

# HOW HOST-MICROBIAL INTERACTIONS SHAPE THE NUTRIENT ENVIRONMENT OF THE MAMMALIAN INTESTINE

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■ **Abstract** Humans and other mammals are colonized by a vast, complex, and dynamic consortium of microorganisms. One evolutionary driving force for maintaining this metabolically active microbial society is to salvage energy from nutrients, particularly carbohydrates, that are otherwise nondigestible by the host. Much of our understanding of the molecular mechanisms by which members of the intestinal microbiota degrade complex polysaccharides comes from studies of *Bacteroides thetaiotaomicron*, a prominent and genetically manipulatable component of the normal human and mouse gut. Colonization of germ-free mice with *B. thetaiotaomicron* has shown how this anaerobe modifies many aspects of intestinal cellular differentiation/gene expression to benefit both host and microbe. These and other studies underscore the importance of understanding precisely how nutrient metabolism serves to establish and sustain symbiotic relationships between mammals and their bacterial partners.

## CONTENTS

INTRODUCTION .....	284
MICROBIAL ECOLOGY OF THE GASTROINTESTINAL TRACT .....	285
GERM-FREE ANIMALS AS TOOLS FOR STUDYING MICROBIAL CONTRIBUTIONS TO NUTRITION .....	285
HOST-MICROBE NUTRIENT EXCHANGE .....	286
MICROBIAL CONTRIBUTIONS TO CARBOHYDRATE METABOLISM .....	287
Microbial Degradation of Starch .....	287

Microbial Degradation of Host-Derived Glycans .....	290
Induction of Host-Derived Glycans by <i>Bacteroides thetaiotaomicron</i> .....	292
Host Utilization of Microbial Fermentation Products .....	295
Microbial Contributions to Amino Acid Homeostasis .....	297
Vitamin Synthesis by the Gut Flora .....	298
MICROBIAL MANIPULATIONS OF THE HOST METABOLIC MACHINERY .....	298
PROBIOTICS AND PREBIOTICS: MANIPULATING THE MICROBIAL COMMUNITY THROUGH DIET .....	300
THE FUTURE .....	300

## INTRODUCTION

Nearly 130 years ago Louis Pasteur developed the Germ Theory of disease. In addition to laying the foundations for our understanding of microbial pathogenesis, he was also among the first to postulate that our health is intertwined with the lives of our resident nonpathogenic microorganisms (96). We now know that our mucosal surfaces are colonized by an enormously large, complex, and dynamic collection of microorganisms. In fact, adult humans are numerically more prokaryotic than eukaryotic: estimates are that 90% of our cells are microbial, whereas only 10% are human (90). The impact of these indigenous microbial communities on our physiology is likely to be most pronounced in the intestine, because this organ harbors the vast majority of our bacteria. Microbial densities in the proximal and middle small intestine are relatively low but increase dramatically in the distal small intestine (approximately  $10^8$  bacteria/ml of luminal contents) and colon ( $10^{11}$ – $10^{12}$ /g) (90).

Gut microbes conduct a multitude of biochemical reactions and can be collectively thought of as a metabolically active “organ.” This metabolic entity plays a critical role in nutrition, degrading a number of dietary substances that are otherwise nondigestible (91). Teleologically, this arrangement makes a great deal of sense. By recruiting a society of resident microbes with metabolic capabilities that allow them to break down these compounds, the host is relieved of the need to evolve such functions. The host also achieves a degree of metabolic adaptability that can help it deal with changes in diet and nutrient availability. In return, members of the gut microbiota are given a “protected,” nutrient-rich niche in which they can multiply.

In this review we discuss the nature and regulation of nutrient exchange between mammalian hosts and their intestinal microorganisms. We focus on recent insights about the molecular mechanisms by which intestinal bacteria help the host degrade dietary carbohydrates and how they impact the host’s ability to absorb and metabolize these and other nutrients. In addition, we consider how advances in functional genomics are creating new opportunities for understanding how hosts and their microbes collaborate to shape the nutrient environment of the gut.

## MICROBIAL ECOLOGY OF THE GASTROINTESTINAL TRACT

The term “microflora” refers to the collection of microbes colonizing a particular host niche and has been used interchangeably with “microbiota.” “Microbiome” refers to all of the genes embedded in the genomes of species represented in a microbiota (40). The designation “normal” microflora denotes species commonly encountered in healthy individuals. However, the microbial ecology of the intestine exhibits an astonishing degree of spatial and temporal complexity, making defining normal a daunting task. At least 500 bacterial species colonize the adult intestine, with 30–40 species comprising up to 99% of the total population (35, 56, 70, 90, 92). If the average genome size and gene density of the species represented in the gut microbiota are similar to those in *Escherichia coli* (5 mega base pairs, 4000 genes), the microbiome would have a complexity of 2.5 giga base pairs and contain 2 million genes.

The true extent of biodiversity in the gut is not known because most organisms cannot be cultured *ex vivo*. Culture-independent molecular methods for enumerating the microbial population exploit sequence diversity in the variable regions of 16S ribosomal RNA (rRNA) genes of Eubacteria (and Archaea). These methods are beginning to be systematically applied (54, 102, 116, 117), although efforts have been largely limited to analyses of the fecal flora.

The intestinal tract is sterile at birth. The composition of the human gut microflora undergoes dramatic changes during postnatal development (13). Denaturing gradient gel electrophoresis or temperature gradient gel electrophoresis of polymerase chain reaction products encompassing variable regions of 16S rDNA genes represented in fecal bacteria have been used to define microbial succession over time within individuals (28).

The adult (fecal) “climax community” is influenced by environmental factors as well as by host genotype. Zoetendal et al. (116) reported recently that the fecal 16S rRNA profiles of monozygotic twins are significantly more similar than those of unrelated individuals or marital partners. The same methods have also revealed that the predominant bacterial species associated with the colonic mucosa are significantly different from the predominant fecal community (115). Despite these variations in the composition of the microbiota between and within individuals, there is a “functional stability” that allows the microbiota to maintain its capacity to carry out a basic set of biochemical reactions, including degradation of carbohydrates, fermentation, and synthesis of vitamins.

