

The guts of ruminants and termites are well-studied examples of bioreactors "programmed" with anaerobic bacteria charged with the task of breaking down ingested polysaccharides, the most abundant biological polymer on our planet, and fermenting the resulting monosaccharide soup to short-chain fatty acids. In these mutualistic relationships, the hosts gain carbon and energy, and their microbes are provided with a rich buffet of glycans and a protected anoxic environment (*2*). Our distal intestine is also an anaerobic bioreactor that harbors the majority of our gut microorganisms; they degrade a varied menu of otherwise indigestible polysaccharides, including plant-derived pectin, cellulose, hemicellulose, and resistant starches.

Microbiologists from Louis Pasteur and Ilya Mechnikov to present-day scientists have emphasized the importance of understanding the contributions of this microbiota to human health (and disease). Experimental and computational tools are now in hand to comprehensively characterize the nature of microbial diversity in the gut, the genomic features of its keystone members, the operating principles that underlie the nutrient foraging and sharing behaviors of these organisms, the mechanisms that ensure the adaptability and robustness of this system, and the physiological benefits we accrue from this mutualistic relationship. This Review aims to illustrate these points and highlight some future challenges for the field.

Microbial Diversity in the Human Gut Bioreactor

The adult human gastrointestinal (GI) tract contains all three domains of life—bacteria, archaea, and eukarya. Bacteria living in the human gut achieve the highest cell densities recorded for any ecosystem (*3*). Nonetheless, diversity at the division level (superkingdom or deep evolutionary lineage) is among the lowest (*4*); only 8 of the 55 known bacterial divisions have been identified to date (Fig. 1A), and of these, 5 are rare. The divisions that dominate—the Cytophaga-Flavobacterium-Bacteroides (CFB) (e.g., the genus *Bacteroides*) and the Firmicutes (e.g., the genera *Clostridium* and *Eubacterium*)—each comprise ~30% of bacteria in feces and the mucus overlying the intestinal epithelium. Proteobacteria are common but usually not dominant (*5*). In comparison, soil (the terrestrial biosphere's GI tract, where degradation of organic matter occurs) can contain 20 or more bacterial divisions (*6*).

Our knowledge of the composition of the adult gut microbiota stems from culture-based studies (*7*), and more recently from culture-independent molecular phylogenetic approaches based on sequencing bacterial ribosomal RNA (16*S* rRNA) genes. Of the >200,000 rRNA gene sequences currently in GenBank, only 1822 are annotated as being derived from the human gut; 1689 represent uncultured bacteria. rRNA sequences can be clustered into relatedness groups based on their percent sequence identity. Cutoffs of 95 and 98% identity are used commonly to delimit genera and species, respectively. Although these values are somewhat arbitrary and the terms "genus" and "species" are not precisely defined for microbes, we use them here to frame a view of human gut microbial ecology. When the sequences (n = 495 greater than 900 base pairs) are clustered into species, and a diversity estimate model is applied, a value of ~800 species is obtained (Fig. 2). If the analysis is adjusted to estimate strain number (unique sequence types), a value of >7000 is obtained (Fig. 2). Thus, the gut microbiota, which appears to be tremendously diverse at the strain and subspecies level, can be visualized as a grove of eight palm trees (divisions) with deeply divergent lineages represented by the fan(s) of closely related bacteria at the very top of each tree trunk.

Diversity present in the GI tract appears to be the result of strong host selection and coevolution. For example, members of the CFB division that are predominantly associated with mammals appear to be the most derived (i.e., farthest away from the common ancestor of the division), indicating that they underwent accelerated evolution once they adopted a mutualistic lifestyle. Moreover, a survey of GenBank reveals that several subgroups in CFB are distributed among different mammalian species (Fig. 1B), suggesting that the CFB-mammal symbiosis is ancient and that distinct subgroups coevolved with their hosts.

Center for Genome Sciences, Washington University School of Medicine, St. Louis, MO 63108, USA.

