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Understanding the gut microbiota by considering human evolution: a story of fire, cereals, cooking, molecular ingenuity, and functional cooperation

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SUMMARY The microbial community inhabiting the human colon, referred to as the gut microbiota, is mostly composed of bacterial species that, through extensive metabolic networking, degrade and ferment components of food and human secretions. The taxonomic composition of the microbiota has been extensively investigated in metagenomic studies that have also revealed details of molecular processes by which common components of the human diet are metabolized by specific members of the microbiota. Most studies of the gut microbiota aim to detect deviations in microbiota composition in patients relative to controls in the hope of showing that some diseases and conditions are due to or exacerbated by alterations to the gut microbiota. The aim of this review is to consider the gut microbiota in relation to the evolution of *Homo sapiens* which was heavily influenced by the consumption of a nutrient-dense non-arboreal diet, limited gut storage capacity, and acquisition of skills relating to mastering fire, cooking, and cultivation of cereal crops. The review delves into the past to gain an appreciation of what is important in the present. A holistic view of "healthy" microbiota function is proposed based on the evolutionary pathway shared by humans and gut microbes.

KEYWORDS gut microbiota, microbiome, human evolution, cereals, starch, hemicelluloses, non-starch polysaccharides, bacterial consortia

THE HUMAN GUT MICROBIOTA

Microbial DNA is detected in the stomach, duodenum, and jejunum of humans, but biomass is low. *Helicobacter pylori* colonizes the stomach and duodenum of humans, but socioeconomic factors influence prevalence (1–4). Some DNA signatures detected in upper gastrointestinal tract samples may represent transient organisms entering the gut in water, food, or saliva (1–4). Further research is needed to clarify

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the situation. Microbial numbers are appreciable in the distal ileum (about 10^8 per milliliter of contents), but study of this microbiota is hampered by limited access and artefacts produced by sampling methods (ileostomy waste has been examined mostly) (4–6). The human colon, however, is inhabited by a diversity of microbial species, about 99% of which are bacteria, totaling around a trillion cells (7). They are predominantly members of the phyla *Firmicutes* (~56% relative abundance) and *Bacteroidota* (~28%), although there are large variations in proportions between individual humans. Two bacterial families belonging to the *Firmicutes*, *Lachnospiraceae*, and *Ruminococcaceae*, each comprise 20%–30% of colonic microbiotas (8). The phyla *Actinobacteriota* and *Proteobacteria* are also represented (about 5% and 8%, respectively) but are of greater proportions in neonates compared to adults (9–11). Knowledge of the colonic microbiota, hereafter referred to as the "gut microbiota," is mostly inferred from the study of feces because these are relatively easily obtained samples. Dwayne Savage, a pioneer of research about the gut microbiota, warned that, "Feces are processed waste … investigators must be cautious about attempting to describe the ecosystem in terms of their (fecal) findings and should endeavor where possible to amplify the findings by examining samplings taken from all areas of the system" (12). This sage advice has largely been ignored, so for most studies, gut microbiota equals fecal microbiota. Fortunately, John Cummings and George Macfarlane studied functional outputs (particularly production of short-chain fatty acids) of the gut microbiota *in situ* and demonstrated the importance of the proximal colon as the site where much of the microbial fermentation of carbohydrates derived from food occurs (13). The bacterial cells detected in feces belong to 75–160 bacterial species per human, although about 1,000 species are probably represented in humans in general because taxonomic composition of the microbiota is highly individualistic (14–17). Metagenomic analysis of bulk DNA extracted from feces has provided detailed knowledge of the gut microbiota in terms of taxonomy and biochemical pathways (functional capacity) (14) but is dependent on the "depth" of sequencing, which relies on the number of sequencing "reads" that are generated per sample. Biochemical information derived from metagenomic annotations may also be unreliable, however, due to mistakes in automated annotations, inappropriate annotation in the biological context, and unknown and orphan proteins (18–20). Current research mostly aims to establish causality between the composition of the gut microbiota and various disease states (21–23). Indeed, alleged bioinformatic associations between microbiota composition and diseases have spread to social media, where the microbiota is commonly posted as optimizing physical well-being and mental health. These are tantalizing propositions, but medical treatments to capitalize on possible causative relationships remain elusive. Deficits of gut microbiota research include emphasis on observational and descriptive studies, comparisons at a high taxonomic level (for example, *Firmicutes*/*Bacteroidota* ratio), ignorance of confounding factors such as habitual dietary intake and variable gut transit times of humans, and lack of appreciation of microbial ecology (17, 24–29). Development of an evolutionary perspective of the human gut microbiota association could provide a basis for an improved understanding of the relationship between microbiota and health. This review aims to provide just such an evolutionary perspective, delving into the past to provide an appreciation of what is important in the present.

THE PATHWAY TO *HOMO SAPIENS*

Hominids diverged evolutionarily from the last ape ancestor about six to eight million years ago (30). Early humans (*Homo*) had a smaller sized gut compared to forest-dwelling, ape-like predecessors because diets were different. Early humans such as *Homo erectus* favored foods with a high density of nutrients such as meat and fat, whereas ape relatives eat diets containing large amounts of fibrous material (leaves and fruits) (31). Hence, the gut of apes contains large, expanded regions (cecum, colon) where bulky food can be stored and digested slowly. Although humans also have an expanded large bowel, it has little storage capacity: less than 60% of the mass expected for a primate of

human body weight and an only slightly greater capacity to that of carnivores (32). The stomach of humans is small and capable of mechanical, enzymatic, and acid breakdown of food. The small intestine is much longer in humans compared to apes, enabling rapid digestion and absorption of lipids, simple carbohydrates, and protein (Fig. 1A) (33). The smaller gut of omnivore meat eaters compared to herbivores aided walking upright because the rib cage no longer flared outward to encase the large gut organs. Arms could be swung more easily when walking and running, and center of gravity of the body was different (34).

More proficient, bipedal posture combined with binocular vision gave a new outlook on life for early humans, and this assisted in hunting food, which, initially, was probably more like scavenging than hunting. Human walking is slow compared to most other mammals, but if they have water, humans have better physical endurance (aided by the spring-like, energy-saving, action of the Achilles tendon) (38). Potential prey could outrun human hunters but had to stop after a relatively short time to cool down and restore energy levels (39). Humans, however, are capable of dogged pursuit and eventually will wear down the quarry. Nevertheless, the remains of animals left over from the activities of other predators were probably important as food. Bush fires—adventitious killing and cooking of prey—provided roasted flesh which was much more easily eaten and digested than raw meat (39, 40). Later (about 1.5 million years ago), early humans learned to manage fire so that it could be carried between campsites or started anew by striking flints or rubbing pieces of wood together (41, 42). This meant that meat could be cooked at will, making consumption easier and aiding the release of nutrients once ingested (41, 43). Eventually, carcasses were butchered in community sites using stone

