Genomics application for the developing world_ The effect of diet on gut microbiota in human living in different

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Chapter 17
The Effect of Diet on Gut Microbiota in Humans Living in Different Environments: A Metagenomic Approach

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New Technologies to Describe Gut Microbiota

In the twentieth century, our knowledge of the gut microbiota was constrained by the ability to describe and study the biological functions of less than a 100 cultivable bacteria (Finegold et al., 1983). The species we described until 2000 were also the most abundant ones, and given the special attention of funding agencies toward pathogens, we fundamentally ignored the vast majority of commensal microorganisms, except in the case of a handful of bacterial species used in food production, such as bifidobacteria and lactobacilli.

In the beginning of this century, a limited number of studies focused on cloning and sequencing of 16S rRNA libraries started opening a window with a view on the astonishing diversity of gut microbiota. Despite their importance, these initial noncultivation-based diversity studies were limited by the costs and complexity of Sanger sequencing methods. In the past 10 years, the picture of our gut microflora has rapidly passed from black and white to a surprising explosion of bright colors, thanks to the advent of Next Generation Sequencing technologies (NGS).

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Metagenomics

The molecular study of both the taxonomic and functional gene composition of a microbial community in an environmental sample is today strongly supported and strengthened by the NGS platforms (Metzker, 2010), including Roche/454, Illumina/Solexa, and Applied Biosystems (SOLiD). Advances in DNA sequencing have dramatically reduced costs and markedly increased capacity, allowing culture-independent metagenomic methods to be readily deployed to characterize microbial communities (microbiota) associated with human body habitats at various stages of the human life cycle and in various populations (Turnbaugh et al., 2007). Initially, remarkable progress in the taxonomic classification of bacteria and their phylogenetic relationships has been achieved based on the partial or full sequencing of ribosomal RNAs (23S, 16S, and 5S; Winker and Woese, 1991). The 16S rRNA gene is widely considered the major molecular marker in the taxonomic classification of bacteria; the reason for this is that it can be sequenced faster, it contains regions varying at different rates, and its sequence is available for thousands of bacteria in large databases (Cole et al., 2009). The 16S rRNA gene also allows one to monitor, in a sufficiently trustworthy manner, time and space variations in the composition of the microbial community under study without sequencing other loci. The 16S rRNA bacterial gene contains nine hypervariable regions (V1–V9, Petrosino et al., 2009). The V2, V3, V5, and V6 regions have a greater discriminating power in identifying bacterial genus and species (McKenna et al., 2008; Andersson et al., 2008). The 454 Genome Sequencer FLX Titanium series (Roche) platform is particularly suited for 16S rRNA-based surveys. This platform can produce in a single run, about one million "reads" of high quality with an average size of 400 bp that is now increasing to 700 bp, thanks to the last 2011 platform upgrade. Basically, using this platform, researchers will be able to sequence the 16S rRNA gene without cloning and without problems in assembling incomplete partial sequences from short reads.

A significant step further has been made possible by approaches enabling sequencing of bacterial genomes in the gut, like Illumina-based NGS techniques. Illumina HiSeq2000 technology is based on a proven chemical technology useful for the sequencing of whole bacterial genomes (Qin et al., 2010; Arumugam et al., 2011). In metagenomic approaches, the analysis of the large amount of information contained in sequences generated by microbial "high throughput" sequencing from a complex environmental sample is now a major challenge. Importantly, Illumina produces short reads at a significantly reduced cost. The real challenge of this technology is the heavy reliance on hardcore bioinformatics and computation required to assemble short reads from mixed samples by blasting the reads to databases of known sequences. The sequencing and subsequent bioinformatics analysis of all DNA belonging to a microbial community is a great way to get information about its content and its potential functional genes (Qin et al., 2010; Arumugam et al., 2011). These new metagenomic surveys not only capture the microbial, organismal, and genetic diversity associated with humans but also make it possible to investigate gene functions.

The combination of 16S rRNA and whole metagenome-based approaches holds the key to understand the functional contributions that our microbes make to our physiologic phenotypes, our health, and our disease predispositions. The ability to reconstruct metabolic pathways active in the gut requires the combination of information on gene and protein sequence with knowledge on metabolism and metabolic functions, as well as correlation with the measurement of the levels of associated metabolites.