*These authors contributed equally to this work.
†To whom correspondence should be addressed. E-mail: jgordon@molecool.wustl.edu

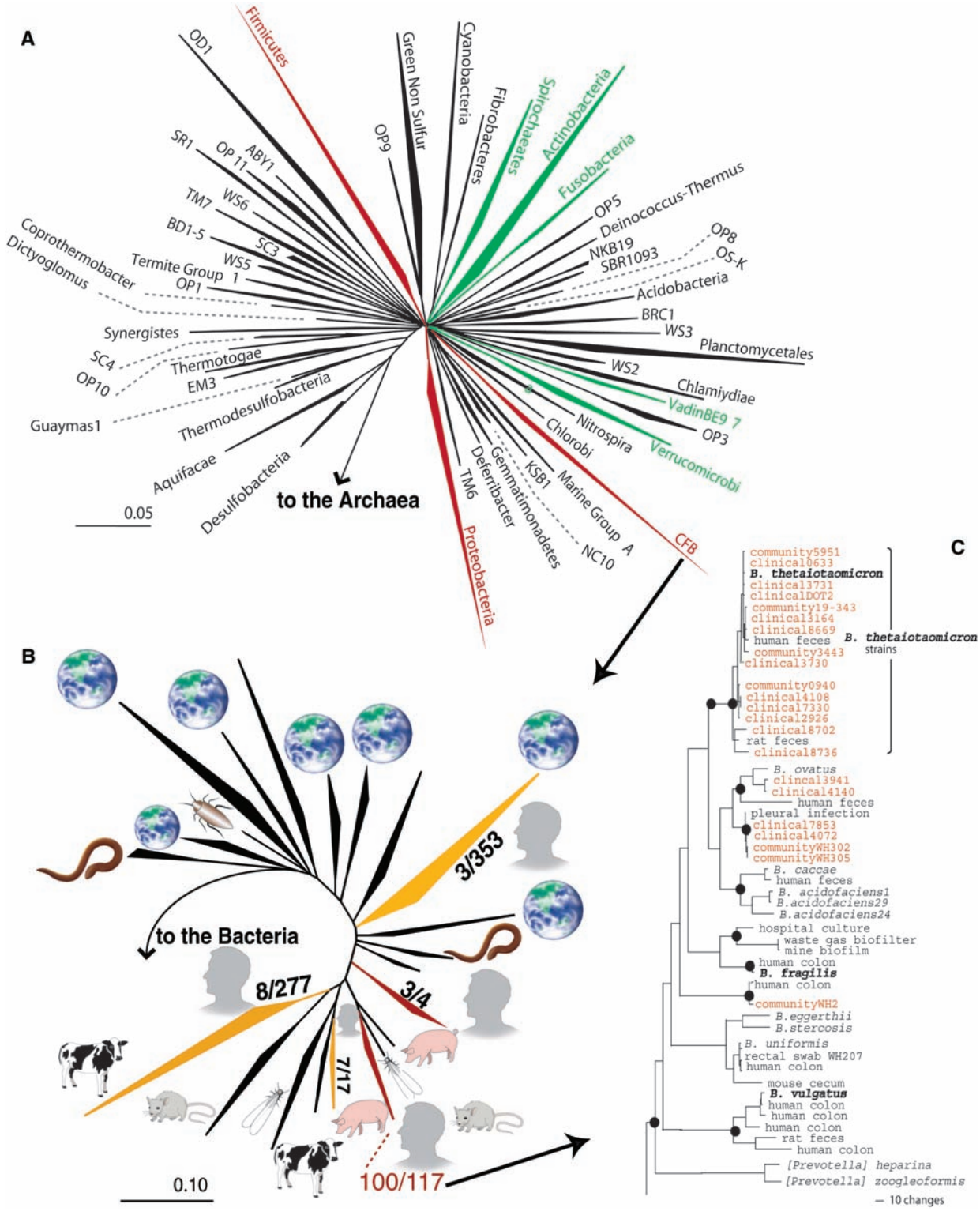

Fig. 1. Representation of the diversity of bacteria in the human intestine. (**A**) Phylogenetic tree of the domain bacteria based on 8903 representative 16*S* rRNA gene sequences. Wedges represent divisions (superkingdoms): Those numerically abundant in the human gut are red, rare divisions are green, and undetected are black. Wedge length is a measure of evolutionary distance from the common ancestor. (**B**) Phylogenetic tree of the CFB division based on 1561 sequences from GenBank (>900 nucleotides) and their ecological context. Wedges, major subgroups of CFB; symbols, source of the sequences [Earth, environmental; cow, ruminants; rodent, rat and/or mouse; person, human GI tract; others are termite, cockroach, worm (including hydrothermal), and pig]. Ratios are the number of sequences represented in the human gut relative to the total number in the subgroup; red, yellow, and black indicate majority, minority, and absence of sequences represented in human GI tract, respectively. (**C**) Phylogenetic (parsimony) tree of *Bacteroides*. Strains classified as *B. thetaiotaomicron* based on phenotype are in red; 16*S* rRNA analysis did not confirm this classification for all strains. *Bacteroides* spp. with sequenced genomes are in bold. Black circles indicate nodes with high (>70%) bootstrap support (*41*). Scale bars indicate the degree of diversity (evolutionary distance) within a division or subgroup [(A) and (B), respectively] in terms of the fraction of the 16*S* rRNA nucleotides that differ between member sequences; in (C), the evolutionary distance between organisms is read along branch lengths, where scale indicates number of changes in 16*S* rRNA nucleotide composition.