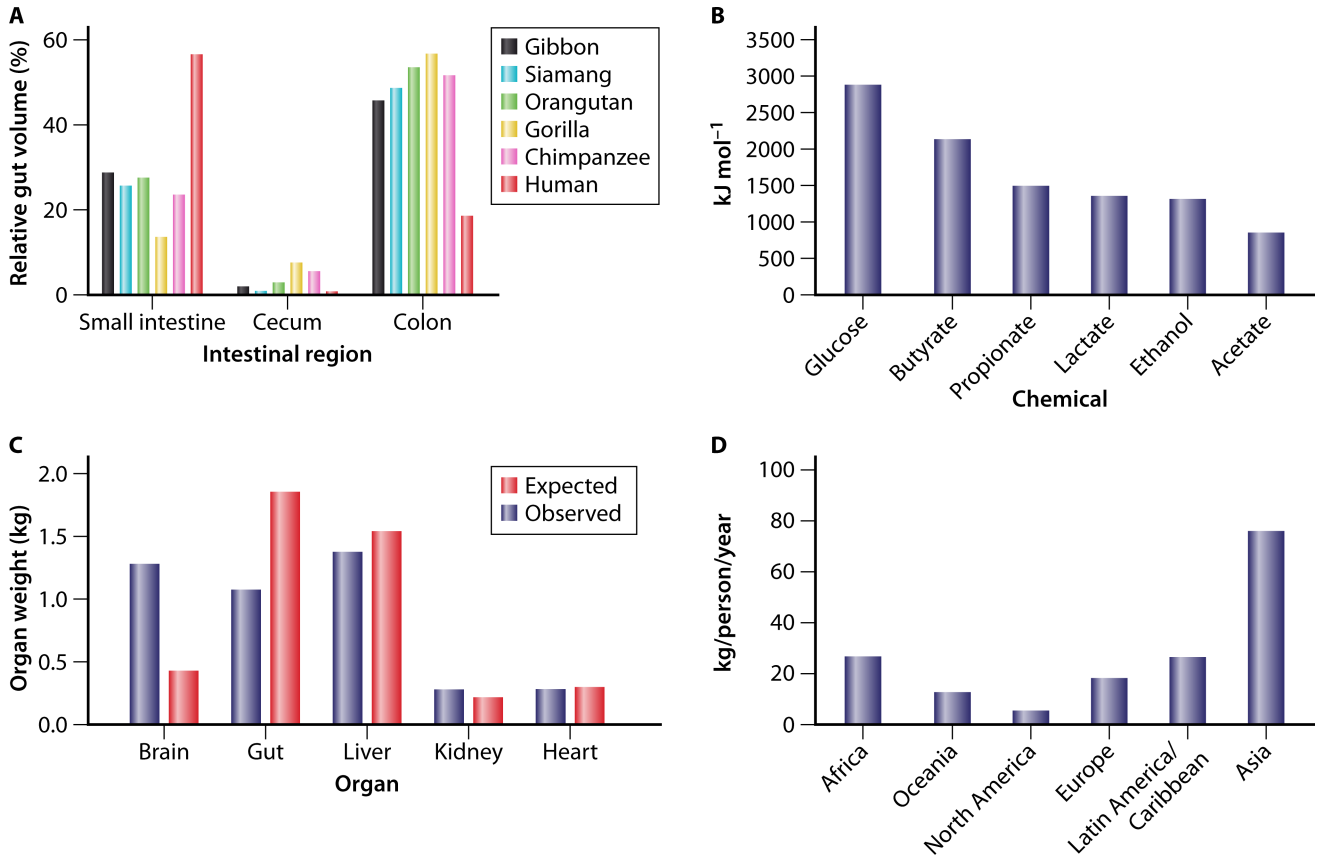

FIG 1 (A) Relative volume of gut regions of primates. Data from reference (33). (B) Comparison of energy yield from combustion of glucose and bacterial fermentation products with oxygen at 1 atm and 37°C. Data from references (35) and (36). (C) Observed and expected organ mass for a 65-kg human. Data from reference (37). (D) Rice per capita consumption 2019–2021. Data from OECD/FAO (2022), "OECD-FAO Agricultural Outlook," OECD Agriculture statistics (database), http://dx.doi.org/10.1787/agr-outl-data-en.

cutting tools and later stone axes for slicing meat and smashing bones to extract marrow which was easy to eat and highly nutritious (39). Dentition changed because incisors for slicing meat were more important than reliance on canines to split open fruits, and large molars to grind leaves and chew coarse fruits were less significant (44, 45). Large, strong jaw muscles became less important as well, especially once humans became cooks and could tenderize their food by this means (45). As jaw muscles diminished in size, more space was freed within the skull, the balance of the skull was altered, with a rise in height and an increase in frontal lobe areas. This made possible new frontal sections of the brain capable of higher intellectual processes. The different balance of the modified skull altered the attachment to the spinal column, assisting upright posture (46). The better-quality diet of humans meant that glucose became the basic body fuel rather than short-chain fatty acids (SCFAs) derived from microbial fermentation of plant fibers, which is the case for herbivores. The relative amounts of energy derived from glucose compared to SCFAs (Fig. 1B) emphasizes the advantage of high-density diets for humans (35). Nevertheless, foraging for roots and tubers of plants to provide additional calories was important, as evidenced by the activities of the few remaining hunter-gatherer societies (47). However, the dietary fiber eaten by hunter-gatherers, although of sizable daily intake compared to that of an urbanized human today (70–130 grams versus 20–40 grams), was coarse and fibrous. The fruits and vegetables that were eaten were unlike the sweet cultivars that have been developed for consumption by modern humans because they did not contain much sucrose and fructose (33, 47, 48). The important nutritional components in the roots and tubers of the hunter-gatherer diet are starch and hemicelluloses, which will be discussed in more detail later.

Modern humans, *Homo sapiens*, arose in Africa 300,000 to 200,000 years ago and eventually spread to all parts of the world, outcompeting other human species such as the Neanderthals which consequently became extinct (39). The most striking feature of *H. sapiens* in relation to earlier humans is brain size (*H. sapiens* cranial volume, ~1,400 mL; *Homo erectus*, ~900 mL; *Homo habilis*, ~650–800 mL) and organization. Development of a larger, two-hemisphere brain is the secret to *H. sapiens* success because abstract thought and ability to learn, store information, conceptualize, plan, empathize, consider the needs of the community, and articulate and communicate the results of all these intellectual activities using complex language developed (41, 49). However, all this brain power requires a high input of energy. The brain is an energy-expensive tissue, and it becomes a question of how much brain power the body can afford. The metabolic requirement of a relatively large brain was offset by a corresponding reduction in the size of another energy expensive organ: the gut (Fig. 1C) (37). A smaller gut still needs to extract and absorb as many nutrients per body mass as a larger gut, and this can only be achieved by consumption of a high-quality (energy-rich) diet. Rapid increase in cranial capacity seems to have occurred from around the time of *H. erectus*, which is 1.9 to 1.8 million years ago, so from that it may be inferred that gut size and, hence, habitual diet changed around this point (41). If diet changed, then, it may be supposed that adaptation of gut inhabitants, through changes in genetic features and/or abundances of taxa, was set in train at about this time because we know that the microbial community, for the most part, lives on dietary components (47, 48, 50).

EVOLUTION OF THE GUT MICROBIOTA

Prokaryotic life began 3 to 4 billion years ago, so there was ample time for the evolution of fermentative bacterial species capable of life in anaerobic habitats like the colon before *Homo sapiens* came on the scene. Anaerobic bacteria capable of deriving carbon and energy by the degradation and fermentation of plant materials were probably ingested by animals with food, and continuing fermentation in the gut resulted in a chemically reduced environment. Hence, anaerobic ecosystems within the gut, such as the rumen, caecum, and colon of herbivorous mammals developed, and a preponderance of obligate anaerobes inhabited these sites (8, 13, 14, 51–54). Gut architecture and habitual (preferred) diets encouraged the development of gut microbiotas characteristic

of animal groups; differences are still recognizable today (55–58). In other words, there were opportunities for the co-divergence of bacterial communities recognizable as being characteristic of the primate or other gut. Indeed, using genetic comparisons, a parallel evolutionary history of some members of modern microbiotas with that of humans can be recognized. *H. pylori* provides the clearest example of genetic diversity in relation to human migrations (59–64).

Charles Darwin recognized that extant animal species on isolated islands were the result of an adaptive radiation of a single ancestral lineage. It is probable that the same kind of process occurred with bacteria in the human gut. Adaptive radiation is a process of evolutionary divergence in which single phylogenetic lineages diversify rapidly into multiple new forms that co-exist by occupying different niches, particularly when a change in the environment makes new resources available (65–67). The "founder" lineages must be able to acquire new properties in order to occupy the novel niches provided by the new habitat (evolvability), but they must also have a set of attributes that permit initial establishment in that habitat (ecological opportunity) (66). Although founders benefit from priority of establishment, there is no evidence of monopolization by them in mature (adult) gut microbiotas (68). Using *in vitro* cultures of *Escherichia coli* as a model, it was shown that the ancestral lineage adapted to the growth-limiting resource (for example, glucose) but then diverged into multiple specialties by partitioning of the limited resource through emergence of glycerol and acetate utilizers (metabolites produced by the ancestral lineage from glucose) (69). Cross-feeding (syntrophy) of short-chain fatty acids and vitamins is common among bacterial species inhabiting the human gut, providing support for theoretical explanations of the evolution of a biodiverse microbiota through "public good" activities (70–72). Thus, subsequent co-evolution, the process of reciprocal evolutionary change that occurs between pairs of species or among groups of species as they interact with one another, underpins the evolution of the biodiversity that we see in nature today. The activity of each species that participates in the interaction applies selection pressure on the others (66). The evolution of diversity of the gut microbiota doubtless also benefits from the abundance of niches associated with plant structures in the diet, which enable bacterial species with biochemical specialties and different surface-binding proclivities to co-exist (73–75).

Experimental and analytical evidence for not only gradual but also sweeping genetic changes in bacterial species inhabiting human colons is available, supporting estimates that *de novo* mutations occur abundantly [estimated as 2×10^9 to 6×10^{12} single-nucleotide polymorphisms (SNP)/microbiota/day] (76). For example, intra-individual human adaptation of lineages by mutation and mobile element diversity occurring over years has been preserved in gene lineages of 602 *Bacteroides fragilis* strains cultured from numerous fecal samples collected over extended periods from 12 humans, as well as metagenomic studies of this bacterial species (76–78). Sixteen genes of *B. fragilis* showed evidence of parallel evolution in different individuals, including genes associated with polysaccharide utilization and cell surface features. These studies are supported by analysis of metagenomic data of 40 commonly detected bacterial species present in feces collected temporally from numerous humans of diverse origins. Acquired gene mutations and gene gains (horizontal gene transfer/recombination) and losses rapidly swept through resident strains in microbiotas on a 6-month timescale. However, in the longer term (several decades), strain replacement was evident (79, 80). Thus, taking these several studies together, short-term evolutionary changes, extensive horizontal gene transfer, and strain replacement, presumably based on ecosystem fluctuations, play a role in the development of the gut microbiota. This seems to be a continuous process despite an association with mammalian digestive tracts, which perhaps has already lasted for hundreds of thousands of years.

The dispersion and acquisition of the microbiota between human generations further confound the evolution of the human gut microbiota. This process is somewhat like the story of microbiota evolution all over again because there is no evidence of obligate,

high-fidelity, vertical transmission of microbes in humans such as occurs in some insect symbioses (10, 81, 82). Detection of bacterial DNA in amniotic fluid and fetal tissues (skin, lung, thymus, spleen, and gut) and placenta has been reported with suggestions that an "amniotic microbiota" might bathe the fetus *in utero*. However, very low biomass in samples (suggesting contamination) and inadequacies in PCR procedures used to search for bacterial DNA in samples may be responsible for these results (83). Milk expressed from the breast contains bacteria, but these are mainly bacteria characteristic of cutaneous or oral habitats. It is notable that milk obtained before the infant feeds contains bacteria characteristic of the maternal skin microbiota, but bacteria characteristic of the oral microbiota of the infant are the most common after feeding (84). The preponderance of evidence points to transmission of fecal bacteria from the mother to neonate as the most important early mechanism of seeding the gut during the first year of life. However, bacteria from adults other than the mother and from the environment are also sources of gut bacteria (85–88). This is reflected in the individuality (idiosyncrasy) of gut microbiota compositions in which, even at the phylum level, marked variation in relative abundances is evident between human subjects (14). Regardless of taxonomy, transmission of core symbiotic functions is probably the critical factor in establishing a "healthy gut microbiota," about which more will follow.