## GERM-FREE ANIMALS AS TOOLS FOR STUDYING MICROBIAL CONTRIBUTIONS TO NUTRITION

The intestinal ecosystem is shaped by interactions between its microbes (both intra- and interspecies communications), epithelium, mucosal immune system, microvasculature, and enteric nervous system (36). The complexity and dynamic

nature of these combinatorial interactions present a unique set of problems for the experimentalist. For example, the presence of numerous bacterial species in the gut makes it exceedingly difficult to determine the specific contributions of individual species to a particular biological process. One useful experimental approach has been to use continuous-flow culture systems (32, 49, 104, 110) to model interactions among defined bacterial populations (23) and to examine microbial metabolism of specified components of the diet (58). Nonetheless, the reciprocal interactions alluded to above are virtually impossible to reproduce *in vitro*. An alternative is to use germ-free inbred strains of mice. These animals are raised without any resident microorganisms. As such, they represent a genetically defined, simplified *in vivo* assay system for defining the impact on host functions of colonizing the gut with specified members of the microflora (26).

Comparisons of conventionally raised mice (or rats) with their germ-free counterparts have revealed a series of anatomic (37), biochemical, and physiological phenotypes collectively known as microflora associated characteristics (MACs) (65). For example, the presence of a microflora increases intestinal epithelial turnover (93). The microflora also deconjugates and dehydroxylates bile acids (64), metabolizes bilirubin (94), reduces cholesterol to coprostanol (66, 81), and degrades mucus glycoproteins produced by the intestinal epithelium's goblet cell lineage (see below).

## HOST-MICROBE NUTRIENT EXCHANGE

Interactions between gut bacteria and their hosts can be viewed in terms of a continuum that spans symbiosis, commensalism, and pathogenicity. Symbiotic relationships are mutually beneficial for both partners, whereas pathogenic relationships benefit one partner at the expense of the other. Commensal relationships are generally defined as those that benefit one partner without necessarily being detrimental to the other.

Symbiotic/commensal relationships are typically centered on metabolic capabilities that allow either or both partners to exploit an otherwise unavailable or poorly utilizable nutrient foundation. These types of relationships are a dominant theme of life in our biosphere, affecting an enormous range of phyla including protozoa (e.g., the interaction of X-bacteria with *Amoeba proteus*), Cnidaria (*Symbiodinium* algae and corals), plants (nitrogen-fixing *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, and *Rhizobium* and legumes), vertebrates (cellulose digestion by *Bacteroides* and *Ruminococcus* spp in ruminants), and Arthropoda (the metabolic capabilities supplied to insect orders by bacteria has been a driving evolutionary force for species diversification in varied habitats) [reviewed in (101)].

The term commensal derives from the Latin *commensalis* and literally means "at table together." This definition is particularly apropos when applied to the intestinal microflora because substrates for both microbial and host metabolism ultimately come from the diet ingested by the host. Intuitively, it would seem that host and microflora, feasting on the same host-ingested fare, would engage in competition for

nutrients. However, conventionally raised animals require 30% less caloric intake to maintain their body weight than their germ-free counterparts (114), indicating that the microbiota aids its host in extracting maximum nutritional value from the diet. As discussed below, there is evidence that two complementary mechanisms are at work. First, microbial metabolism is responsible for the conversion of many dietary substances into nutrients that can be absorbed and utilized by the host. Second, the presence of microbes can alter the intrinsic metabolic machinery of host cells, resulting in more efficient nutrient uptake and utilization.

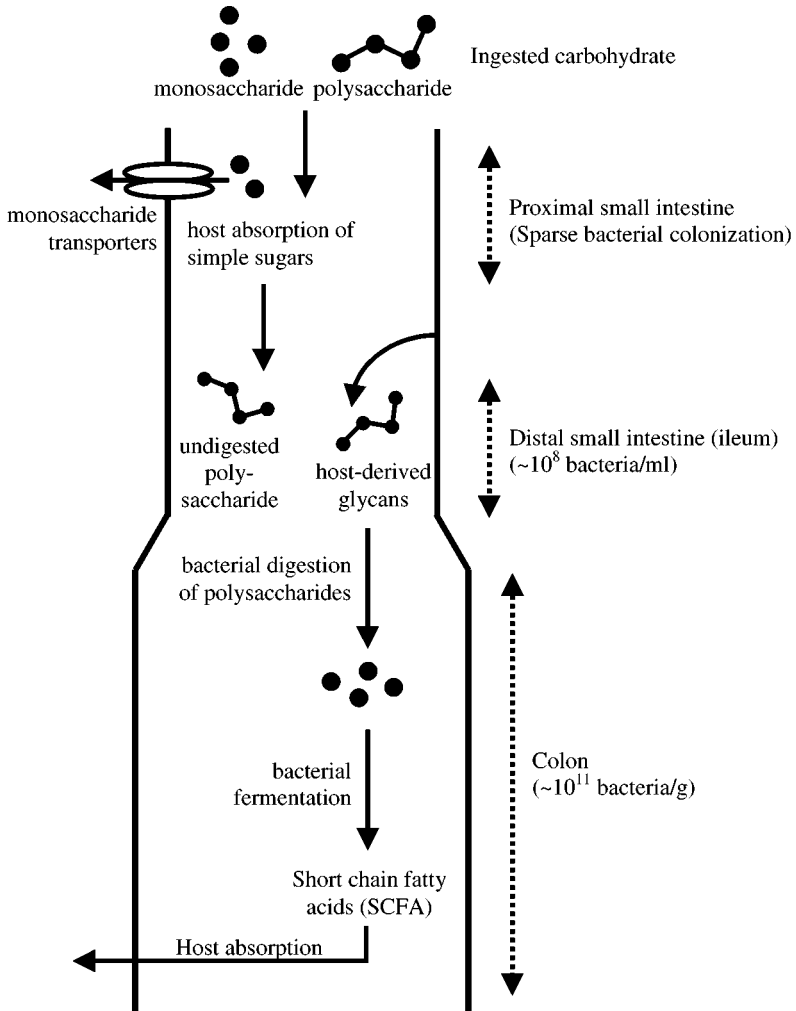
## MICROBIAL CONTRIBUTIONS TO CARBOHYDRATE METABOLISM

Carbohydrates form the bulk of most human and animal diets and thus are critical nutrients for both host and microbiota. Mammals are well equipped to absorb simple sugars such as glucose and galactose in the proximal portion of their small intestine (29). They can hydrolyze certain disaccharides (e.g., sucrose, lactose, and maltose) to their constituent monosaccharides. They can also degrade starch to glucose monomers but are generally limited in their capacity to hydrolyze and utilize other polysaccharides. Therefore, a large quantity of undigested dietary carbohydrate passes into the distal portion of the gastrointestinal tract each day. This material includes polysaccharides from components of plant cell walls (including cellulose, xylan, and pectin) as well as undigested starch. The host's problem of gaining nutritional benefit from these polysaccharides has been solved, in part, by adopting a microbial population that has the ability to degrade these biomolecules.