The structure and composition of the gut microbiota reflect natural selection at two levels: at the microbial level, where lifestyle strategies (e.g., growth rate and substrate utilization patterns) affect the fitness of individual bacteria in a competitive ensemble; and at the host level, where suboptimal functionality of the microbial ensemble can reduce host fitness. Microbial consortia whose integrated activities result in a cost to the host will result in fewer hosts, thereby causing loss of their own habitat. Conversely, microbial consortia that promote host fitness will create more habitats. Thus, the diversity found within the human GI tract, namely, a few divisions represented by very tight clusters of related bacteria, may reflect strong host selection for specific bacteria whose emergent collective behavior is beneficial to the host. This hypothesis has two important implications: (i) A mechanism exists to promote cooperation, and (ii) the structure promotes functional stability of the gut ecosystem.

To benefit the host, bacteria must be organized in a trophic structure (food web) that aids in breaking down nutrients and provides the host with energetic substrates. Cooperative behavior that imposes a cost to the individual while benefiting the community can emerge within groups of bacteria (*8*) and can be maintained by group selection as long as consortia are isolated and new consortia form periodically (e.g., new GI tracts). Furthermore, selection must act simultaneously at multiple levels of biological organization (*9*). These criteria are met in the human GI tract where new acts of colonization occur at birth, with a small founding population of noncheaters from the mother, and selection occurs both at the microbial and host level.

Diversity is generally thought to be desirable for ecosystem stability (*10*). One important way diversity can confer resilience is through a wide repertoire of responses to stress [referred to as the insurance hypothesis (*11*)]. In man-made anaerobic bioreactors used to treat wastewater (a system analogous to the gut but where no host selection occurs), rates of substrate degradation can remain constant, whereas bacterial populations fluctuate chaotically as a result of blooms of subpopulations (*12*). Functional redundancy in the microbial community ensures that key processes are unaffected by such changes in diversity (*13*). By contrast, in the human gut, populations are remarkably stable within individuals (*14*), implying that mechanisms exist to suppress blooms of subpopulations and/or to promote the abundance of desirable bacteria. A study of adult monozygotic twins living apart and their marital partners has emphasized the potential dominance of host genotype over diet in determining microbial composition of the gut bioreactor (*15*). The role of the immune system in defining diversity and suppressing subpopulation blooms remains to be defined. One likely mediator of bacterial selection is secretory immunoglobulin A (*16*).

The human gut is faced with a paradox: How can functional redundancy be maintained in a system with low diversity (few divisions of bacteria), and how can such a system withstand selective sweeps in the form of, for example, phage attacks? [The estimated 1200 viral genotypes in human feces (*17*) suggest that phage attack is a powerful shaper of the gut's microbial genetic landscape (*18*, *19*)]. Enough diversity of genome and transcriptome must be represented at the subspecies level to lend resilience to the gut ecosystem. Ecological studies of macroecosystems have shown that less diversity is required to maintain stability if individual species themselves have a wide repertoire of responses (*11*). In the following section we discuss recent genome-based studies exploring how a presumed keystone bacterial species in our gut is able to adapt to (i) changing dietary conditions in ways that should stabilize the microbiota's food web and (ii) changing immune and phage selective pressures in ways that should stabilize the ecosystem.