The ability of bacteria to produce spores doubtless facilitates transmission between humans. Search of genomic and metagenomic data for a "spore gene signature" has revealed its phylogenetically widespread existence (50% to 60% of bacterial genera) within the human gut microbiota (89). This indicates that transfer of a large proportion of the gut microbiota is probably facilitated by resistant, metabolically inert forms that enhance survival under the aerobic conditions that are likely to pertain to transmission between humans. Factors that determine colonization of recipient humans by bacteria are likely to be numerous and include matching of nutritional resources and bacterial catabolic capacity in the potential, new habitat, which is succinctly expressed in the Baas Becking hypothesis that "everything is everywhere, but the environment selects" appropriately favored bacterial species (90). Further studies of the features that underpin niche fitness of bacterial species in the human gut may help to explain the phenomenon of "colonization" (20).

The evolution of the gut microbiota was likely to be complicated in early humans by the effects of seasonal variation in the availability of different plants, as described for the microbiotas of the great apes, and reinforced by studies of human hunter-gatherers (47, 48, 91–93). It is, of course, tempting to think that we can learn about the gut microbiota of humans by reference to apes such as gorillas and chimpanzees to which we are closely related genetically. However, the diet of apes depends on an arboreal habitat and is largely composed of fruits and leaves which is overall rather coarse and bulky (31). Baboons (Old World monkeys), on the other hand, resemble humans in being relatively large-bodied, largely terrestrial, intelligent, omnivorous primates that live in groups with social hierarchy and have adapted to a wide variety of environments in Africa including the savannah, so their gut microbiotas might be informative of that of early humans (94). Wild baboons show seasonal fluctuations in gut microbiota composition, but overall, the results of metagenomic comparisons between the gut microbiotas of wild baboons and modern humans show little overlap in bacterial species-level compositions (95, 96). Indeed, hundreds of bacterial clades that co-diversified in parallel with apes are no longer detectable in human populations (97). Of course, baboons and apes do not cook their food, but humans do.

HUMANS BECOME FARMERS AND COOKS

Farming, the managed growing and harvest of domesticated plants for consumption by humans and domesticated animals, probably first developed about 10,000 years ago in the fertile crescent between Palestine and Lower Mesopotamia. Farming (the "neolithic revolution") arose independently in the Old and New Worlds and perhaps about 7,000 years ago in Papua New Guinea (98, 99). Farming provided a greater and more constant

supply of food compared to that available to hunter-gatherers and, indeed, led to the abandonment of nomadic lifestyles and the establishment of settled communities. Long before, food variety would have benefited by the independent evolution (8–5 million years ago) of a modified form of photosynthesis (C4) in numerous plant lineages (mostly dicots) that enabled them to produce biomass under conditions (warmer temperature, more arid, lower atmospheric carbon dioxide level) that were marginal for the prevailing C3 photosynthetic plants (100–102). Thus, a greater diversity of crops including maize, sugar cane, and sorghum were eventually available in some regions of the world. However, wheat, rice, and oats which are mainstay crops for human consumption carry out C3 photosynthesis.

The adoption of an agrarian lifestyle doubtless altered the composition of the human gut microbiota. Studies of remaining hunter-gatherer microbiotas and those of agrarian, non-industrialized people show them to be different (48, 103–105). Notable differences in microbiotas of people inhabiting non-industrialized and industrialized regions are the higher prevalence and relative abundance of *Prevotella copri* (106) and the presence of members of the genus *Treponema* as members of the gut microbiota (103, 107) of non-industrialized people. This may reflect the ingestion of coarser dietary fiber. The fermentation products produced from carbohydrates by the treponemes are formate, acetate, succinate, and lactate (108). These acids are commonly produced by members of "industrialized" gut microbiotas even though devoid of *Treponema*. Indeed, gut treponemes produce similar fermentation products to *Ruminococcus*, *Eubacterium*, and *Butyrivibrio* that are commonly present in gut microbiotas (32). Unless the treponemes had some other function in the gut, they are probably not missed by humans in industrialized regions.

Seeds from wild grasses were components of the diet of early humans, but the nutritious parts of the grain "berry" are the endosperm and germ that are within a hard protective, multilayer coat. The seeds can be ground between the molars to release the berry contents, but the texture of chewed grains is unpleasant and the yield of nutritive material is small. Flat-surfaced stones used in pairs to grind, pound, or rub small quantities of grain to extract flour appeared about 75,000 years ago and enabled more efficient separation of seed components: coatings could be picked or blown away, retaining the flour. Stone mortars and then saddlestones-metates were developed later (109). These had better grinding actions and retained the substances being pounded/ground until the desired degree of fineness was reached. Around 8,000 years ago, the managed cultivation of cereal grains developed, and wheat cultivation migrated throughout Europe and to China by 3,000 years ago (109). Wheat became the dominant cereal crop in Europe (rye in Central and Eastern areas). Rice, on which a large proportion of the world population is dependent for nutrition, was domesticated in China about 9,000 years ago (109). Fascinatingly, domestication of wheat and rice has each occurred in a single-origin step from wild plants (109, 110). From then on, human ingenuity devised more and more improvements to the large-scale cultivation, harvesting, storing, grinding, milling, and utilization of grains (109). Root vegetables such as the potato, sweet potato, and manioc were domesticated by South American populations about 8,000 years ago (111). Humans had moved from food gathering to food raising.

Capturing fire (rubbing sticks together or making sparks from striking flints) and preparing and cooking grains, nuts, acorns, roots, and tubers to release edible content with most nutritive value and hunger-quelling effect were a giant leap forward in human evolution. These are starchy foods, and starch in its native forms is digested by human salivary and pancreatic (small intestinal) amylases, with finishing touches by maltase-glucoamylase and sucrase-isomaltase at absorptive surfaces (112). Salivary alpha-amylase gene (*AMY1*) copy number correlates with salivary amylase activity, and individuals from populations with higher starch content diets have, on average, more *AMY1* copies than those eating lower starch diets (113). This may be the result of positive selection due to shifts from hunter-gatherer to farming lifestyles or an earlier selective, genetic sweep in

Africa (114). *AMY1* copy number may influence the composition of the gut microbiota, but more research on this topic is required (115). Starch is made up of two polymeric components, amylose and amylopectin. Amylose is a straight chain of glucose molecules connected by α (1–4) bonds. Amylopectin contains linear glucose chains, but there are also "branch points" created by α (1–6) bonds. Theoretically, amylose should be easier to digest because there are no branch points to produce steric hindrance of hydrolysis, but amylose can form a very compact physical structure, which inhibits diffusion of enzyme into starch granules and thus delays digestion. Therefore, amylopectin is digested better than amylose (112). Hydrolysis of starch releases glucose which is absorbed in the small bowel to give a characteristic rapid increase and then decline in plasma glucose concentration: the glycemic response (112).

Having reliable supplies of starchy foods through farming was progress so long as the carbohydrate could be easily extracted, eaten, and digested. Starch in its native form consists of dense, semi-crystalline granules. Cooking starch with moisture present results in structural order-to-disorder changes (gelatinization) that include leaching of amylose, dissociation of amylopectin double helices, melting of starch crystallites, and destruction of granule morphology (116). The extent to which these changes occur depends on moisture content, temperature, duration, and mixing during cooking. Boiling, soaking, and mashing tend to release starch granules into aqueous media where they form an unbound matrix that can interact with other food components such as lipid or protein (117). The digestibility of starch can be measured *in vitro* using the Englyst method (alpha-amylase hydrolysis), among others, which can be related to digestion rate in the small intestine (118). Thus, "rapidly digestible starch" is hydrolyzed within 20 minutes of incubation, "resistant starch" (RS) is the portion remaining after 120 minutes, and "slowly digestible starch" is the portion digested between 20 and 120 minutes. In general, a continuum exists, and almost all ingested starch is digested before it reaches the colon (118). RS (which includes "retrograded starch" formed when some starch is cooked and cooled) and alpha limit dextrins (short-chain remnants of amylopectin) are the only forms that reach the colon (119). Due to the relatively large amount of starch consumed daily by humans, even a low proportion of RS (say 2%–5%) in the diet becomes significant as a growth source for the colonic microbiota (118, 120). Accessing starch by pre-processing and cooking food enabled humans to consume less bulky food yet improve energy gain from faster digestion (121). In another innovation, cooked cereals and the invention of bone spoons meant that a mother could more easily feed a toddler plus a new baby with the help of supplementation of breast milk with solid food for the older child (122). This would have been important in population growth of the long-gestating human species.