Metabolomics

The human metabolome is the complete set of metabolites, end products of cellular processes, present in human biofluids at a give time. Metabolite profiling approaches represent the key to understanding the functional contribution of the gut microbiota to the host organism, and its coevolution with diet. Metabolomics allows us to characterize the metabolite profile within a human biological sample such as stool, blood, and breath, by analytical chemistry methods, such as gas chromatography-mass spectrometry (GC-MS) or nuclear magnetic resonance (NMR). GC-MS analysis represents a robust method for quantification of selected metabolites, with more satisfactory sensitivity and resolution than the conventional NMR approach (Elango et al., 2009; Gao et al., 2009). This analysis is applied in metabolomics research of urinary and serum samples, brain tissue extracts, and fecal samples and allows the analysis of certain class of metabolites such as fatty acids, phenolic acids, and amino acids in fecal water. The variation in metabolic profiles could depend on host genotype and disease status and also on the intake of fruit, vegetables, dietary proteins, and other foods or on composition of intestinal microbiota. Dietary carbohydrates, specifically resistant starches and dietary fiber contained in fruits, vegetables, and whole grain cereals, are readily fermented by the colonic microbiota to short-chain fatty acids (SCFAs), primarily acetate, propionate, and butyrate. These metabolites and their biologically active compounds modulate human nutrition and health, by decreasing risk of developing gastrointestinal (GI) and autoimmune disorders, allergy, and inflammatory bowel disease (IBD).

Another important set of metabolites are amino acids and the by-products of their metabolism. The proportion of dietary amino acids, which are metabolically available to the human body is influenced by digestibility, absorption, and by genes of the gut microbiota, involved in metabolism of amino acids. Amino acid availability is important in humans for protein synthesis, modulation of gene expression, and intestinal integrity. Amino acid composition of foods varies greatly. In cereals and legumes, respectively, lysine and methionine concentrations are significantly lower compared to foods rich in animal protein (Elango et al., 2009). Uniting metagenomics data with analyses of the products of microbial community metabolism (metabolomics), it is possible to shed light on how microbial communities function in a variety of human populations with different dietary habits (Tuohy et al., 2009).

The Human Gut Microbiota in the Next-Generation Sequencing Era

The human gut microbiota is a complex consortium of trillions of microorganisms, whose collective genomes or metagenomes contain at least 100 times as many genes as our own genome (Gill et al., 2006). This essential "organ," the microbiome, provides the host with enhanced metabolic capabilities, protection against pathogens, education of the immune system, and modulation of GI development (Bäckhed et al., 2005). The role of the resident microbiota in the human gut and its profound effects on host health and daily well-being is now recognized as crucial. This microbial community provides energy, nutrients, bioactive compounds and aids in detoxification, and resistance to infectious diseases. The reconstruction of the evolution of our dietary habits is crucial for understanding the evolutionary context of our modern diets and the diseases often associated with them. The gut microbiota recovers energy and biologically active molecules from food that would otherwise be discharged from the intestinal tract, thanks to its microbiome enriched with genes involved in metabolic pathways, such as the breakdown of indigestible plant polysaccharides, the biosynthesis of essential vitamins and isoprenoids, or amino acid and xenobiotics metabolism. This set of genes has been shown to be enriched in the gut microbiome and is identified as a "core gut microbiome" (Turnbaugh et al., 2009a). Close symbiotic integration of functions from the intestinal cells with bacterial cells suggests that human beings should be considered as "superorganisms" whose metabolism involves integration of metabolic processes coded in the host genome with those of the microbiome (Nicholson et al., 2005). Initial work showed that the exact human microbiome composition varies between healthy people (Gill et al., 2006; Nicholson et al., 2005; Eckburg et al., 2005) and also between lean and obese individuals, and moreover, that the microbiome composition is responsive to dietary modulation for weight reduction (Ley et al., 2006). Eckburg's research team (2005) discovered that there were high levels of strain variation, but far fewer intermediate and deep lineages, supporting the idea that gut bacteria evolution is a classic case of adaptive radiation, where a few successful colonists gave rise to a variety of descendants, and thus there was diversification of an initial population bottleneck into various strains that likely correspond to ecotypes.