A very efficient, mutually beneficial arrangement has evolved from a nutritional perspective (see Figure 1). In the proximal gut, bacterial competition for absorbable monosaccharides could be detrimental to the host, but in nonruminants colonization is sparse in this region. Microbial digestion of carbohydrate polymers that reach the distal gut could benefit the host by helping it extract nutrient value from otherwise poorly utilized dietary substrates. Correspondingly, the distal regions of the gut are heavily colonized (90). By recruiting a microbiota that can hydrolyze these carbohydrates, mammals avoid the need to evolve the complex repertoire of glycosylhydrolases that would be required to break down the wide variety of linkages that occur in dietary polysaccharides. The host also acquires flexibility in its capacity to adapt to shifts in the diet. The microbes, in turn, gain access to abundant, readily fermentable carbon sources that would otherwise be worthless to the host.

### Microbial Degradation of Starch

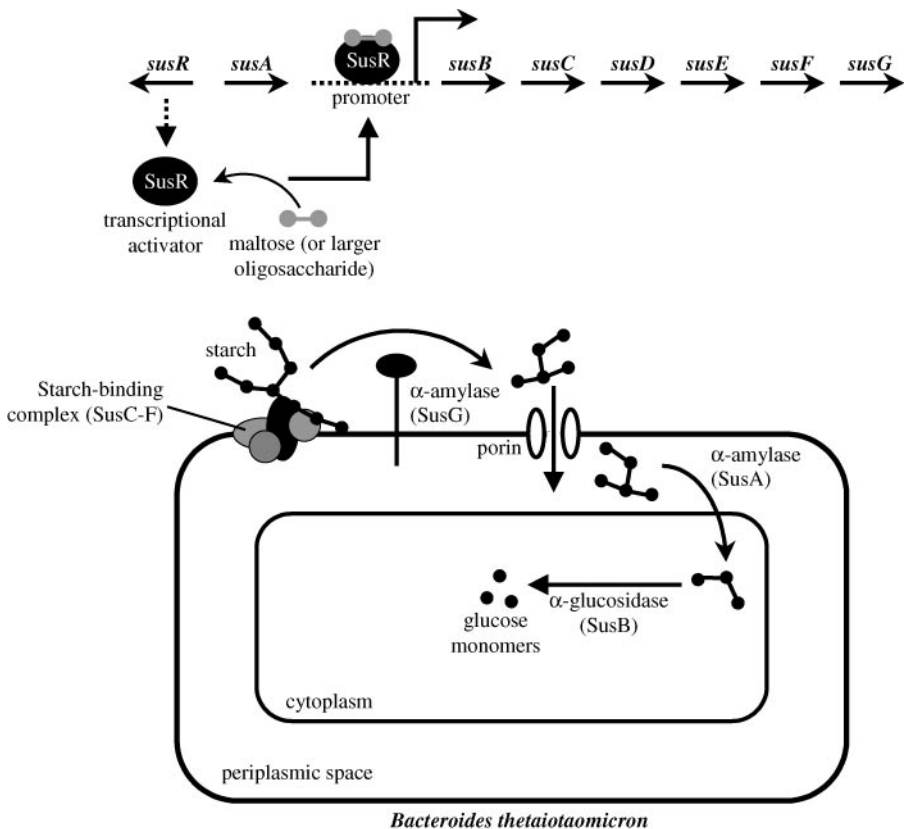
Members of the genus *Bacteroides* are among the most abundantly represented species in the distal human intestine (70). This group of anaerobes can degrade and ferment a wide variety of polysaccharides, including xylan (83), psyllium hydrocolloid (84), and numerous other plant polysaccharides (88). Such saccharolytic capacity may explain, at least in part, why *Bacteroides* species are such



**Figure 1** Overview of host and bacterial contributions to carbohydrate utilization in the intestine. Mammals absorb simple sugars, such as glucose and galactose, via active transport in the proximal regions of their small intestine (29). However, they have limited intrinsic capacity to digest dietary polysaccharides. Undigested polysaccharides such as cellulose, xylan, and undigested starch, as well as host-derived glycans (mucins and glycosphingolipids) pass into the distal regions of the small intestine (ileum) and colon where they are degraded by resident microbes. Bacteria ferment the resulting monosaccharides, and the byproducts of this fermentation (short chain fatty acids) are absorbed and utilized by the host.

successful gut colonizers. Analyses of the molecular details of how *Bacteroides* utilize these polysaccharides provides a useful paradigm for understanding how intestinal microbes sense and exploit nutrients in their environment.

The starch utilization system (*sus*) of *Bacteroides thetaiotaomicron* is the best understood example of polysaccharide utilization. *B. thetaiotaomicron* is a prominent, genetically manipulatable member of the normal mouse and human distal intestinal microflora (70, 82, 106). Eight *B. thetaiotaomicron* genes have been identified that participate in starch metabolism (Figure 2). Seven of these genes (*susA*–*susG*) encode proteins that mediate the initial utilization steps (77). *SusC*, *SusD*,



**Figure 2** Model of *Bacteroides thetaiotaomicron* starch utilization. Eight starch utilization genes have been identified. *SusC*–*SusF* are outer membrane proteins that form a complex (11, 77) that mediates binding of starch to the bacterial cell surface. Bound starch is then digested by  $\alpha$ -amylases encoded by *susA* and *susG* (17). *SusB* is an  $\alpha$ -glucosidase that breaks down oligosaccharides released by *SusA* and *SusG* into glucose (17). *SusR* encodes a transcriptional activator that binds to maltose or larger oligosaccharides and increases transcription of *susA*–*susG* (18).

SusE, and SusF are all outer membrane proteins that interact to form a complex that mediates binding of starch to the bacterial cell surface (11, 77). Bound starch is then hydrolyzed by  $\alpha$ -amylases encoded by *susA* and *susG*. The products of these genes cleave starch at  $\alpha$ 1,4 and  $\alpha$ 1,6 linkages to yield smaller oligosaccharides. SusG is an outer membrane protein, whereas SusA is soluble and is located in the periplasmic space (17). These two proteins act in concert with an  $\alpha$ -glucosidase (SusB) that breaks down their oligosaccharide products to glucose monomers (17). By binding starch to its outer membrane prior to initiating hydrolysis, *B. thetaiotaomicron* can degrade a large polymer into oligosaccharides small enough to pass through outer membrane porins without losing these digestion products to nearby microbial competitors (98). An eighth gene, *susR*, encodes a transcriptional activator that responds to the presence of maltose or larger oligosaccharides by increasing transcription of *susA*–*susG* (18). Thus, the starch binding and degrading activities in *B. thetaiotaomicron* are expressed only if cells are exposed to glucose oligomers. This arrangement is not unique. In this and other examples discussed below bacteria expend energy to express genes involved in nutrient metabolism only when the particular nutrient is available in the gut ecosystem.