In some geographical regions, change from a nomadic lifestyle of hunter-gathers to a sedentary farming of domesticated plants and animals may have been associated with a gradual decrease in stature and body mass of humans between 10 and 6 thousand years ago (123). This may reflect a less nutritious diet (less protein, more carbohydrate) through the transition to dependence on arable farming, at least in regions where introduced cultivars grew poorly compared with growth in their centers of origin (124). Contact with domesticated animals and life at a higher population density in settlements facilitated the spread of infectious diseases (including zoonoses) that also decrease growth and development (124). Increase of stature and body mass rebounded from at least four thousand years ago to the present (123, 125). The domestication of cattle and the consumption of cow's milk may be linked to this rebound effect in some human populations through the provision of additional fat and protein in the diet (123). Milk consumption was, nevertheless, limited because of lactose intolerance. Human infants transcribe the *LCT* gene encoding the small intestinal enzyme lactase during early life to facilitate the utilization of lactose in human milk, but transcription of the gene ceases for the majority of the world's children after weaning. From this time, only limited volumes of milk can be consumed per day without experiencing abdominal discomfort due to the fermentation of lactose by the microbiota in the distal gut (lactose intolerance)

(126). In some human populations, however, genetic variants of the *LCT* gene were selected, seemingly in waves of genetic drift that passed through pastoral populations in Europe and East Africa and which resulted in lactase persistence throughout life (123). In lactose-intolerant populations, fermented milk (yoghurt, cheese) is useful in nutrition because lactose is utilized by bacteria during yoghurt fermentation, thus lowering the concentration in the fermented product, and lactose is partitioned to the whey in cheese manufacture. These foods go together with dairying and are also associated with the development of pottery to prepare and store the fermented foods, although pottery was first integral to storing and cooking grains. Interestingly, members of the bacterial genus *Bifidobacterium* seem to be more abundant in the feces of lactose-intolerant people (127–129). This has been thought to indicate a biological link between human genotype and gut microbiota. It has been proposed that this bacterial association reduces the concentration of undigested lactose in the distal gut with beneficial results. However, it has been reported recently that lactose intolerance symptoms are more severe if bifidobacteria are components of the gut microbiota (130). Milk has undoubtedly been and remains an important source of nutrients for some human populations, but the importance of starch as a major dietary component is universal. Cooked starchy foods such as bread, pasta, rice, and potatoes continue to be major sources of carbohydrates from foods in the modern world (Fig. 1D and 2).

MICROBIAL MOLECULAR INGENUITY ASSOCIATED WITH THE UTILIZATION OF GLYCANS FROM CEREALS

Starch is the principal energy storage substance in cereal grains and continues to be a common component of human diets. As indicated previously, some of this dietary starch reaches the colonic microbiota. A range of Gram-positive and Gram-negative species can hydrolyze starch using α-glucosidases belonging to CAZyme (carbohydrate-active enzymes) GH13 (Table 1), GH31, and GH97 (132). CAZymes are enzymes grouped as families based on similarities in their predicted protein sequences and structures (many are only known from genomic sequences), but a family should contain at least one biochemically characterized member (133, 134). They are involved in the assembly or breakdown of oligosaccharides and polysaccharides. Glycosyl hydrolases (GHs) that break glycosidic linkages constitute a large group (GH1-156) important in the catabolism

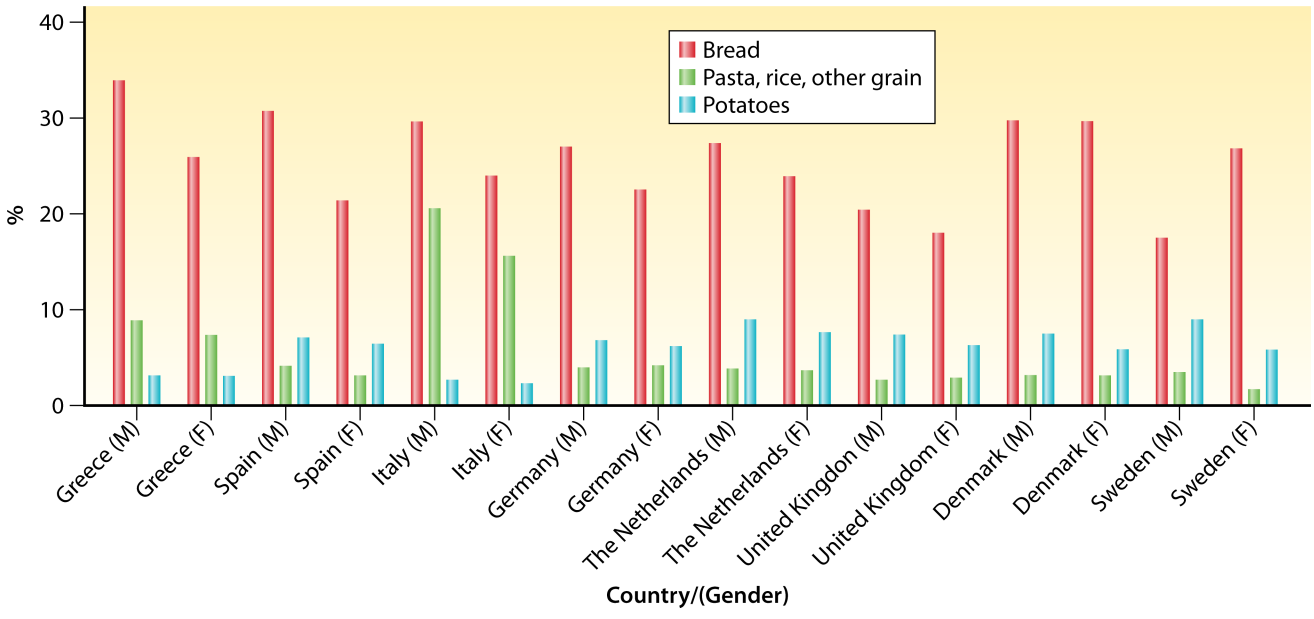

FIG 2 Starch-rich foods as proportion of total carbohydrates consumed by Europeans. Data by countries and gender (M, males; F, females) are shown. Data from reference (131).

TABLE 1 Examples of starch-degrading bacterial species inhabiting the human colon[a]

Bacterial strain	Starch hydrolysis	Resistant starch hydrolysis	Genomic abundance of GH13 loci
Ruminococcus bromii L2-63	+	+	15
Bifidobacterium adolescentis P2P3	+	+	17
Bifidobacterium choerinum FMB-1	+	+	14
Eubacterium rectale DSM 17629	+	w	13
Prevotella distasonis ATCC 8503	+	w	7
Bacteroides thetaiotaomicron VPI5482	+	w	8
Bifdobacterium breve UCC 2003	+	–	14
Bifidobacterium pseudolongum DSM 20092	+	–	17
Bifidobacterium angulatum DSM 20098	+	–	13
Roseburia intestinalis L1-82	+	–	13
Butyrivibrio fibrisolvens 16/4	+	–	10

[a]Information from reference (132). +, hydrolysis; w, weak hydrolytic activity; -, no hydrolysis. GH13 is a major glycoside hydrolase family active on substrates containing α-glucoside linkages. There is particular emphasis on α-amylases [α(1-4) glucoside linkage hydrolysis] and pullulanases [α(1-6) glucoside linkage hydrolysis].

of plant glycans, as are polysaccharide lyases and carbohydrate esterases (32). The metagenome of the human gut microbiota has one of the highest densities of GHs per kilobase of DNA of all natural environments, and CAZyme genes are often grouped in genetic loci associated with catabolism of a specific polysaccharide (135). For example, the genome of *Bacteroides thetaiotaomicron* encodes a "starch utilization system" (Sus) which was one of the first "polysaccharide utilization locus" (PUL) detected in bacterial genomes (136). PULs are genetic loci that encode colocalized and coregulated proteins for the highly specific capture, degradation, and importation of plant glycans: each kind of PUL generally targets a single glycan, although some PULs may have coordinated activity for catabolism of particularly complex glycan structures (137–139). Sus was aptly referred to by Abigail Salyers as a "sequestration mechanism" because it enables starch hydrolysis products to be trapped and utilized exclusively by the *Bacteroides* cell instead of the potentially risky export of extracellular hydrolytic enzymes into the external milieu which might make hydrolytic products accessible to other types of bacteria (32). Sus includes several cell surface proteins (SusDEF), a TonB-dependent transporter (SusC), and three hydrolytic enzymes (SusABG). Overall, the Sus proteins work together to capture and degrade starch at the cell surface and subsequently import the liberated malto-oligosaccharides into the periplasm for further hydrolysis (137). Nevertheless, *B. thetaiotaomicron* has only weak ability to degrade RS without the assistance of other bacteria but, as is also true of some other members of the phylum *Bacteroidota*, has an armory of other PULs that facilitate the utilization of plant cell wall glycans (hemicelluloses) which will be discussed later (119).