Diet and Human Metagenome Evolution

Evolutionary changes in the length and compartmentalization of the digestive tract have enabled vertebrates to occupy diverse habitats and exploit different feeding strategies. Spectacular numbers of microbes live in mammalian guts and provide their hosts with important nutritional functions. The coevolution of gut anatomy, microorganisms, and diet has been first proposed by studies on mammals (Ley et al., 2008) in which the authors have made a comparative metagenomic study of the fecal

microbiota of human beings and 60 other mammalian species, living in zoos and in the wild, to see how taxonomic position and diet affect the composition of the commensal microbiota and to understand how these relationships have coevolved. The authors found that both diet and phylogeny influence the increase in bacterial diversity from carnivore to omnivore to herbivore. Although there is a general trend to herbivores harboring the most diverse communities and carnivores the least, overall, the relationship between gut microbiota and its host is species specific: Baboons in the St. Louis Zoo have much the same gut microbiota as wild baboons in Namibia.

Through cultural innovation and changes in habitat and ecology, there have been a number of major dietary shifts in human evolution, including meat eating, cooking, and those associated with the introduction of agriculture and animal domestication. The analysis performed on the gut microbiota of humans living a modern life-style revealed that the microbial community is typical of omnivorous primates (Ley et al., 2008).

Humans are genetically adapted to the environment in which their ancestors survived, which is known to have conditioned their genetic makeup. While humans range from being almost completely carnivorous as in the Eskimo groups in northern temperate and arctic regions, to being largely vegetarian, as in the hunter gatherers in southern Africa, the majority of societies effectively balance their diet with an omnivorous mix of meat and vegetables (Luca et al., 2010). Most people depend on plant foods as the main reliable sources of calories, and many plant food staples are also good sources of high-quality protein, such as wheat, nuts, and legumes. However, others plant foods, like manioc and rice, are not protein rich and require supplementary proteins or amino acids to support nutritionally balanced diets.

Decades of anthropological research have been devoted to elucidating dietary history, in part because dietary shifts were likely associated with major anatomical and cultural changes (e.g., the increase in relative brain size and the advent of modern civilization via agriculture).

Dietary habits are considered one of the main factors contributing to the diversity of the human gut microbiota (Bäckhed et al., 2005). Profound changes in diet and lifestyle conditions began with the so-called "Neolithic revolution" with the introduction of agriculture and animal husbandry approximately 10,000 years ago (Cordain et al., 2005). The origin and spread of agriculture and animal husbandry over the past 10,000 years, with centers of domestication in Asia, Europe, South America, and Africa, represent the most important major shift in human diets. After that time, food resources became more abundant and constant. The food production and storage technologies associated with this dietary shift led to population densities that are orders of magnitude greater than what is possible under hunter–gatherer subsistence economies. The concentration of large human populations in limited areas created selective pressures that favored pathogens specialized in colonizing human hosts and probably produced the first wave of emerging human diseases (Blaser, 2006). We may hypothesize that bacteria specialized in human-associated niches, including our gut commensal microbiota, underwent intense transformation during the social and demographic changes that took place with the first Neolithic settlements (Mira et al., 2006; Strachan, 1989). However, on the whole, the spread of agriculture was associated with an astounding relative reduction of nutritional

intake diversity. For example, 50–70% of the calories in the agricultural diet are from starch alone. In addition to a reduction in nutritional diversity, agricultural diets may also have been associated with a caloric availability that exceeds growth and energetic requirements, as observed among the most developed contemporary agricultural economies. Western developed countries successfully controlled infectious diseases during the second half of the last century, by improving sanitation and using antibiotics and vaccines. At the same time, a rise in new diseases such as allergic, autoimmune disorders and IBD both in adults and in children has been observed (Blaser, 2006). It is hypothesized that improvements in hygiene together with decreased microbial exposure in childhood could be responsible for this increase (Mira et al., 2006; Rook and Brunet, 2005).