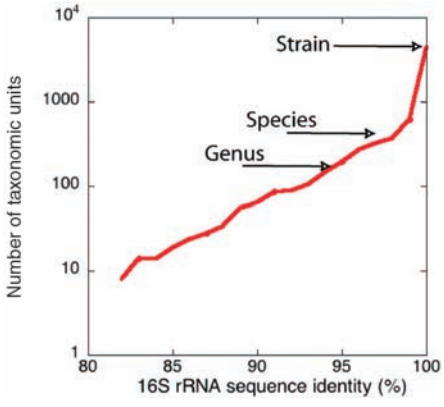

Fig. 2. Taxon richness estimates for bacteria in the human GI tract. Taxon richness estimates (*41*) for varying levels of 16*S* rRNA sequence identity, ranging from below the "genus" level (95% identity), to the "species" level (98% identity), to the strain level (unique sequences). Estimates are based on sequences available in GenBank, annotated as derived from the human GI tract, after alignment and clustering into taxonomic units ranging from 80 to 100% identity (*41*).

Bacteroides thetaiotaomicron—A Highly Adaptive Glycophile

Bacteroides thetaiotaomicron is a prominent mutualist in the distal intestinal habitat of adult humans. It is a very successful glycophile whose prodigious capacity for digesting otherwise indigestible dietary polysaccharides is reflected in the fully sequenced 6.3-Mb genome of the type strain (ATCC 29148; originally isolated from the feces of a healthy adult human) (*20*). Its "glycobiome" contains the largest ensemble of genes involved in acquiring and metabolizing carbohydrates yet reported for a sequenced bacterium, including 163 paralogs of two outer membrane proteins (SusC and SusD) that bind and import starch (*21*), 226 predicted glycoside hydrolases, and 15 polysaccharide lyases (*22*). By contrast, our 2.85-Gb genome only contains 98 known or putative glycoside hydrolases and is deficient in the enzyme activities required for degradation of xylan-, pectin-, and arabinose-containing polysaccharides that are common components of dietary fiber [we have one predicted enzyme versus 64 in *B. thetaiotaomicron* (table S1)].

The carbohydrate foraging behavior of *B. thetaiotaomicron* has been characterized during its residency in the distal intestines (ceca) of gnotobiotic mice colonized exclusively with this anaerobe (*23*). Scanning electron microscopy studies of the intestines of mice maintained on a standard high-polysaccharide chow diet, containing xylose, galactose, arabinose, and glucose as its principal monosaccharide components, revealed communities of bacteria assembled on small undigested or partially digested food particles, shed elements of the mucus gel layer, and exfoliated epithelial lining cells (*23*). Whole-genome transcriptional profiling of *B. thetaiotaomicron* (*23*) disclosed that this diet was associated with selective up-regulation of a subset of SusC and SusD paralogs, a subset of glycoside hydrolases (e.g., xylanases, arabinosidases, and pectate lyase), as well as genes encoding enzymes involved in delivering the products of mannose, galactose, and glucose to the glycolytic pathway and arabinose and xylose to the pentose phosphate pathway. In contrast, a simple sugar (glucose and sucrose) diet devoid of polysaccharides led to increased expression of a different subset of SusC and SusD paralogs, glycoside hydrolases involved in retrieving carbohydrates from mucus glycans, as well as enzymes that remove modifications that make these host glycans otherwise resistant to degradation (O-acetylation of sialic acids and sulfation of glycosoaminoglycans) (*23*).

These findings provide insights about how functional diversity and adaptability are achieved by a prominent member of the human colonic microbiota (Fig. 3). Dining occurs on particulate nutrient scaffolds (food particles, shed mucus, and/or exfoliated epithelial cells). For a bacterium such as *B. thetaiotaomicron*, which lacks adhesive organelles, seating at the "dining table" is determined in part by the repertoire of glycan-specific outer membrane–binding proteins it produces, and this repertoire is itself shaped by the menu of available glycans (*23*). Attachment to nutrient platforms helps avoid washout from the intestinal bioreactor, in much the same way as dense, well-settling, granular biofilms help oppose elimination from engineered (man-made) an-

aerobic upflow bioreactors (*24*). Attachment also presumably increases the efficiency of oligo- and monosaccharide harvest by adaptively expressed bacterial glycoside hydrolases and their subsequent distribution to other members of the microbiota whose niche overlaps that of *B. thetaiotaomicron*. In this conceptualization, microbial nutrient metabolism along the length of the intestine is a summation of myriad selfish and syntrophic relationships expressed by inhabitants of these nutrient platforms. Diversity in these microhabitats and mutualistic cooperation among their component species (including the degree to which sanctions must be applied against cheats) are reflections of a dynamic interplay between the available nutrient foundation and the degree of flexible foraging (niche breadth) expressed by microhabitat residents. *Bacteroides* spp., such as *B. thetaiotaomicron*, impart stability to the gut ecosystem by having the capacity to turn to host polysaccharides when dietary polysaccharides become scarce. The highly variable outer chain structures of mucus and epithelial cell surface glycans are influenced by host genotype and by microbial regulation of host glycosyltransferase gene expression. Coevolution of host glycan diversity and a large collection of microbial glycoside hydrolases that are regulated by nutrient availability provides insurance that the "system" (microbiota and host) can rapidly and efficiently respond to changes in the diet, and maximize energy harvest, without having to undergo substantial changes in species composition. Rather than minimizing genome size, a keystone species such as *B. thetaiotaomicron* has evolved an elaborate and sizable genome that can mobilize functionally diverse adaptive responses.