Ruminococcus bromii, in contrast, is a "resistant starch specialist" because although of Gram-positive cell structure, it has a special cell surface adaptation known as the "amylosome" (Fig. 3) that confers the exceptional ability of these bacteria to degrade particulate RS (140, 141). *R. bromii* is effective in the hydrolysis of both cooked (boiled) and raw starches (140). The amylosome complex is reminiscent of the "cellulosome" of *Ruminococcus champanellensis* (human colon) and *Ruminococcus flavefaciens* (rumen) that degrade cellulose derived from plant cell walls (8, 142). Although cellulose is ubiquitous in plant cell walls, only small quantities are usually present in the human diet (3–4 grams per day), and although 80% is degraded in the colon, it may have little impact on the gut microbiota relative to RS and the hemicelluloses (143, 144). Daily dietary fiber intake may have increased since publication of this UK data, but the consumption of more refined and processed diets probably means that cellulose intake has not changed significantly. Studies using recombinant dockerins and cohesins from amylosome proteins have revealed that some dockerins, for example, of amylases Amy4 and Amy9, bind several different cohesins, while dockerins from other proteins, such as pullulanase Amy12, are more specific (141, 145). Additionally, the *R. bromii* genome encodes extracellular GH13s (such as Amy5) that lack cohesins or dockerins but which

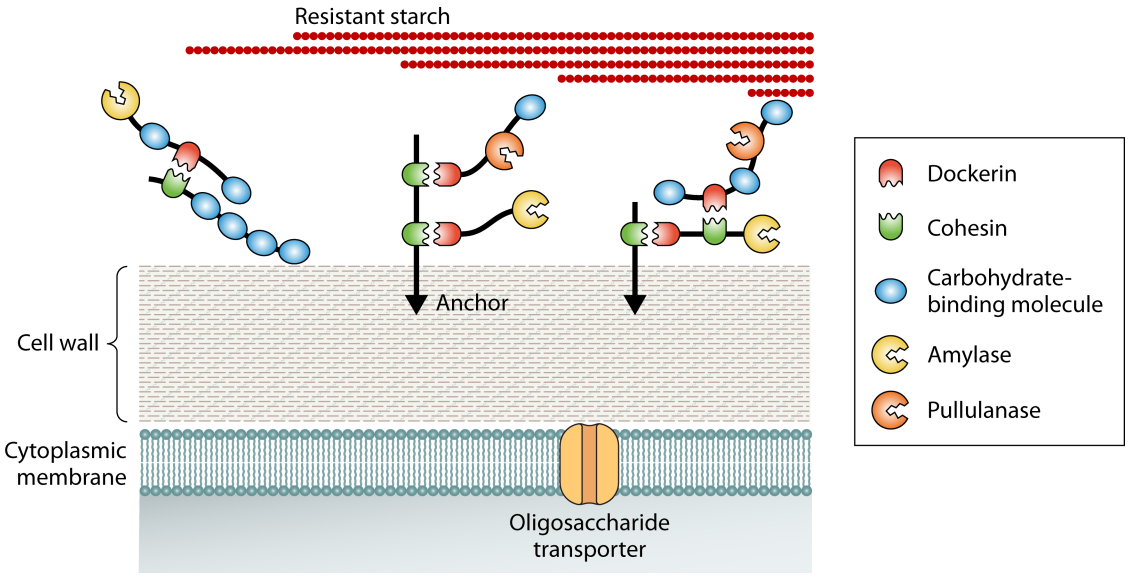

FIG 3 The "amylosome." A simplified explanation of the multiprotein complex known as the amylosome of *R. bromii*. Scaffoldin proteins with cohesion domains and complementary dockerin domains interact through calcium-dependent reactions to produce a strongly linked, multipronged starch-degrading apparatus containing carbohydrate-binding proteins and amylases/pullulanases on the cell surface (141). The pullulanases cleave α (1-6) linkages (147). The amylosome proteins of *R. bromii* are constitutively expressed, unlike the inducible starch-degrading mechanisms of other bacteria. Additional proteins with a role in amylosome structure and function continue to be investigated (148). [Adapted from reference (32) with permission from Springer Nature (SNCSC).]

are also likely to contribute to RS degradation (146). *Ruminococcus bromii* probably acts as a keystone species in consortia (guilds) that facilitate starch hydrolysis and fermentation of hydrolytic products (140). The mere presence of *R. bromii* in co-cultures, for example, even if unable to replicate in a vitamin-deficient medium (absence of rumen fluid), leads to the release of glucose, maltose, and oligosaccharides into the starch-containing medium. These substrates stimulate the growth of *E. rectale*, *B. adolescentis*, and *B. thetaiotaomicron* in co-cultures in media containing RS.

Bifidobacteria that degrade RS have multiple cell surface GH13s. The genome of *B. adolescentis*, for example, encodes seven extracellular, multi-modular GH13 enzymes and multiple carbohydrate-binding modules (CBMs), including CBM74 which is common in *Bifidobacterium* species (119). The CBM74 domain is often present on the same polypeptides as starch-binding domains CBM25 or CBM26 and may function synergistically for attachment to starch granules. Complete starch degradation requires hydrolysis of both the α (1-4) and α (1-6) linkages of starch, so it is interesting to note the presence of an amylopullulanase enzyme, ApuB, in *Bifidobacterium breve* UCC2003 which has activity on potato starch, amylopectin, glycogen, and pullulan. ApuB is composed of an *N*-terminal α-amylase and a *C*-terminal pullulanase separated by a CBM25 and two CBM41 domains (149).

There is often an increased relative abundance of members of *Clostridium* cluster XIVa (for example, *B. fibrisolvens* and *E. rectale*) in the fecal microbiotas of humans fed RS (150–153). These bacteria do not seem to be major degraders of RS but utilize the metabolites produced by RS degraders such as *R. bromii*. Nevertheless, *Clostridium* cluster XIVa species have GH13 amylase with CBM domains anchored to the cell surface. These are starch-inducible functions that probably play a structural role in starch binding and degradation. The arrangement of the CBM and catalytic domains is different between species (154). The specificity and affinity of individual CBMs associated with the cell wall of *E. rectale* probably explain the differential binding to corn but not potato starch granules by this species (155). Based on *in vitro* and *in vivo* observations, a mechanistic framework has been proposed where primary degradation of RS governed largely by *R. bromii* in the colon provides fermentable substrates and increased acetate

concentrations that, through syntrophy, enable the growth of some butyrate producers as well as hydrogen-scavenging sulfite reducers and acetogens (156).

The hemicelluloses [non-starch polysaccharides (NSP)] are plant glycans that are also abundant in the human diet. They provide the matrix in which cellulose fibrils are embedded within the plant cell wall (8, 157). They include xylans, glucans, and pectins (Table 2) that vary greatly in chemical composition and structure, even within specific glycan groups derived from different plant sources (158). Members of the gut microbiota that belong to the genus *Bacteroides* are adept at capture and degradation of hemicelluloses, and the bacterial genomes collectively encode thousands of CAZymes responsible for these functions (159). The PULs of Gram-negative bacteria such as *Bacteroides* species are generally identified in genomes by the presence of adjacent *susC* and *susD* homologs. The *susC* and *susD* homologs, TonB-dependent transporter and cell surface glycan-binding protein (Fig. 4), together form an active trapping and transport complex (160). Characterization of neighboring CAZyme genes in PULs can reveal the glycan-degrading capacity of a species. For example, *B. thetaiotaomicron* and *B. ovatus* both have PULs for the utilization of pectin, but *B. ovatus*, due to unique PULs, is a hemicellulose-degrading "expert" because it can degrade xyloglucan, xylan, mixed-linkage β-glucan, and galactomannan, phenotypes lacking in *B. thetaiotaomicron* (160, 161). Interestingly, the *B. thetaiotaomicron* genome has PULs involved in metabolism of host mucin *O*-glycans, which is a characteristic of only some *B. ovatus* strains (158). When presented with a variety of utilizable plant glycans, gut bacteria prioritize the degradation of these complex substrates. *B. ovatus*, for example, preferentially uses unsubstituted pectin (homogalacturonan) before β-glucan and then, in the order, substituted pectin (rhamnogalacturonan) and arabinoxylan (162, 163). Thus, presumably as the result of a long evolutionary association with humans consuming plant glycans, *Bacteroides* species are core symbionts that use a spectrum of specialized molecular processes to adapt to potential day-to-day changes in the hemicellulose composition of the host diet.

The hemicellulose-degrading mechanisms of Gram-positive members of the gut microbiota are not close homologs of *Bacteroides* PULs and vary between *Firmicutes* in terms of specific GH genes. In general, capture of hydrolysis products is due to extracellular solute-binding proteins and transport of glycan fragments into the cell is

TABLE 2 Hemicelluloses commonly present in the human diet

Hemicellulose	Description	Reference
Pectin	A complex polysaccharide rich in galacturonic acid and composed of heterogeneous branched components such as homogalacturonan (HG), rhamnogalacturonan I (RGI), and rhamnogalacturonan II (RGII). HG is a linear polymer of (1-4)-linked α-D-GalpA. RGI consists of a repeating disaccharide [-4)-α-D-GalpA- (1-2)-α-L-Rhap-(1-] with arabinan, galactan, and/or arabinogalactans attached to rhamnose residues. RGII has a backbone of HG to which are attached complex side chains.	(164)
Mixed-link (β1-3, β1-4) glucans	Mixed-linkage glucan, sometimes referred to as beta-glucan, consists of β-D(1-3)- and β-D(1-4)-linked glucosyl residues. A common component of the endosperm (75% of the soluble non-starch content) and aleurone layer of cereal grains.	(139, 165, 166)
Xyloglucan	Xyloglucan has a backbone of β(1-4)-linked glucose residues, most of which are substituted with 1-6-linked xylose sidechains. The xylose residues are often capped with a galactose residue. The specific structure of xyloglucan differs between plant families (for example, [arabinogalacto]xyloglucan (tomatoes, peppers, eggplant, olives); [fucogalacto]xyloglucan (leafy vegetables)).	(167)
Xylan	Xylans are present in some plant cell walls and have highly variable structures depending on plant source (glucuronoxylans, glucuronoarabinoxylans, arabinoxylans). Arabinoxylans are common components of cereal-derived foods. They consist of a linear backbone of β(1-4)-linked D-xylopyranosyl units to which α-L-arabinofuranosyl residues are attached through *O*-2 and/or *O*-3; some ferulic acid residues may be esterified to arabinose residues at *O*-5. They probably explain the "dietary fiber effect" of shorter bowel transit time.	(168–170)