The interplay between diet and microbiota probably shaped the immune system itself. It is possible to hypothesize that our immune system changed profoundly with the transition to agriculture and husbandry into villages and cities. In particular, the production of the first fermented foods and cheeses could have selected alleles for tolerance to milk and at the same time favored the recognition of lactic bacteria and *bifidobacteria* or bread yeasts as "friends." In this perspective, we can speculate that studies on the evolution of host–microbe interaction, a field currently in its infancy, will witness rapid progress in the coming years.

Symbiotic Interaction Between Gut Microbes and Host: A Source of Complexity in Organisms to Exploit Different Feeding Strategies

Molecular phylogenetic studies have greatly extended our understanding of the origins and evolution of animal symbioses, validating and extending Buchner's thesis that many of these associations have long evolutionary histories (Buchner, 1965). Such studies have shown repeatedly that nutritional symbionts have evolved in parallel with their hosts, starting with studies of aphids and *Buchnera* and extending to whiteflies, scale insects, psyllids (Baumann, 2005), tsetse flies (Chen et al., 1999), stinkbugs (Hosokawa et al., 2006), carpenter ants (Schroder et al., 1996), and cockroaches (Lo et al., 2003).

Animals generally require a dietary supply of various nutrients (vitamins, essential amino acids, etc.) because their biosynthetic capabilities are limited. The capacity of aphids to use plant phloem sap, with low essential amino acid content, has been attributed to their symbiotic bacteria, *Buchnera aphidicola*, which can synthesize these nutrients (Shigenobu et al., 2000). In insects, there is significant evidence on how symbiosis with microorganisms has enabled this group of animals to escape from the constraints of requiring a balanced dietary supply of amino acids.

Our microbial partners have coevolved with us to forge mutually beneficial (symbiotic) relationships. These relationships are typically founded on nutrient sharing. In the mammalian gut, the availability of several nutrients is dependent on our microbiota; supplementation of vitamins, in particular vitamin K, is dependent on microbial metabolism.

Gene transfer between bacteria the in the gut has recently been shown to be influenced by the interactions between food and the gut microbiota in Japanese individuals, whose diet includes regular consumption of sushi. Hehemann and coworkers (2010), working on *Zobellia galactanivorans*, a member of the marine Bacteroidetes, discovered an enzyme (porphyranase) responsible for breaking down porphyran, an abundant polysaccharide in the red algae species *Porphyra*, on which *Z. galactanivorans* is often found. While searching gene-sequence databases, investigators came across predicted porphyranase sequences in metagenomes derived from human feces and in the genome of the resident human gut bacterium *Bacteroides plebeius*, suggesting that *B. plebeius* acquired the genes laterally from marine bacteria. Because it turned out these sequences were present in Japanese individuals but not in residents of the United States, the authors concluded that *Z. galactanivorans* were introduced via *Porphyra* ("nori"), the traditional seaweed used to wrap sushi and a common component of the Japanese diet. The researchers hypothesize that by constantly consuming seaweeds, Japanese communities produced a selective force that led to retaining the beneficial porphyranase genes in their gut microbiomes (Hehemann et al., 2010; Sonnenburg, 2010). Such interplay between food and gut microbiota underlines the importance of food microbial composition in terms of both quality (e.g., presence of probiotic species) and safety (e.g., ratio of probiotics/pathogens).

A prominent role of the gut microbiota is energy extraction from fiber and complex carbohydrates. Plant materials that are in fact indigestible to humans are a complex carbohydrate known as dietary fiber. Our bodies need roughage to properly digest food and eliminate waste because the bulky substance cleans the colon as it makes its way through our digestive tract. Dietary fiber comes from vegetables, fruit, grain, and legumes. The plant polysaccharides are rich in xylan-, pectin-, and arabinose-containing carbohydrate structures. The human genome lacks most of the enzymes required for degrading these glycans (see http://afmb.cnrs-mrs.fr/CAZY/). However, the distal gut microbiome provides this capacity. At least 81 different glycoside hydrolase families are represented in the microbiome, many of which are not present in the human "glycobiome" (Bäckhed et al., 2005). Host mucus provides a consistent reservoir of glycans for the microbiota and thus, in principle, can serve to mitigate the effects of marked changes in the availability of dietary polysaccharides. Dietary carbohydrates, specifically resistant starches and dietary fiber, contained in fruits, vegetables, and whole grain cereals are readily fermented by the colonic microbiota to SCFAs, primarily acetate, propionate, and butyrate. Gnotobiotic mouse models of the human gut microbiota have indicated that linked terminal fucose in host glycans is an attractive and accessible source of energy for various members of the microbiota such as the Bacteroidetes (Turnbaugh et al., 2009b).