## Microbial Degradation of Host-Derived Glycans

In addition to their ability to hydrolyze numerous plant polysaccharides, *Bacteroides* species have also evolved the capacity to degrade a variety of host-derived glycoconjugates (glycans) including chondroitin sulfate (85, 87), mucin (88), hyaluronate (9), and heparin (8). The chondroitin sulfate and hyaluronate degradation pathways in *B. thetaiotaomicron* (Figure 3) have features resembling those of the starch utilization system. As with starch, chondroitin sulfate and hyaluronate are too large to enter the cell through porins. There is evidence that both of these host glycans are first bound to the bacterial cell surface prior to degradation. *csuF* encodes an outer membrane protein that is a candidate receptor for these glycoconjugates: a transposon insertion in *csuF* abrogates growth on intact chondroitin sulfate or hyaluronate but does not disrupt growth on their disaccharide components (10). *B. thetaiotaomicron* degrades chondroitin sulfate and hyaluronic acid to disaccharides via the action of two chondroitin lyases, both of which are located in the periplasm (86). The  $\beta$ -glucuronidase that cleaves the resulting disaccharides to monosaccharides is located in the cytoplasm (86).

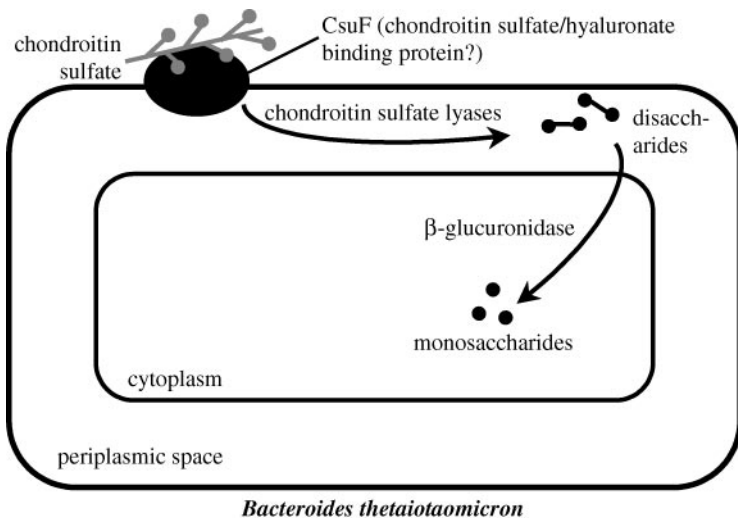
The chondroitin sulfate and heparin utilization pathways are biochemically distinct. However, they are linked by *chuR*, which controls expression of the genes involved in utilization of both glycans (8). Like components of the starch utilization pathway, the hydrolases involved in breakdown of chondroitin sulfate and heparin are inducible: they are only produced when *B. thetaiotaomicron* is grown in the presence of chondroitin sulfate or heparin, or in the presence of their component disaccharides (85). *B. thetaiotaomicron*'s ability to utilize these host glycans appears to be critical for its survival in the intestinal ecosystem. For example, an insertion in *chuR*, which abolishes growth on both chondroitin and heparin, prevents



the mutant strain from successfully competing with an isogenic wild-type strain in cocolonization studies of germ-free intestines (9).

Mucins and glycosphingolipids represent two other classes of host glycans degraded by secreted bacterial hydrolases. Mucins are high molecular weight, heavily glycosylated glycoproteins produced by goblet cells. They serve the host by helping to maintain the integrity of the mucosal barrier (20). Indigenous bacteria have evolved a number of enzymes for breaking down and utilizing the oligosaccharide side chains of host mucins (14). These side chains typically have very heterogeneous and complex structures, with diverse monosaccharide components connected by a variety of glycosidic linkages. Mucin degradation frequently requires the participation of several bacterial species, each of which expresses some but not all of the required glycosidases. However, some members of the *Bacteroides*, *Ruminococcus*, and *Bifidobacterium* genera are able to support complete side chain degradation (67).

Glycosphingolipids constitute another group of host glycans that can be degraded by secreted bacterial glycosidases (27, 55). These glycans are found on gut epithelial cells and have oligosaccharide side-chains attached to a lipid ceramide group. Glycosphingolipids on shed epithelial cells are progressively degraded in the lumen of the intestine (44). There is evidence of an *in vivo* selection for



**Figure 3** Model of *Bacteroides thetaiotaomicron* chondroitin sulfate/hyaluronate utilization. Both chondroitin sulfate and hyaluronate are likely first bound to the bacterial outer membrane prior to degradation. CsuF, an outer membrane protein, is a candidate receptor (10). Chondroitin sulfate and hyaluronic acid are degraded to disaccharides in the periplasm by chondroitin lyases I and II (86). A cytoplasmic  $\beta$ -glucuronidase cleaves the disaccharides to yield monosaccharides (86). These three hydrolases are induced only when *B. thetaiotaomicron* is grown in the presence of chondroitin sulfate or hyaluronate (85).

bacteria that are able to hydrolyze the oligosaccharide chains of glycosphingolipids produced by the individual they colonize. Hoskins & Boulding found that the fecal flora of adult humans with the histo-blood group A phenotype degrade A but not B antigens, whereas the fecal flora of individuals with the histoblood group B phenotype degrade B but not A (45, 46). Members of the *Bifidobacterium* and *Ruminococcus* genera were responsible for this activity of their assay system.

In summary, host glycans appear to be a critical nutrient source for *B. thetaiotaomicron* and other bacterial species. The advantages of being able to utilize these glycans are readily apparent: they are constantly replenished due to epithelial cell turnover; once degraded they are readily fermented to yield carbon and energy; and competition for these glycans is limited, because they are degraded only by some bacterial species (89).