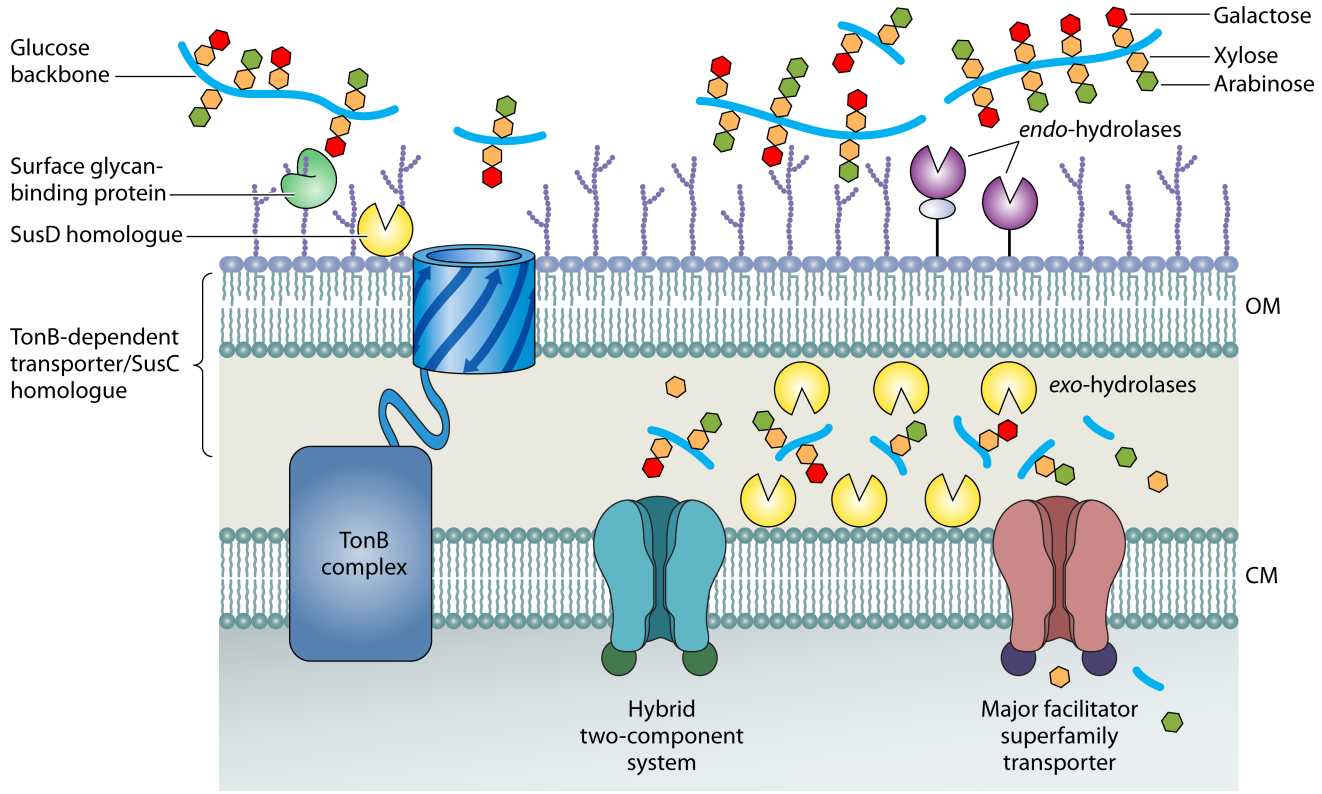

FIG 4 XYGUL, an example of a polysaccharide utilization locus. A simplified explanation of the multiprotein complex known as the XYGUL system of *B. ovatus*. Endo-xyloglucanases attached to the cell surface hydrolyze long xyloglucan chains into smaller fragments which are captured by a surface glycan-binding protein (SGBP-A) and a Sus C homolog (SGBP-B). The oligosaccharides are imported into the periplasm through the SusC/TonB-dependent transporter. Two arabinofuranosidases, a galactosidase and a xylosidase, and two β-glucosidases remove xylose, arabinose, galactose, and glucose residues. The liberated saccharides are imported into the cell. A two-component regulatory system is associated with regulation of PUL transcription (upregulated in presence of specific plant glycan). One or more XYGULs were detected in metagenomes of the fecal microbiota of 92% of 250 humans from North America, Europe, and Japan (171). [Adapted from (161) with permission from Springer Nature.]

accomplished by ATP-binding cassette transporters, phosphoenolpyruvate:carbohydrate phosphotransferase system transporters, or major facilitator superfamily transporters (160).

Brewing developed about the same time as the farming of cereals and for thousands of years beer provided a safer alternative to drinking water. Most of this brewing was carried out in the home. Yeast cell wall mannans are present in beer as a result of autolysis and were probably ingested by humans in greater quantity when brewing was a less sophisticated process than it is today. They are variable in structure according to source but are mainly linear polymers of (1–6)-linked D-mannopyranose units with short side chains of mannose units attached to the backbone by α(1-2)-linkages and to each other by α(1-2) and α(1-3) linkages (172). Suffice it to say that sufficient yeast mannans ingested in beer and leavened bread reached the colon to influence the evolution of the gut microbiota because *Bacteroides thetaiotaomicron* (and a few other *Bacteroidota* species) have α-mannan PULs that encode the biochemical machinery to trap and degrade these substrates. These PULs are commonly detected in human gut metagenomes (173).

Bacteroides species are abundant in the human fecal microbiota, probably due to the ingenious molecular attributes that enable them to exploit a diversity of plant glycans as nutrition. *Firmicutes* species are also abundant in the microbiota, and while they also have capacity to utilize dietary carbohydrates for growth, they may owe their dominance to the outcomes of syntrophy in which cross-feeding of metabolites

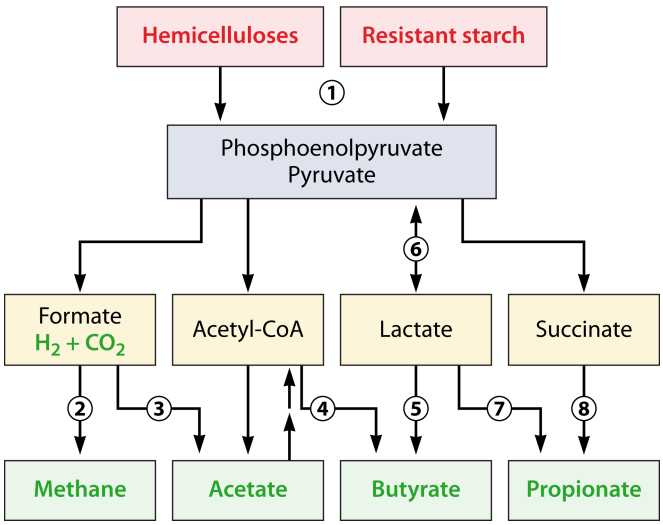

FIG 5 Major features of the integrated metabolism of the colonic microbiota. RS and hemicelluloses (red) comprise the main dietary components that are utilized by bacteria. The principal emergent properties of the total microbiota are shown (green), together with bacterial functional groups that produce them. 1, primary degraders/fermenters such as *Bacteroides* spp. and *R. bromii*; 2, methanogens such as *Methanobrevibacter smithii*; 3, acetogens such as *Blautia hydrogenotrophica*; 4, butyrate producers such as *E. rectale*, *Roseburia* spp., and *F. prausnitzii*; 5, butyrate producers that can grow on lactate if acetate is present such as *Eubacterium hallii* and *Anaerostipes* spp.; 6, lactate producers such as *Bifidobacterium* spp. and *Colinsella aerofaciens*; 7, propionate producers such as *Veillonella* spp. and *Megasphaera elsdenii*; 8, acetate/propionate/succinate producers such as *Bacteroides* spp. Data from references (32) and (71). Note: there is a wealth of biochemical pathway information in the appendix to reference (71).

between bacterial species has led to a metabolic integration that supports the ecosystem (Fig. 5) (71). A critical, yet little studied aspect of the gut microbiota concerns the formation, sources, and functions of bacterial consortia (guilds) which could be critical to metabolic integration. Mathematical models reveal that even in a uniform environment, metabolic competition generally leads to the consistent coexistence of distinct microbes, collectively called a "consortium" (174). In a consortium, distinct microbes organize themselves to create a community-level metabolism that best exploits the nutrients present. The models shows that while growing, a consortium depletes the available pool of nutrients to such low levels that only members of the consortium can survive. The findings suggest that the benefit of metabolic diversity stems from the ability of a consortium to automatically deplete nutrients to levels at which no other microbes can invade the ecosystem, creating a "barrier effect" ("colonization resistance") (175). Models also indicate that consortia that arise under conditions where there is a steady supply of nutrients produce the maximum mass of microbes. It may be helpful in future culture-based research to envisage the microbiota as a jigsaw puzzle and to fit co-cultured bacterial species (the jigsaw pieces) together to form consortia, each consortium based on the utilization of a specific glycan, and to measure the kinetics of the integrated metabolism resulting from nutritional networks within each consortium (176–178). Consortia that carry out the same function could differ in terms of constituent bacterial species because of metabolic redundancy in the bacterial world. This could help to explain the taxonomic individuality of human microbiota compositions (179).