Microbiome Variation in Human Populations

The awareness of the importance of work on human cohorts is stimulating the boost of comparative metagenomics studies in individuals from different countries. However, addressing comparative metagenomics studies directly in humans is

challenging because of numerous uncontrolled variables, such as genetic variation, geographical and environmental differences, and dietary differences.

The constitution of enteric microbiota may be a consequence of country of origin. Yet, one of the most prominent differences between various populations prior to world globalization or "Westernization" was diet. Examples included the so-called Mediterranean diet, characterized by the traditional abundance of olive oil and legumes, or the fish and fat diet of Eskimo populations. The geographical diversity of diet could therefore profoundly explain geographically different microbiota as a result of the interplay of the above mentioned variables.

Several studies analyzed variables such as different geographical locations, diets, and age. Because of the importance of the gut microbiota on health, age-dependent changes in its composition could be of major significance. Several studies, mainly conducted on Asian populations, indicate higher numbers of enterobacteria and lower numbers of anaerobic bacteria, including *Bifidobacteria* in the elderly compared to adult subjects. The data might however be of limited relevance due to the use of classical microbiological methods (Mitsuoka and Hayakawa, 1973).

An earlier study (Holdeman et al., 1976) describes the distribution of 101 bacterial isolates from 25 fecal specimens from three men on (a) their normal diet and normal living conditions, (b) normal living conditions but eating the controlled metabolic diet designed for use in the Skylab simulation and missions, and (c) the Skylab diet in simulated Skylab conditions (isolation). Analyses of the kinds of bacteria from each astronaut during the 5-month period showed more variation in the composition of the flora among the individual astronauts than among the eight or nine samples from each person. The authors suggest that the variations in fecal flora more certainly reflect real differences (and not daily variation) in the types of bacteria maintained by individual people. The proportions of the predominant fecal species in the astronauts were similar to those reported earlier from a Japanese-Hawaiian population (Moore and Holdeman, 1974) and were generally insensitive to changes from the normal North American diet to the Skylab diet; only two of the most common species were affected by changes in diet. However, one of the predominant species (*Bacteroides fragilis* subsp. *thetaiotaomicron*) appeared to be affected during confinement of the men in the Skylab test chamber. Evidence is presented suggesting that an anger stress situation may have been responsible for the increase of this species simultaneously in all of the subjects studied. Another cross-sectional study on intestinal microbiota composition was performed on 230 healthy subjects (two age groups: 20–50 years and > 60 years) living in four European locations in France, Germany, Italy, and Sweden (Mueller et al., 2006). The analysis used a set of 14 group- and species-specific16S rRNA-targeted oligonucleotide probes to analyze fecal samples by fluorescence in situ hybridization coupled with flow cytometry. Age-related differences in the microbiota makeup were detected but differed between the study populations from the four countries, each showing a characteristic colonization pattern. Marked country–age interactions were observed for the German and Italian study groups. These interactions were inverse for the predominant bacterial groups *Eubacterium rectale–Clostridium coccoides* and *Bacteroides–Prevotella*. Differences between European populations were observed

for the *Bifidobacterium* group only. Proportions of *Bifidobacteria* were two- to threefold higher in the Italian study population than in any other study group, and this effect was independent of age. Higher proportions of enterobacteria were found in all elderly volunteers independent of the location. Gender effects were observed for the *Bacteroides–Prevotella* group, with higher levels in males than in females.