### Induction of Host-Derived Glycans by *Bacteroides thetaiotaomicron*

*B. thetaiotaomicron*'s remarkable capacity to hydrolyze host-derived glycans is accompanied by an ability to actively shape the nature of glycans produced in its host's intestinal epithelial cells. By serving as a nutrient source, these microbe-induced host glycans may help establish a mutually beneficial host-bacterial relationship. This point is illustrated by molecular analyses of fucosylated glycan production in the NMRI inbred strain of mice (7, 43). In conventionally raised NMRI mice, glycoconjugates terminating with  $\text{Fu}\alpha 1,2\text{Gal}\beta$  are first detectable in a small subset of distal small intestinal (ileal) epithelial cells beginning at weaning (postnatal days 14–17). Production of these glycans increases dramatically over the next 7 days to yield the adult pattern of generalized expression in all ileal enterocytes (7). In contrast, the sparse initial expression of enterocytic  $\text{Fu}\alpha 1,2\text{Gal}\beta$  glycans at weaning is completely extinguished by early adulthood in germ-free mice. The weaning period is associated with a dramatic change in the composition of the intestinal microflora from a predominance of facultative anaerobes to a predominance of obligate anaerobes (90). Together, these observations raise an intriguing hypothesis. By matching host carbohydrate structures with the capacity of bacterial species to produce appropriate glycosidases and to utilize the resulting sugars, glycans may serve as a nutrient foundation that helps organize initial colonization of different regions of the postnatal intestine. Bacterial-host and bacterial-bacterial interactions could then shape the metabolic environment in a fashion that permits establishment of more diversified populations of bacterial species. Species diversification could benefit the host by providing new metabolic capabilities at critical developmental/dietary transitions, by supplying microbial factors that influence other aspects of host physiology, and by creating resistance to colonization by potential pathogens that cannot effectively compete with established residents of the microbial community for available nutrients.

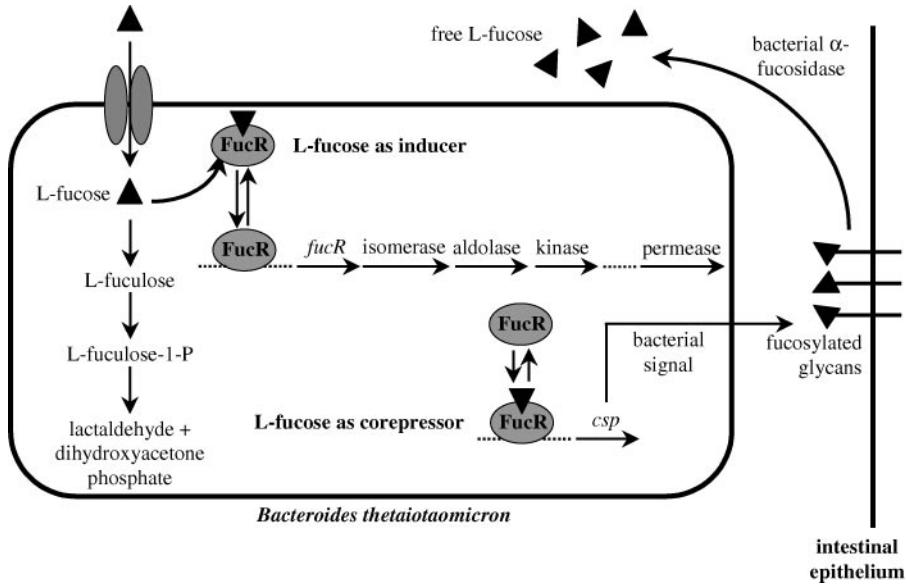
This hypothesis was tested, in part, by examining fucosylated glycan production in adult germ-free NMRI mice in which the capacity to produce ileal enterocytic  $\text{Fu}\alpha 1,2\text{Gal}\beta$ -containing glycans had been extinguished. When an unfractionated

ileal microflora was introduced into these adult germ-free animals, a generalized pattern of Fuc $\alpha$ 1,2Gal $\beta$ -glycan expression was elicited. The time course of induction, as well as the spatial and cellular patterns of fucosylated glycan production, phenocopied what occurs in the distal small intestines of normal conventionally raised mice during weaning.

These results support the idea that sustained production of enterocytic Fuc $\alpha$ 1,2Gal $\beta$ -glycans is dependent on one or more components of the microbiota (7). Colonization of adult germ-free mice with *B. thetaiotaomicron* alone can induce a pattern of epithelial Fuc $\alpha$ 1,2Gal $\beta$ -glycan expression that recapitulates the effects of a complete microflora (7). *B. thetaiotaomicron*'s ability to evoke production of these glycans is dependent on the density of colonizing organisms. It is also not a general effect of intestinal colonization: i.e., some other prominent members of the distal intestinal microbiota are unable to direct production of ileal Fuc $\alpha$ 1,2Gal $\beta$ -containing glycoconjugates (7).

Because *B. thetaiotaomicron* is genetically manipulatable (82), some of the molecular details of how this bacterium influences host glycan expression are now apparent. A combination of genetic and biochemical studies of the *B. thetaiotaomicron* fucose utilization pathway disclosed a functional connection between bacterial fucose utilization and stimulation of host Fuc $\alpha$ 1,2Gal $\beta$ -glycan production (43). The fucose utilization gene cluster in this organism consists of five genes, four of which encode proteins directly involved in fucose uptake (FucP, a permease) and metabolism (an isomerase, aldolase, and kinase; FucIAK) (see Figure 4). The first open reading frame in the gene cluster specifies a transcriptional repressor (FucR). Monomeric fucose functions through FucR as an inducer of the isomerase, kinase, and aldolase genes, as well as the gene encoding FucR itself. In the absence of fucose, FucR represses transcription of these genes by binding to sequences in a promoter located directly upstream of the *fucRIAK* operon (43). The *fucP* (permease) gene is not regulated by FucR and is constitutively expressed in the presence or absence of fucose.

Colonization of germ-free mice with mutant strains of *B. thetaiotaomicron* harboring engineered disruptions of these fucose pathway genes revealed that FucR is the critical link between bacterial fucose utilization and bacteria signaling of host enterocytic Fuc $\alpha$ 1,2Gal $\beta$ -glycan production. Disruption of the isomerase gene (*fucI*) prevents conversion of fucose to L-fuculose and blocks the ability of the bacterium to signal its host to manufacture fucosylated glycans. In contrast, isogenic mutant strains with disruptions of genes encoding any one of the other enzymes involved in fucose breakdown are capable of initiating but not completing degradation of fucose and retain the ability to signal the host to synthesize Fuc $\alpha$ 1,2Gal $\beta$ -glycans. Importantly, a strain containing a polar mutation of *fucR* that blocks transcription of *fucR* plus downstream genes in the operon (including *fucI*), is able to elicit host fucosylated glycan synthesis. This result suggested that, in addition to regulating transcription of the fucose pathway enzymes, FucR mediates transcriptional repression of a second genetic locus that is distinct from the fucose utilization operon. The product of this second locus is postulated to play a role in generating the bacterial signal that elicits Fuc $\alpha$ 1,2Gal $\beta$ -glycan synthesis.