Diet-associated changes in the glycan foraging behavior of *B. thetaiotaomicron* are also accompanied by changes in expression of its capsular polysaccharide synthesis loci (*CPS*), indicating that *B. thetaiotaomicron* is able to change its carbohydrate surface depending upon the nutrient (glycan) environment. This could be part of a strategy for evading an adaptive immune response. Whole-genome genotyping studies of *B. thetaiotaomicron* isolates, with the use of GeneChips designed from the sequenced genome of the type strain, disclose that their *CPS* loci differ, whereas their housekeeping genes are conserved (*25*). Because selective sweeps are most likely to come from the immune system and phages, both of which respond to surface structures, the associated genes are likely to be the most diverse in the genome. Accordingly, *B. thetaiotaomicron* has a remarkable apparatus for altering its genome content. The sequenced type strain contains a plasmid, 63 transposases, 43 integrases, and four homologs of a conjugative transposon (*20*). Gene transfer and mutation mechanisms endow strains of bacterial species with the (genetic) versatility necessary to withstand selective sweeps that would eradicate more clonal populations (*26*).

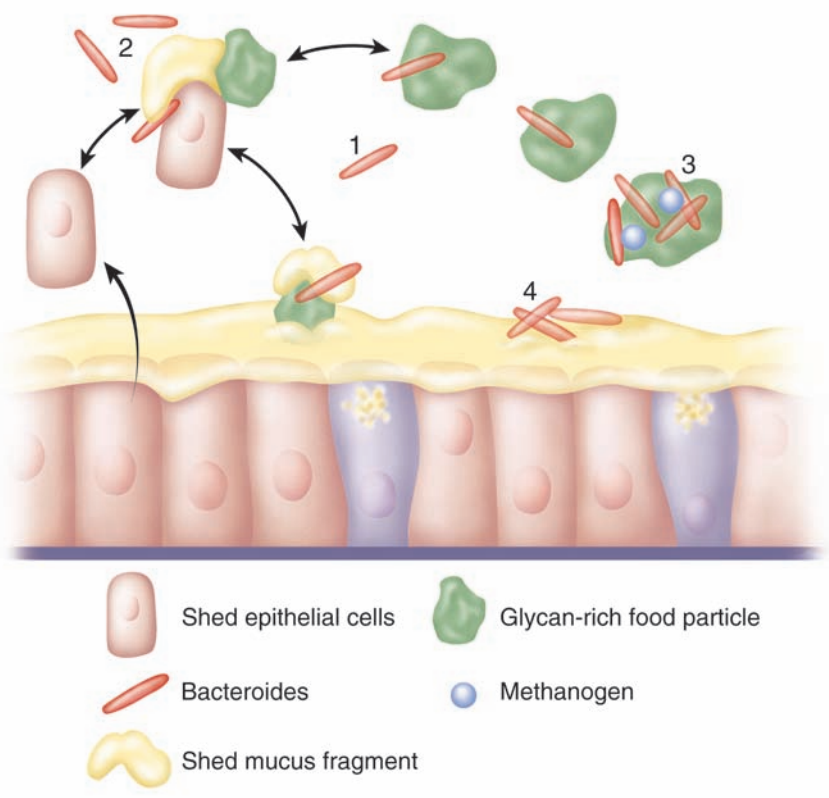

Fig. 3. Lessons about adapative foraging for glycans obtained from *B. thetaiotaomicron*. (1) *B. thetaiotaomicron* does not have adhesive organelles. Without outer membrane polysaccharide-binding protein-mediated attachment to glycan-rich nutrient platforms, it is at risk for being washed out from the intestinal bioreactor. Substrate access is limited under these conditions. (2) Small nutrient platforms are composed of undigested or partially digested food particles (e.g., dietary fiber), shed host epithelial cells, and/or mucus fragments. These platform elements may be in dynamic equilibrium with one another and with the mucus layer overlying the intestinal epithelium. Microbial fermentation of otherwise indigestible polysaccharides in these platforms is made possible by induced expression of substrate-appropriate sets of bacterial polysaccharide-binding proteins and glycoside hydrolases. (3) Mesophilic methanogens drive carbohydrate utilization by removing products of fermentation (H_2 and CO_2 are converted to methane), thereby improving the overall efficiency of energy extraction from polysaccharides. (4) When dietary polysaccharides are scarce, *B. thetaiotaomicron* turns to host mucus by deploying a different set of polysaccharide binding proteins and glycoside hydrolases. This adaptive foraging reflects the coevolved functional versatility of *B. thetaiotaomicron*'s glycobiome and the structural diversity of the host's mucus glycans.

The Gut Microbiota as a "Host" Factor That Influences Energy Storage

Comparisons of mice raised without exposure to any microorganisms [Germ-Free (GF)] with those that have acquired a microbiota since birth [Conventionally Raised (CONV-R)] have led to the identification of numerous effects of indigenous microbes on host biology (table S2), including energy balance. Young adult CONV-R animals have 40% more total body fat than their GF counterparts fed the same polysaccharide-rich diet, even though CONV-R animals consume less chow per day (*27*). This observation might seem paradoxical at first but can be explained by the fact that the gut microbiota allows energy to be salvaged from otherwise indigestible dietary polysaccharides (*28*). "Conventionalization" of adult GF mice with cecal contents harvested from CONV-R donors increases body fat content to levels equivalent to those of CONV-R animals (*27*). The increase reflects adipocyte hypertrophy rather than hyperplasia and is notable for its rapidity and sustainability (*27*).