The utilization of whole microbiome sequencing moved microbial populations studies beyond a description of microbial species or genes present in particular habitats, to linking the structure and dynamic operations of microbial communities reciprocally to human biology and pathobiology. The METAHIT consortium published a paper describing metagenomic data sets that were generated with Illumina sequencing technology, containing almost 200-fold more gut microbial sequences from fecal samples of 124 European individuals, including healthy, overweight, and obese adults as well as patients with IBD (Qin et al., 2010). The functional analysis of the gene catalogue identified a core set of genes that seem to be essential for bacterial survival in the gut. These include typical housekeeping genes and others that may encode products involved in adhesion to host proteins or in harvesting the sugars that are carried on blood or epithelial cells. Some functions were present in all 124 individuals included in the study cohort but detected in only a small proportion of previously sequenced genomes of gut bacteria. Therefore, the genes encoding these functions may be essential for the functioning of the gut ecosystem as a whole but may be encoded across different bacterial species. Recently, the METAHIT consortium (Arumugam et al., 2011) described the worldwide microbiome variation according to a series of distinct enterotypes. This study increases the number of individuals and populations from which gut microbiomes have been sequenced. The authors surveyed the gut metagenomes of 22 European individuals and combined the findings with existing data from Japanese and American individuals. They found that the microbiome clusters into distinct phylogenetic groups (enterotypes) which do not reflect country or continent of origin, body mass index (BMI), age, or gender. Each of these three enterotypes are identifiable by the variation in the levels of one of three genera: *Bacteroides* (enterotype 1), *Prevotella* (enterotype 2), and *Ruminococcus* (enterotype 3). The authors affirmed that enterotypes appear complex, are probably not driven by nutritional habits, and cannot simply be explained by host properties such as age or BMI, although there are functional markers such as genes or modules that correlate remarkably well with individual features. Yet, though these studies infer differences related to different diets, they can hardly describe in detail the actual dietary variables.

Children from different human populations provide an attractive model to study the role of geography and diet on microbiota variation. Infants living in developing countries have been shown to be colonized at younger ages with fecal bacteria and have more rapid transfer of enteric microbial strains than infants living in developed countries (Adlerberth et al., 1998).

In our recent work (De Filippo et al., 2010), investigating diet-gut microbiota interaction, we discuss how the ability of modern *H. sapiens* to live in different environments and to follow a wide range of different diets has affected our gut microbial ecology. We characterized the fecal microbiota of 14 African children (BF) living in

Fig. 17.1 Life in a rural village of Burkina Faso, Boulpon. Millet and sorghum (basic components of Mossi diet) are produced locally and are ground into flour on a grinding stone to produce a thick porridge called Tô

a rural village (Boulpon, Burkina Faso, Africa, Fig. 17.1) and of 15 European children (EU) living in an urban area (Florence, Italy) by sequencing the 16S rRNA gene, with the aim of elucidating the effects of different diets on gut microbiota. The experimental design includes a "natural" control set, breast-fed children from Burkina and Italy. There are five major advancements. The first key finding is that Burkina children cluster separately from Florence children, and breast-fed toddlers form a third cluster in between (Fig. 17.2a). This can be solely the result of diet. Contributions from sanitation could play a role, but this is indeed minor; in fact, if sanitation or hygiene did cause the difference, then one would expect that breast-fed children would as well cluster differently. While we clearly observed that mother's milk significantly reduces the differences and that is why breast-fed children cluster separately, the cluster contains both Florence and Burkina toddlers and is apart from the other Burkina and Tuscan children. The second key finding is that the BF microbiota was significantly enriched in Bacteroidetes and depleted in Firmicutes compared to their EU counterparts, suggesting a coevolution of intestinal bacteria with their diet, rich in plant polysaccharides (Fig. 17.2b). The third key finding is that the fecal samples from children in BF especially differed from the Italian subjects because of the presence of *Prevotella* and *Xylanibacter* (Bacteroidetes), *Treponema* (Spirochaetes), and *Butyrivibrio* (Firmicutes); all appeared in the African but were not found in the Italian samples. This peculiar microbiota is at risk of being lost in urbanized Africans. We hypothesize that these distinctive bacterial genera