**Figure 4** Model of how *Bacteroides thetaiotaomicron* induces production of Fuc $\alpha$ 1,2Gal $\beta$ -containing glycans in the distal intestinal epithelium. L-fucose induces the bacterial fucose utilization operon that encodes FucR plus the enzymes involved in fucose metabolism (note that fucose permease is constitutively expressed and is not a member of the operon). In this model FucR also functions as a corepressor at another locus, control of signal production (*csp*), that regulates signaling to the intestinal epithelium. Signaling induces Fuc $\alpha$ 1,2Gal $\beta$ -glycans in absorptive enterocytes. These glycans are postulated to function as a nutrient source for *B. thetaiotaomicron*.

This second locus, designated control of signal production (*csp*), has not yet been directly identified, although its existence is inferred from the genetic experiment.

The ability of FucR to coordinate fucose consumption by the microbe and fucosylated glycan production by the host appears to occur through a distinctive regulatory mechanism. Figure 4 presents a model consistent with the observed fucose utilization and host signaling phenotypes of the various isogenic mutant *B. thetaiotaomicron* strains described above. In the model, fucose acts through FucR to induce transcription of the fucose utilization operon and to repress *csp*. This explains the nonsignaling phenotype of *fucI* mutants. Because this enzyme catalyzes the first enzymatic step in the breakdown of fucose, its absence leads to an accumulation of fucose in the bacterium. Fucose accumulation, in turn, increases the proportion of fucose-bound FucR and thereby silences *csp*. The model predicts that lowering intracellular fucose levels will shift FucR to its fucose-unbound form and release *csp* from inhibition so signaling can occur. In fact, disruption of the constitutively expressed fucose permease gene reduces import of fucose into *B. thetaiotaomicron* and promotes signaling (43).

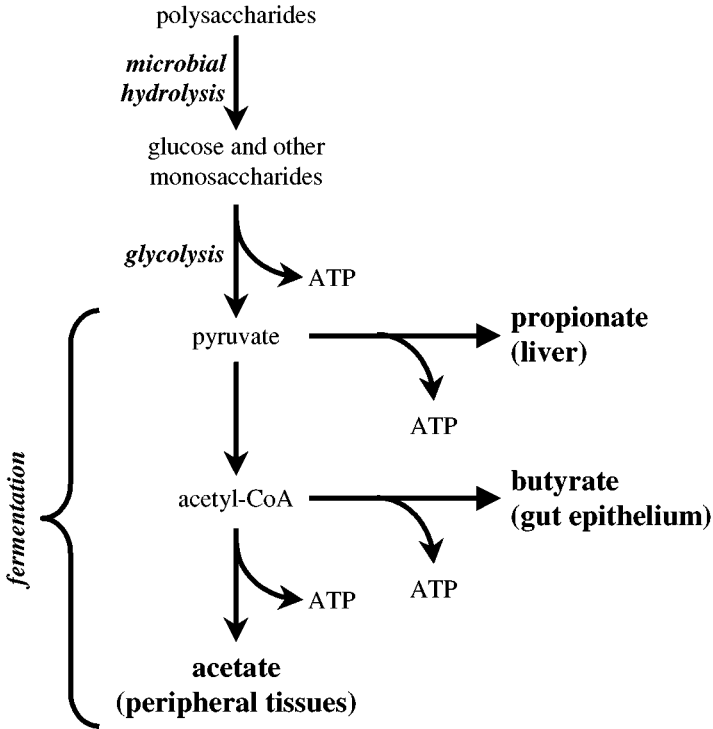
This model postulates that FucR acts as a molecular sensor of environmental fucose availability and matches bacterial demands for fucose with supply. When the bacteria have ample fucose, fucose-bound FucR allows expression of bacterial genes required for breakdown of the available pentose sugar, and turns off production of the *csp*-dependent signal that tells intestinal cells to synthesize more Fuc $\alpha$ 1,2Gal $\beta$ -glycans. Conversely, when fucose is scarce, unbound FucR allows transcription of *csp* to occur, resulting in a “request” to the host for more fucose.

Engineering production of its own nutrient source in the intestinal epithelium makes sense for a gut commensal such as *B. thetaiotaomicron*. Like other strict anaerobes, it colonizes that intestine at weaning, when this ecosystem is densely populated with a preweaning microflora that limits the availability of nutrient sources. The sparse preformed pool of fucosylated glycans present at the beginning of weaning may provide sufficient hydrolyzable fucose to promote initiation of *B. thetaiotaomicron* colonization. Once a critical density of organisms is attained, the population can signal the host to provide a sustained supply of host Fuc $\alpha$ 1,2Gal $\beta$ -glycans. Consistent with this view, *B. thetaiotaomicron* secretes an  $\alpha$ -fucosidase activity that can cleave the terminal fucose residues present in these Fuc $\alpha$ 1,2Gal $\beta$ -glycans (43). The bacterium is able to conserve energy by signaling only when fucose supplies are low. The host, in turn, synthesizes only as much glycan as is necessary to support the nutritional needs of at least one of its commensals. The host benefits by gaining some control over the composition of its microflora and may be able to use localized expression of such glycoconjugates to “direct” colonizing organisms to the distal portions of the intestine, thus avoiding competition for absorbable monosaccharides in the proximal regions of the gut.

The stage is set to directly test these notions by loss-of-function or gain-of-function experiments conducted in genetically engineered germ-free mice. Knock-out mice that lack a glycosyltransferase normally expressed in the gut (e.g., the  $\alpha$ 1,2 fucosyltransferases encoded by *FUT1*, *FUT2*) (21, 22), or transgenic mice with engineered gut-specific expression of glycosyltransferases that allow novel carbohydrate structures to be produced in specified regions of their intestines (25), could be colonized with one or more members of the gut microflora (including isogenic strains of bacteria with and without the ability to utilize the relevant host glycan). The distribution and selection of these bacterial species could then be followed in the intestines of the resulting ex-germ-free animals.

## Host Utilization of Microbial Fermentation Products

Allowing indigenous microbes to assume some of the responsibility for carbohydrate breakdown presents a potential problem: How does the host derive nutritional benefit from the products of this breakdown? Monosaccharides released from carbohydrate polymers are converted in bacterial cells to pyruvate via glycolysis, resulting in net production of adenosine triphosphate (ATP) (Figure 5). Because the distal intestinal lumen is a highly anaerobic environment, additional carbon and energy is extracted from pyruvate by microbial fermentation. To recover some



**Figure 5** Overview of bacterial fermentation in the intestine. Monosaccharides released from carbohydrate polymers are converted in the bacterial cytoplasm to pyruvate via glycolysis, which results in net production of ATP. In the highly anaerobic environment of the distal intestine, additional carbon and energy is extracted from pyruvate by microbial fermentation. The predominant end-products of fermentation are acetate, propionate, and butyrate. To recover some of the nutritional value of polysaccharides degraded by gut microbes, mammalian hosts absorb and utilize these short chain fatty acid species.

of the nutritional value of microbially degraded polysaccharide, mammals have evolved mechanisms for absorbing and utilizing products of bacterial fermentation.