The mutualistic nature of the host-bacterial relationship is underscored by mechanisms that underlie this fat-storage phenotype. Colonization increases glucose uptake in the host intestine and produces substantial elevations in serum glucose and insulin (*27*), both of which stimulate hepatic lipogenesis through their effects on two basic helix-loop-helix/leucine zipper transcription factors—ChREBP and SREBP-1c (*27*, *29*). Short-chain fatty acids, generated by microbial fermentation, also induce lipogenesis (*30*). Triglycerides exported by the liver into the circulation are taken up by adipocytes through a lipoprotein lipase (LPL)–mediated process. The microbiota suppresses intestinal epithelial expression of a

circulating LPL inhibitor, fasting-induced adipose factor (Fiaf, also known as angiopoietin-like protein-4) (*27*). Comparisons of GF and conventionalized wild-type and *Fiaf*$^{-/-}$ mice established Fiaf as a physiologically important regulator of LPL activity in vivo and a key modulator of the microbiota-induced increase in fat storage (*27*).

The caloric density of food items is portrayed as a fixed value on package labels. However, it seems reasonable to postulate that caloric value varies between individual ''consumers'' according to the composition and operation (e.g., transit time) of their intestinal bioreactors, and that the microbiota influences their energy balance. Relatively high-efficiency bioreactors would promote energy storage (obesity), whereas lower efficiency reactors would promote leanness (efficiency is defined in this case as the energy-harvesting and storage-promoting potential of an individual's microbiota relative to the ingested diet).

The idea that individual variations in bioreactor efficiencies may be a significant variable in the energy balance equation is supported by several observations. First, individual variations in the composition of the microbiota occur and are influenced by host genotype (*15*). Second, small but chronic differences between energy intake and expenditure can, in principle, produce major changes in body composition [e.g., if energy balance is +12 kcal/day, >0.45 kg of fat could be gained per year if there are no compensatory responses by the host; this is the average weight increase experienced by Americans from age 25 to 55 (*31*)]. Third, the microbiota is a substantial consumer of energy. One group estimated that individuals on a ''British Diet'' must ferment 50 to 65 g of hexose sugars daily to obtain the energy required to replace the 15 to 20 g (dry weight) of bacteria they excrete per day (*32*).

These considerations emphasize the need to assess the representation of species with large capacities for processing dietary polysaccharides, such as *Bacteroides*, in lean versus morbidly obese individuals, and in cohorts of obese individuals before, during, and after weight reduction achieved by high-polysaccharide/low-fat versus high-fat/low-polysaccharide diets [or by bariatric (gastric bypass) surgery]. The results, coupled with coincident assessments of energy extraction from the diet, should provide a proof-of-concept test of whether differences in the composition of the microbiota are associated with differences in gut bioreactor efficiency (and predisposition to obesity).