CONSEQUENCES OF HARBORING A GUT MICROBIOTA

As considered earlier, comparison of the structure of the digestive tracts of mammals shows adaptations in the form of expanded gut regions where bulky diets rich in plant materials can be stored (180). These anatomical features are apparent in the fetus, so adaptations of gut structure are evolutionary features programmed in the germline.

Much of the stored "food" cannot be digested by animal genome-encoded processes, so the colonization of the gut with microbial species that degrade and ferment plant glycans became an associated feature of food retention. The advantage of microbial degradation of complex carbohydrates by the gut microbiota on the digestion of food is obvious in ruminants because about 80% of the caloric requirement of these animals comes from microbial fermentation products (32). Germ-free mice consume 20%–30% more laboratory chow than conventional or conventionalized animals (181–183). So, in the presence of microbial associates, gut microbiota activity allows conventional mice to consume less food but still harvest sufficient energy from the diet to satisfy their bodily requirements. The evolution of this digestive trait must have been a pivotal, evolutionary event because, as seen in comparing the distribution of CAZyme families of mammalian (mouse, rumen, human) gut microbiotas, bacterial functional adaptation has converged (53, 184). These adaptations have resulted in the formation of consortia of bacterial species that power the overall metabolism of the gut microbiota.

In humans, the bacterial fermentation products are SCFAs (mostly acetate, propionate, and butyrate) as well as gases (hydrogen, carbon dioxide, and methane), minor amounts of branched-chain fatty acids (isobutyrate and isovalerate) derived from fermentation of amino acids in the distal colon, a minor amount of the SCFA valerate, and volatiles such as indoles (derived from tryptophan), hydrogen sulfide, and thiols (derived from sulfur-containing amino acids such as methionine) (13, 185). The release of "phytochemicals" (polyphenols, anthocyanins, phenolics, and flavins) through the degradation of plant-derived substances by the microbiota also occurs (186). SCFAs are absorbed from the colon, and some are carried by the portal vein to the liver. Acetate then passes to the peripheral circulation and can be detected throughout the body and is a source of acetyl-CoA that supports fatty acid synthesis (187). Propionate is transformed to glucose in the liver (gluconeogenesis) (188). Butyrate seems to be mostly used as energy source by the cells forming the colonic epithelium (189).

Microbial harvest of additional calories from plant materials in the diet is commonly used to indicate the importance of the gut microbiota to humans: microbial activity enables additional energy harvest from food (183, 190, 191). However, Harry Flint has assessed this aspect thoroughly, and a summary of his conclusions provides a balanced view of the situation (32).

1. Digestible carbohydrates are estimated to contribute 3.8 kcal/g to energy supply through metabolism of glucose in body tissues.
2. The contribution of energy from carbohydrates in plant glycans must inevitably be less than this because gut bacteria use up about a quarter of the energy for their own growth.
3. SCFA absorption is highly efficient (up to 95%), but only 50%–75% of the glycans arriving in the human colon is fermented. Fermentation and SCFA absorption are less complete the more rapid the passage of digesta through the colon. About 2 kcal/g glycans is the most that could be gained.
4. An alternative, indirect, approach to estimating how much human diet-derived energy is gained has been to ask how much energy is needed to account for the mass of bacteria present in the gut and then to deduce how much total glycans would have to be fermented to produce this much energy. However, it is not known precisely how much ATP is released by carbohydrate fermentation by anaerobic bacteria or how much ATP is required to produce a gram of bacterial cells in the gut. Confident assertions can often be found in the literature about the importance of microbiota fermentations (for example, 10% daily energy gain is derived from non-digestible carbohydrates), but these calculations are based on very few primary sources (190).

Flint concludes that, except in the case of very high plant glycan intakes characteristic of hunter-gatherer humans, the contribution of the microbial energy harvest to total caloric intake is probably relatively small, and under conditions of consuming a constant

amount of food, more dietary fiber compared to digestible carbohydrates results in a net decrease in total energy gain because there is less net energy yield per gram from plant glycans (32, 192). Given the restricted storage capacity of the *H. sapiens* gut, alluded to earlier, energy harvest by the microbiota may merely be an evolutionary remnant from our ape ancestors. However, plant glycans (dietary fiber) increase fecal bulk, gut transit time, and satiety, all of which have relevance to good health (193).

Of course, microbial products produced in the gut may have additional roles. They could be signaling molecules and/or mediators of the barrier effect that reduces the likelihood of pathogen proliferation in the gut. Research about microbial signaling in the gut is ongoing, and the results might increase understanding of human physiological dynamics (194–200). However, the magnitude of these effects on human physiology has not been quantified and seems speculative. For example, reduction in SCFA production has been invoked in the etiology of Crohn's disease (CD) because human studies indicate that there is generally a depletion of bacterial species in the microbiota that characteristically produce butyrate (201, 202). This is of interest because butyrate is an energy source for colonocytes and has anti-inflammatory effects (203, 204). Thus, modulation of the microbiota to produce more butyrate remains an attractive treatment option. However, CD patients tend to have low fiber intakes (https://www.crohnscolitisfoundation.org/diet-and-nutrition/what-should-i-eat). In some patients, this lower intake of dietary fiber may be the actual reason for reduced production of butyrate. Increasing dietary fiber intake may, therefore, be beneficial for some IBD patients but may not be appropriate for patients with bowel strictures where there is potential for blockage. Ironically, the best current nutrition-based treatment for CD (mainly pediatric CD) is exclusive enteral nutrition (EEN), in which patients consume a liquid diet that provides all of their energy and nutrient needs. EEN results in lower butyrate production by the gut microbiota because the diet does not contain dietary fiber (205–207).

Comparison of gut wall features between germ-free and conventional animals shows a thinner lamina propria in the former due to less reticuloendothelial cells (phagocytic cells such as macrophages): the conventional gut, relative to germ free, is in a state of mild inflammation (182). Epithelial cell turnover is more rapid in conventional animals (208). In general, partitioning of immunological mechanisms between gut mucosa and systemic organs (lymph nodes, spleen) has enabled the development of tolerance towards the multiplicity of food, environmental, and microbiota antigens in the gut while retaining an effective defense against invasive pathogens (209). Other symbiogenic effects of the microbiota include chemical modification of host secretions. For example, conjugated bile acids synthesized in the liver from cholesterol and released in bile are essential for the emulsification and hydrolysis of dietary lipids. Although 95% of bile acid output is recycled by enterohepatic circulation, a small proportion is deconjugated, and some is further modified through 7-α-dehydroxylation by the microbiota in the colon and lost in the feces (210, 211). Some gut bacteria, such as *Akkermansia muciniphila*, have their nutritional requirements satisfied by the components of intestinal mucus, which covers the surface of the mucosa and forms a protective and lubricating blanket (212–214). The mucus layer is in a dynamic state, constantly liberated from association with the mucosal surface and replaced by secretion from goblet cells. Glycoproteins known as "mucins" make up 2%–10% of the mucus, with the remainder being mostly water. The mucins are high-molecular-weight linear molecules, heavily glycosylated with *O*-glycans and lesser amounts of *N*-glycans. They consist of a protein backbone containing regions of tandem repeats of threonine/serine, to which the oligosaccharides are attached (215, 216). Intriguingly, paleofeces and feces collected from members of nonindustrial societies have less representation of *A. muciniphila* and lower abundance of genes associated with mucin degradation compared to industrial microbiotas. It is speculated that these differences are due to higher dietary intake of plant glycans in the ancient and nonindustrial societies with concomitant lower utilization by the microbiota of mucins as alternative growth substrates (217). Therefore, the greater abundance of *A. muciniphila* in the gut microbiota of humans inhabiting industrial countries may be a

recent evolutionary event resulting from reduced plant glycan consumption. Based on experiments with gnotobiotic mice fed plant glycan-deficient food, mucin hydrolysis by bacteria may decrease resistance to infection by invasive pathogens because the mucus layer normally protecting the colonic surface might be compromised (218).

Certainly, chemical transformation of human-derived substances by the microbiota means that there is an energy demand to balance microbiota depredation of human resources (219). The "growth-promoting" effect of the administration of subtherapeutic concentrations of antimicrobials in the food of farm animals (poultry, pigs, calves) may, in part, be due to the suppression of bacterial populations that stimulate the inflammatory response or influence cell replacement rates (219). More rapid turnover of intestinal epithelium and immune cells could require that the host divert energy from "growth" (muscle gain) to maintenance of gut tissues. The growth-promoting effect of antibiotics is certainly due to an effect on the microbiota because germ-free animals do not show a growth response when fed antibiotics (220). Also notable is that the metabolic rate of germ-free mice is lower than that of conventional animals (183). Together, these phenomena indicate that there is a detrimental impact of the gut microbiota on vertebrate energy balance.

CONTINUING EVOLUTION OF THE GUT MICROBIOTA

Although plant glycans continue to provide the major sources of carbon and energy for the gut microbiota, much of the food now consumed, at least in western countries, is processed, packaged, and pre-prepared on huge commercial scales. This means that the gut microbiota probably continues to evolve because it is now exposed to novel substrates introduced through new technologies by the food industry (for example, lecithins and plant gums used as thickeners and emulsifiers and artificial sweeteners) (221, 222). The significance of these substances in colonic ecology has hardly been investigated. However, an interesting example of the evolutionary response to "novel" substrates in the colon is provided by evidence of the transfer of genes from marine bacteria (*Zobellia galactanivorans*) to the gut inhabitant *Bacteroides plebius* in Japanese whose diet is rich in seaweed (red algal) polymers. The genes encode enzymes that hydrolyze sulfated polysaccharides (porphyrans) and agaroses (223, 224). While this horizontal gene transfer event may have occurred long ago, it emphasizes the ability of various *Bacteroides* species to respond to environmental change exerted by special features of the diet (225–227).