The predominant end products of bacterial fermentation in the gut are short chain fatty acids (SCFA). The three major SCFA produced in the colon are acetate, propionate, and butyrate (Figure 5). In humans, the molar ratio of these three SCFA is approximately 70:20:10. Their aggregate concentration ranges from 70 to 120 mM (5, 47, 68, 111). However, these ratios and concentrations are influenced by diet. Conversion of pyruvate to any of these SCFA yields an additional molecule of ATP. Even after microbial extraction of ATP, SCFA production appears to represent 60–75% of the energy content of ingested carbohydrate (5).

Much of our understanding of the nutritional value of SCFA comes from studies of ruminants. The evolutionary success of ruminants in the wild is founded on their

ability to feed on cellulose-rich plants. These animals harbor a microbiota in their rumen that is responsible for digestion of carbohydrate polymers [for a review of rumen microbial ecology, see (80)]. Because fermentation occurs in the proximal regions of the ruminant gut, only small amounts of monosaccharide (including glucose) are absorbed by the animal. Thus, SCFA constitute the major source of ruminant energy (as much as 70% of caloric requirements) (5).

Humans and rodents produce considerably less SCFA than ruminants. In humans on European diets, 50–60 g of carbohydrate are typically fermented per day, yielding 0.5–0.6 mol of SCFA, with a total energy value of 140–180 kcal (~10% of the maintenance caloric requirement) (5, 59). However, human diets vary widely with respect to the amount of fiber, and this affects the amount and type of SCFA produced (16). Unfortunately, we have a very limited understanding of the role of SCFA in human nutrition or of the impact of diet on this role.

In nonruminants, SCFA are absorbed in the cecum and the colon by passive diffusion across the epithelium (79). Acetate, propionate, and butyrate are each ultimately taken up by different organs and have different metabolic fates. Butyrate is metabolized primarily by the intestinal epithelium, where it is converted to ketone bodies or oxidized to CO<sub>2</sub> (75). The colonic epithelium derives 60–70% of its energy needs from butyrate (78). In fact, studies of isolated colonocytes have shown that metabolism of butyrate suppresses glucose oxidation (2, 30). Propionate is transported to the liver via the portal vein. In ruminants, intestinal glucose uptake is minimal, and propionate is therefore an important precursor for gluconeogenesis (6). Much less is understood about the role of propionate metabolism in humans. Acetate is taken up primarily by peripheral tissues such as skeletal and cardiac muscle (16) and can also be utilized by adipocytes for lipogenesis (5). The relative levels of acetyl-CoA, propionyl-CoA, and butyryl-CoA synthetases in different tissues appear to determine which tissue metabolizes which SCFA (3, 5).

In addition to their nutritional value, SCFA have important effects on other aspects of gut physiology. For example, SCFA are the predominant anions in the colon, and net absorption of water is coupled with sodium, chloride, as well as SCFA transport (48). SCFA have also been implicated in stimulating intestinal blood flow (73, 74). SCFA, particularly butyrate, affect epithelial proliferation and differentiation (33, 51, 53, 72, 99, 109). Recent studies have sought to identify genes whose expression is impacted by SCFA (4). Coupling these types of functional genomic analyses with enumeration studies of the gut microbiota should help provide a molecular explanation for epidemiological data that suggest that dietary fiber reduces the incidence of colorectal cancer (12, 76).

## Microbial Contributions to Amino Acid Homeostasis

The gastrointestinal microbiota can also contribute to amino acid homeostasis in the host. This contribution is most pronounced in ruminants, where dietary sources of nitrogen are first subjected to ruminal metabolism prior to host digestion and where microbial amino acid synthesis is critical for the nutritional well-being of the host (15). Remarkably, ruminants can survive on diets lacking protein because

their microbiota is able to synthesize the complete complement of amino acids from ammonia or urea (108).

In nonruminants, microbial protein is generally lost unless the animal practices coprophagy (ingestion of feces, which is common in rodents). However, at least some of the human requirement for certain amino acids is met by microbial synthesis. Using a method in which microbial amino acids are specifically labeled with  $^{15}\text{N}$  (105), Metges et al. (62, 63) concluded that 1–20% of circulating plasma lysine and threonine in adult humans is derived from the intestinal microbiota [also see (61)]. Urea generated by host tissues passes into the gut and is hydrolyzed to ammonia by the intestinal microflora (31). For example, high concentrations of urea are found in the colons of germ-free rats (71), indicating that microorganisms play a key role in nitrogen recycling in the intestine.

### Vitamin Synthesis by the Gut Flora

Vitamin synthesis by gut bacteria has been recognized for many years. Germ-free rodents require vitamin K in their diets, in contrast to those that are conventionally raised (113). Some germ-free animals also require higher amounts of certain B vitamins (e.g., B<sub>12</sub>, biotin, folic acid, and pantothenate) than their conventional counterparts (103). These vitamins are synthesized by several intestinal genera, including *Bacteroides*, *Eubacterium*, *Propionibacterium*, and *Fusobacterium* (1, 39). However, the presence of appreciable quantities of these B vitamins in feces suggests that the majority may be associated with bacterial cells and are thus unavailable for uptake by noncoprophagic hosts.

## MICROBIAL MANIPULATIONS OF THE HOST METABOLIC MACHINERY

As discussed above, one driving force for retaining a complex microbiota in the intestine is that commensals and symbionts aid the host in extracting maximal nutritional value from a diet. This extraction not only involves direct microbial degradation of dietary macromolecules but also microbial induction of intestinal genes that facilitate recovery of nutrients.

A combination of high-density oligonucleotide-based microarrays containing probe-sets representing 25,000 mouse genes, laser capture microdissection, and real-time quantitative reverse transcriptase-PCR (qRT-PCR) has been employed to comprehensively profile changes in gut gene expression during the course of colonization with components of the normal microbiota and to determine the cellular basis of the host response (41, 42). mRNA populations in germ-free and ex-germ-free intestines were first compared with DNA microarrays to identify genes that underwent induction or repression during colonization. Laser capture microdissection (24, 100) was then utilized to retrieve specified cell populations from frozen intestinal sections, prior to and after colonization. Use of laser capture microdissection allows the influence of surrounding cells and the luminal environment on cellular gene expression to be preserved. Finally, qRT-PCR assays of RNA



recovered from laser capture microdissected cells permitted quantitative measurements of colonization-associated changes in the levels of specific mRNAs.