Lessons that have been learned by environmental engineers who study how to optimize the efficiency of man-made anaerobic bioreactors (table S3) suggest that these enumeration studies should also include members of archaea. Thermodynamics dictates that the energy obtained from substrate conversions will be higher if low concentrations of products are maintained (*33*, *34*). In the human gut, methanogenic archaea provide the last microbial link in the metabolic chain of polysaccharide processing. Bacteria degrade polysaccharides to short-chain fatty acids, CO_2, and hydrogen gas. Methanogens lower the partial pressure of hydrogen by generating methane, and thereby may increase microbial fermentation rates. Defining the representation of mesophilic methanogens in the colonic microbiota of individuals, sequencing their genomes [as we are currently doing with *Methanobrevibacter smithii*, a prevalent isolate from the human colon (*35*)], and characterizing archaeal-bacterial syntrophy in simplified gnotobiotic mouse models consuming different diets should provide a starting point for defining the role of archaea in shaping the functional diversity, stability, and beneficial contributions of our distal gut microbiota. Devising ways for manipulating archaeal populations may provide a novel way for intentionally altering our energy balance.

Looking to the Future

A comprehensive 16*S* rRNA sequence–based (bacterial and archaeal) enumeration of the microbiotas of selected humans, representing different ethnic groups, living in similar or distinct milieus, would provide an invaluable database for studying normal and diseased populations (*36*). The concept of using the microbiota as a biomarker of impending or fully manifest diseases within or outside of the GI tract and for monitoring responses to therapeutic interventions needs to be explored.

Several groups are embarking on metagenome sequencing projects to define gene content in the human gut microbiome. If we view ourselves as being a composite of many species, this represents a logical continuation of the Human Genome Project. A complementary approach to metagenomic analysis is to determine genome-level diversity among bacterial populations belonging to a specific genus or species residing within a defined gut habitat of a single individual or a few individuals. Members of *Bacteroides* provide a natural experiment for examining the impact of habitat on genome content since they have yet to be encountered in any environment other than animal GI tracts. Figure 1C illustrates how a collection of just 29 isolates phenotyped as *B. thetaiotaomicron* provided a broad range of 16*S* rRNA sequences, including several new species. We are close to producing finished genome sequences for two prominent members of the colonic microbiota, *B. vulgatus* and *B. distasonis* (*37*). *B. fragilis*, a less prominent member, has recently been sequenced (*38*, *39*). The results will allow us to ask how evolutionary history relates to genome content and what constitutes a minimal *Bacteroides* genome.

We also need to obtain a direct view of how the metabolites originating from the microbiome influence host physiology. This will be a formidable task, requiring new techniques for measuring metabolites generated by single and defined collections of symbionts during growth under defined nutrient conditions in single-vessel chemostats, in more elaborate mechanical models of the human gut, and in vivo after colonization of specified habitats of the intestines of gnotobiotic mice. The results should help formulate and direct hypothesis-based investigations of the microbiota's ''metabolome'' in humans.

Databases that connect molecular data with ecosystem parameters are still rare (*40*). A human intestinal microbiome database is needed to organize genomic, transcriptomic, and metabolomic data obtained from this complex natural microbial community, and would provide a substrate for generating testable hypotheses.

Finally, just as microbiotas have coevolved with their animal hosts, this field must coevolve with its academic hosts and their ability to devise innovative ways of assembling interactive interdisciplinary research groups necessary to advance our understanding.

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Supporting Online Material
www.sciencemag.org/cgi/content/full/307/5717/1915/DC1
Materials and Methods
Tables S1 to S3
References

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REVIEW

Immunity, Inflammation, and Allergy in the Gut

Thomas T. MacDonald[1]*† and Giovanni Monteleone[2]

The gut immune system has the challenge of responding to pathogens while remaining relatively unresponsive to food antigens and the commensal microflora. In the developed world, this ability appears to be breaking down, with chronic inflammatory diseases of the gut commonplace in the apparent absence of overt infections. In both mouse and man, mutations in genes that control innate immune recognition, adaptive immunity, and epithelial permeability are all associated with gut inflammation. This suggests that perturbing homeostasis between gut antigens and host immunity represents a critical determinant in the development of gut inflammation and allergy.