Impressive advances in the development of pharmaceutical drugs since the 20th century have occurred. The therapeutic action in the body of some orally administered medicines is due to microbiota metabolism. For example, sulfasalazine, used in the treatment of ulcerative colitis, is inactive until cleaved in the colon by bacterial azoreductase activity that releases the active anti-inflammatory constituent: 5-aminosalycylic acid (228). Pharmaceuticals may be inactivated (for example, digoxin inactivated by *Eggerthella lenta*) or bioaccumulated by bacteria (for example, the antidepressant duloxetine) (228–231). Enterohepatic circulation of medications can involve bacterial metabolism. For example, irinotecan (CPT-11) used in colorectal cancer therapy is glucuronated (detoxified) in the liver and excreted in the urine and intestine. In the intestine, bacterial glucuronidases re-toxify the molecule (232, 233). Other drugs, such as poorly absorbed antibiotics, are potentially toxic to the microbiota in response to which some members of the microbiota acquire resistance mechanisms (234). Conjugative transposons carrying antimicrobial resistance genes have been important in the spread of antibiotic resistance in gut bacteria as evidenced by the pioneering work of Abigail Salyers. Comparison of the frequency of occurrence of antibiotic resistance determinants in *Bacteroides* isolates obtained from human feces and clinical specimens pre- and post-1970 was made. This showed a tripling of incidence in a 25-year period, presumably associated with the increased use of antibiotics in agriculture and human medicine during that time (235). Modern humans are exposed to many chemicals generated by industrialization in air, food, and water, but the magnitude (amounts and constancy)

of the exposures and, therefore, the impact on the functional evolution of the gut microbiota are likely to be highly variable, unknown or unexplored.

Investigations of the mosaic structures of PULs in *Prevotella copri*, *Bacteroides ovatus*, and *B. xylanisolvens* isolates clearly demonstrate strain-to-strain variation in relation to the utilization of plant glycans by members of a bacterial species (236). This is a well-known phenomenon in culture-based, taxonomic investigations where variable fermentation of carbohydrates is a feature of identification schema (237). Thus, the "pan-genome" (the sum of all genes in all strains or the global gene repertoire) of a species which is composed of the "core genome" (the pool of genes shared by all strains) plus the "dispensable genome" (pool of genes present in only some strains) reflects the acquisition (or loss) of metabolic capability in relation to continuing bacterial evolution (238–240).

EVOLUTIONARY KNOWLEDGE HELPS ASSESS A "HEALTHY" GUT MICROBIOTA

Consumption of plant roots, tubers, rhizomes, seeds, and an ~8,000-year association with cooked, domesticated cereals links all the steps in the evolution of *Homo sapiens*. The two major polysaccharide components provided by cooked cereals that cannot be digested by humans in the small bowel are RS and the hemicelluloses such as arabinoxylans, glucans, and pectins from plant cell walls. The constancy of the presence of these carbohydrates in the habitual diets of humans resulted in humans "contracting out" these hydrolytic functions to bacteria because of the paucity of human genome-encoded CAZymes secreted into the gut (171). This functional relationship can be tested using chemical assays in which the amount of undegraded RS or hemicelluloses (NSP) relative to that consumed in the diet is measured in feces (the "processed waste"). An example of this approach was reported by Alan Walker, Harry Flint, and colleagues (241) using analytical procedures developed by Hans Englyst, John Cummings, and colleagues (242, 243). Of 12 out of 14 individuals tested, only 4% of retrograded starch and 10% of NSP (wheat bran) consumed by the study participants in supplemented, standardized diets was detected in the feces. These individuals could be considered to harbor healthily functioning gut microbiotas (even though they were taxonomically distinct in composition) in which most of the ingested RS and NSP was degraded in the colon. In contrast, the gut microbiota of the remaining two subjects was functionally abnormal because a considerable proportion of the RS (~60%) that they had ingested was detected in the feces. These individuals might be considered to have a deficiency of "core symbionts." That is, they lacked consortia of biochemically specialized symbionts that have adaptations that can be linked to food and human evolution. Therefore, it can be proposed that this relatively simple analytical approach, based on chemical assay of the amount of RS and NSP in feces after several days' consumption of specific doses of the carbohydrates, could revolutionize the assessment of microbiotas. The emphasis would move from microbiota taxonomy to assessing collective microbial function. A combination of the common types of dietary RS may need to be included in test diets in case there is a differential functional response by microbiotas (244), but RS3 is probably most relevant because it is retrograded starch found in potatoes, rice, pasta, and bread after cooking and cooling (160). At the least, RS and NSP fecal assays could provide a microbiota screen of functional capacity prior to metagenomic studies. The results of metagenomic studies of the gut microbiota, although highly informative of bacterial taxonomic composition and biochemical capacity of the gut microbiota of humans, are subject to confounding factors such as bowel transit time and geographical origin and require very large numbers of participants to obtain adequate statistical power (17, 25, 29, 245–247). Taxonomic comparisons are difficult because only nine bacterial genera (*Bacteroides*, *Alstipes*, *Blautia*, *Faecalibacterium*, *Dorea*, *Roseburia*, *Subdoligranulum*, *Ruminococcus*, and *Lachnoclostridium*) are common to the microbiotas of 95% of fecal microbiotas (data from ~20,000 humans were examined) (248, 249). Moreover, the taxonomic composition of the microbiota is recorded as "relative abundances" (the percentage of the total microbiota that is made up of the various taxa). This may relegate

taxa of low relative abundance but, nevertheless, of ecological significance to obscurity. Absolute values (the number of cells of the taxon per gram of feces) may be more useful but are seldom measured, even though it is technically possible. All things considered, a focus on the principal, symbiogenic feature of human evolution (the colonic digestion of RS and NSP) could have considerable merit in assessing and modulating the gut microbiota associated with human health.

Restoration of a functionally "normal" microbiota might be possible for people who lack the usual degree of RS and NSP catabolism. Since the highly specialized RS and NSP degraders characteristic of the human gut microbiota are known, restoration of a normally functioning microbiota is feasible by means of intentional inoculation of the gut by ingestion of cultured preparations of these bacteria, much as is proposed in a more general "wellness" context using lactic acid bacteria as probiotics (250). This approach might be assisted by use of cultured consortia and dietary improvements in relation to RS and/or NSP: some individuals with abnormal microbiota function could have low dietary intake of plant glycans. Why the microbiota of some individuals lacks RS and NSP degraders is, of course, a critical question to be answered. There seems to be a wealth of associated research possibilities because measurements of other symbiogenic influences such as chemical transformation of bile acids, utilization of mucins, immunological screens, prevalence of PULs, and colonic transit time could be measured in parallel. Suggestions for priority research (first steps) associated with this new approach are listed in Table 3.

To paraphrase Matt Ridley (253), "A scientist tries to learn about what is in the natural world, how the natural world works, and how the natural world got to be the way it is.

TABLE 3 Priority research

Topic	Comment
In-depth analysis of dietary intake	All studies should include a detailed analysis of the diets of human participants because gut bacteria live for the most part on substrates derived from the diet. For example, habitual food group intake can be obtained using 5-day non-consecutive estimated food records and 30-day, multi-item, semi-quantitative food frequency questionnaires (17). Statistical investigative approaches might use mediation analysis, principal component, and multiple logistic regression analysis to describe linkages between dietary components and gut microbiota (17, 251, 252).
Improve quantification of RS and NSP in feces	Although methods to determine amounts of these substances present in feces relative to a standardized diet already exist (241), the development of newer, preferably simplified methods would be useful. Pioneering efforts to measure RS and NSP catabolism in the human gut used a small number of male participants who were overweight. Further research using men and women of normal weight in diverse geographical locations is required to determine "normal ranges" worldwide. Assays might be developed to investigate digestion of habitual dietary fiber rather than specific RS and NSP included in a test diet.
In-depth analysis of plant glycan structure	The composition and structure of glycan substrates that feed gut bacteria need to be analyzed in greater depth. The structure of plant glycans in foods is poorly advanced mainly due to the lack of rapid, high-throughput technologies. Plant glycans are diverse in chemical content (monosaccharide content) and have different monosaccharide spatial arrangements (sequences) and different glycosidic linkages and stereochemistry. These features are variable between plant species even when considering general categories of polysaccharide (for example, "xylans"). In-depth analytical techniques could enhance studies of the temporal degradation of glycans by bacterial consortia in the gut, reveal substrate preferences, and allow regulation of bacterial enzyme activities to be studied in synthetic and natural communities (20).

Science is not simply a collection of facts; rather, it is a path to understanding." Scientists can cover the first question "what bacteria are in the human gut" with authority because of the taxonomic studies that have been completed. "How the gut microbiota works" can be described in broad terms, but bacterial consortia and function in the gut are poorly understood. The challenging topic of "how the gut microbiota got to be the way it is" emphasizes the advantages of a holistic view of microbiota function and the use of methodologies that are informed by evolutionary knowledge rather than reliance on investigations based on bacterial taxonomic compositions.

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