These approaches disclosed that *B. thetaiotaomicron* colonization of germ-free mice produced changes in expression of a number of host genes involved in the processing and absorption of carbohydrates, as well as the breakdown and absorption of complex dietary lipids. For example, colonization led to increased ileal expression of the Na<sup>+</sup>/glucose co-transporter, the principal mediator of glucose uptake from the lumen into the epithelium (38). There was a concerted increase in expression of at least four components of the host's lipid absorption machinery: pancreatic lipase-related protein 2 (57), which breaks down triacylglycerols; co-lipase, which stimulates pancreatic lipase-related protein 2 activity; L-FABP, a cytosolic fatty acid-binding protein involved in intracellular trafficking of fatty acids; and apolipoprotein A-IV, which mediates export of triacylglycerols once they are resynthesized within the enterocyte. In addition, colonization of germ-free mice with *B. thetaiotaomicron* results in a decrease in expression of fasting-induced adipocyte factor (FIAF). PPAR $\alpha$ , an important regulator of shifts in cellular energy substrate preferences when nutrient environments change, controls FIAF transcription. FIAF transcription is known to be induced in both liver and adipocytes by fasting (50). Its repression in the intestine during colonization with *B. thetaiotaomicron* is thus consistent with an accompanying augmentation of host lipid absorption.

Developmental studies of PPAR $\alpha$  and FIAF expression in the small intestines of germ-free mice revealed that their mRNAs increase dramatically during the suckling-weaning transition and continue to rise to adulthood. In contrast, expression in conventionally raised mice is low and monotonous (L. Hooper, J. Manchester, J. Gordon, unpublished observations). It is tempting to speculate that because shifts in fatty acid metabolism are often accompanied by shifts in carbohydrate metabolism, microbe-induced changes in expression of PPAR $\alpha$  and its FIAF target may be accompanied by concomitant changes in the host's capacity to metabolize sugars. Furthermore, based on the "fasting paradigm," decreases in expression of PPAR $\alpha$  targets (e.g., FIAF) may be accompanied by a corresponding decrease in gluconeogenic capacity of the intestinal epithelium. Colonization of germ-free mice with members of the microbiota such as *B. thetaiotaomicron* provides an opportunity to assess the intestine's role in gluconeogenesis and whether its gluconeogenic activity is modulated by members of its microbiota. One model is that as members of the microbiota liberate simple sugars from more complex dietary carbohydrate polymers, the resulting increased intestinal carbohydrate uptake leads to decreased reliance upon fats as an energy source and a corresponding decrease in the need for gluconeogenic capacity.

The effects of colonization of germ-free mice with *B. thetaiotaomicron* extends to genes involved in regulated absorption of dietary metal ions. There is an increase in expression of an epithelial high affinity copper transporter (CRT1), as well as decreases in expression of metallothionein-I and -II, which sequester heavy metals such as copper and zinc. An increase in the capacity to absorb heavy metals (via CRT1) and a decrease in the capacity to sequester them (via metallothioneins)

suggests greater host demand for these compounds, either due to increased utilization by host metabolic pathways or competition with the microbe.

## PROBIOTICS AND PREBIOTICS: MANIPULATING THE MICROBIAL COMMUNITY THROUGH DIET

At the beginning of the twentieth century, Pasteur's colleague Elie Metchnikoff first proposed manipulating the microflora to benefit human health (60). Since then there have been numerous efforts to shape the species composition and metabolic activities of the gut microflora through diet. In its simplest form, components of the normal microflora are administered as live dietary supplements (probiotics) that confer some benefit to the host. The best-known examples of probiotic supplements are the *Lactobacilli* and *Bifidobacteria* in yogurt and other dairy products. These supplements can alleviate symptoms of lactose intolerance (52, 69, 97).

Additional efforts to beneficially alter the gut microbial community have centered on the use of prebiotics, which are defined as nondigestible food ingredients (usually oligosaccharides) that are selectively utilized by one or more components of the normal microflora. The original hope was that many gastrointestinal disorders could be treated prophylactically by targeting specific bacteria (e.g., *Bifidobacteria*) with compounds such as oligofructose and inulin (34). There have been some intriguing results suggesting that certain prebiotics may have beneficial effects, including increased absorption of minerals (95), reduced serum lipid levels (19), and even anticarcinogenic activity (112). However, without the ability to comprehensively enumerate the microbiota in individuals prior to, during, and after treatment with pre- or probiotics, it is difficult to determine the mechanisms that underlie any of these observed host responses.

## THE FUTURE

At the beginning of the twenty-first century we are poised to explore the contributions of the gut microbiota to our nutrition at a molecular level. The use of germ-free technology and genetically manipulatable model organisms, coupled with functional genomics approaches, has resulted in powerful experimental systems for defining the relative contributions of host factors, the microbiota, and the diet to shaping the nutrient milieu of the intestine.

Despite the importance of the microflora to human nutrition and physiology, the components of the human microbiota remain poorly defined. Molecular methods, such as broad-range sequencing of 16S rRNA genes will undoubtedly facilitate definition of microbial diversity in the normal gut and how its bacterial communities respond to changes in the diet (107). In addition, the capacity to define biodiversity in humans is a prerequisite for any efforts to design, follow, and interpret pre- or probiotic experiments designed to change the digestive properties of the "intestinal bioreactor." Studies of the microflora in lean and morbidly obese human

populations (or in isogenic mice with and without mutations that predispose to obesity) may provide insights as to whether the composition of the microflora is a risk factor for obesity (by promoting augmented extraction of calories from the diet). A related principle is that the caloric value of a diet should not be viewed as a fixed entity but rather as a value influenced by the consumer's intestinal microbiota.

The marriage of germ-free technology (gnotobiotics) and genetically defined mouse models creates an opportunity, unavailable in human populations, to define microbial ecology along the length of the gut, at various stages of development, under rigorously controlled environmental conditions and then to decipher the consequences of dietary/probiotic/prebiotic interventions. The lessons learned could then be utilized for human clinical trials.

Finally, the capacity to enumerate the microbiota represents a first step in understanding molecular contributions of this microbial society to human physiology. To fully define our own metabolic potential, it will be necessary to define the metabolic potential of our microbiota. This effort should include initiation of a systematic effort to sequence the microbiome (40) and to develop methods for monitoring microbial gene expression in vivo (first in gnotobiotic mouse models and later in humans). Over our evolutionary history, components of the intestine's microbiota have endured a stringent selection to become "master physiologic chemists": i.e., they have had to develop chemical strategies for regulating nutrient processing in ways that benefit themselves and us. By identifying these host genes and the microbial effectors of their expression/function, we should be able to identify new molecular targets and new chemical strategies for manipulating nutrient processing, uptake, and utilization.

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