The gastrointestinal tract is the site where the divergent needs of nutrient absorption and host defense collide: The former requires a large surface area and a thin epithelium that has the potential to compromise host defense. Many infectious diseases involve the gut, and the investment by the gut in protecting itself is evident in the abundant lymphoid tissue and immune cells it harbors. In westernized countries, most infectious diseases of the gut are largely under control, yet gastrointestinal food allergies and idiopathic inflammatory conditions have dramatically increased; in other words, we now have inflammation without infection. Although the reason for this remains unknown, a prevailing notion is that the absence of overt gut infection has upset the balance between the normal bacteria that colonize the healthy gut and the mucosal immune system.

The Gut Epithelial Barrier

The primary cellular barrier of the gut in preventing antigens encountering the immune system is the single layer of gut epithelium, the surface area of which is expanded to the order of 400 m^2, largely because it is formed into millions of fingerlike villi in the small bowel. Each epithelial cell maintains intimate association with its neighbors and seals the surface of the gut with tight junctions. In the upper bowel, the bulk of the antigen exposure comes from diet, whereas in the ileum and colon, the additional antigenic load of an abundant and highly complex commensal microflora exists.

Nevertheless, the gut epithelial barrier does not completely prevent lumenal antigens from entering the tissues. Thus, intact food proteins can be detected in plasma (*1*), and a few gut bacteria can be detected in the mesenteric lymph nodes draining the gut of healthy animals (*2*). Antigens can cross the epithelial surface through breaks in tight junctions, perhaps at villus tips where epithelial cells are shed, or through the follicle-associated epithelium (FAE) that overlies the organized lymphoid tissues of the intestinal wall (*3*). Peyer's patches (PP) in the small bowel are aggregates of lymphoid tissue numbering ~200 in the average adult, although tens of thousands of much smaller individual follicles also line the small bowel and colon. FAE contains specialized epithelial cells termed M cells whose function is to transport lumenal antigens into the dome area of the follicle (*3*) (Fig. 1). Antigen-presenting dendritic cells (DC) also send processes between gut epithelial cells without disturbing tight junction integrity and sample commensal and pathogenic gut bacteria (*4*, *5*). The gut epithelial barrier therefore represents a highly dynamic structure that limits, but does not exclude, antigens from entering the tissues, whereas the immune system constantly samples gut antigens through the FAE and DC processes.

Commensal Bacteria in Epithelial/Immune Cell Function in the Gut

Interaction of commensals with gut epithelium. The gut epithelium itself can also directly sense commensal bacteria and pathogens; integral to this are the mammalian pattern recognition receptors (PRRs), which recognize conserved structures of bacteria and viruses and generally activate pro-inflammatory pathways alerting the host to infection (*6*). Two different classes of PRRs are involved. The Toll-like receptors (TLRs) are usually associated with cell membranes and have an external leucine-rich repeat (LRR) recognition domain and an intracellular interleukin-1 receptor (IL-1R)–like signaling domain (*7*). The nucleotide-binding oligomerization domain (Nod) molecules, Nod1 and Nod2 [also known as CARD4 and CARD15 (caspase activation and recruitment domain)], are present in the cytosol of epithelial cells and immune cells. These proteins also have LRRs at the C terminus, a Nod domain, and CARD domains at the N terminus (*8*). There is abundant evidence that signaling through Nod or TLR activates transcription factor NF-κB, leading to pro-inflammatory gene expression (*7*, *8*)

TLR1 to TLR9 and Nod1 and Nod2 are each expressed by gut epithelial cells (*6*, *9*). Nod1 and Nod2 recognize slightly different muropeptide motifs derived from bacterial peptidoglycans (*6*), which suggests that they sense intracellular infection or attempted bacterial subversion of epithelial cells (*10*). TLRs recognize many different components of bacteria and viruses. For example, TLR4 recognizes

[1]Division of Infection, Inflammation, and Repair, University of Southampton School of Medicine, Southampton General Hospital, Southampton, SO16 6YD, UK. [2]Dipartimento di Medicina Interna e Centro di Eccellenza per lo Studio delle Malattie Complesse e Multifattoriali, Università Tor Vergata, Rome, Italy.

*Present address: Barts and the London School of Medicine and Dentistry, Turner Street, London E1 2AD, UK.
†To whom correspondence should be addressed. E-mail: t.t.macdonald@qmul.ac.uk