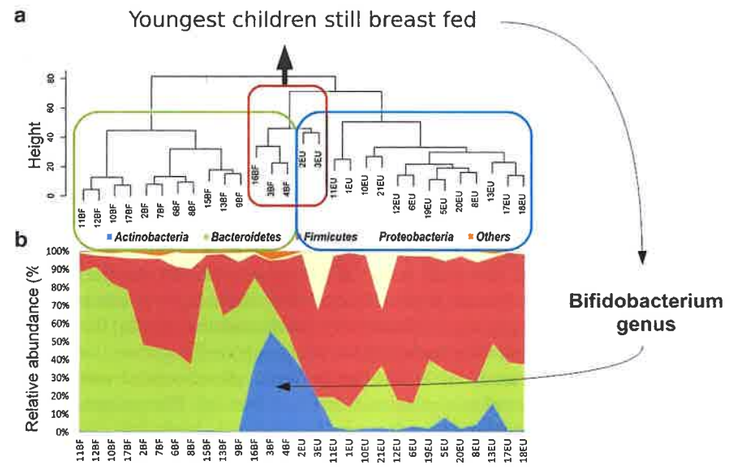

Fig. 17.2 (**a**) Dendrogram obtained with complete linkage hierarchical clustering of the samples from BF and EU populations based on their genera. The subcluster located in the middle of the tree contains samples taken from the five youngest children (1–2 years old) still breast-fed (3 from BF group and 2 from EU group). (**b**) Relative abundances (percentage of sequences) of the four most abundant bacterial phyla in each individual among the BF and EU children. Firmicutes (*red*) and Bacteroidetes (*green*) abundances significantly differentiate the BF from the EU children. *Blue area* in the middle shows abundance of Actinobacteria, mainly represented by *Bifidobacterium* genus, in the five youngest EU and BF children

might help to extract energy from the polysaccharides in the children's heavier fiber diet. These bacteria are capable of fermenting cellulose and xylan through a number of carbohydrate-active enzymes, producing anti-inflammatory effects at the same time. The speculation is that there is a link between these microbes that colonize the human gut and termites as part of the Burkinabé diet. The fourth key finding is that SCFA levels are statistically much higher in Burkina children with respect to the European ones, and the four Burkina specific species (*Prevotella*, *Xylanibacter*, *Treponema*, and *Butyrivibrio*) have the enzymes needed to digest fibers and produce these beneficial molecules. The presence of these species would allow BF children to maximize the energy intake from indigestible components by producing high levels of SCFAs that supply the host with an additional amount of energy. Normal colonic epithelia derive 60–70% of their energy supply from SCFAs, particularly butyrate (Scheppach, 1994). Propionate is largely taken up by the liver and is a good precursor for gluconeogenesis, liponeogenesis, and protein synthesis (Wolever et al., 1991). Acetate enters the peripheral circulation to be metabolized by peripheral tissues and is a substrate for cholesterol synthesis (Wolever et al., 1989; Cummings et al., 1987). Together, they account for 10% of calories extracted from

a Western diet each day (McNeil, 1984) and probably an amount of calories more abundant of a typical rural village of BF diet. In addition, SCFAs have several functions: they can reduce inflammation in colitis, promote tissue renewal (Topping et al., 2001), increase the absorption of sodium and water in diarrhea (Binder, 2010), regulate enteric neurons, and control GI motility (Soret et al., 2010). The last key finding is that biodiversity is significantly reduced in European children with respect to BF children. The different bacterial compositions are likely to have profound influences on another organ as well as the immune system, possibly explaining the absence of inflammatory bowel diseases in African children and adults. Exposure to the large variety of environmental microbes associated with a high-fiber diet could increase the potentially beneficial bacterial genomes, enriching the microbiome. Reduction in microbial richness is possibly one of the undesirable effects of globalization and of eating generic, nutrient-rich, uncontaminated foods. Both in the Western world and in developing countries, diets rich in fat, protein, and sugar, together with reduced intake of unabsorbable fibers, are associated with a rapid increase in the incidence of noninfectious intestinal diseases. The potential protective effects of the diet on bowel disorders was first described by Burkitt (1973) who, working in Africa in the 1960s, noticed the remarkable absence of noninfectious colonic diseases in Africans consuming a traditional diet rich in fiber. We extend this observation proposing that this health improvement is the combined result of a fiber-rich diet and microbiota. The speculation regarding the influence of a Burkina type of diet in reducing IBD and allergies have to be thoroughly tested in the future, but several independent pieces of evidence link Crohn's disease and allergies to alterations in intestinal microbiota and consequently in immune responses, and the increase in SCFAs that we report in rural BF children could explain why such diseases that are increasing in the Western world are virtually absent in Africa.

The increased biodiversity and possibly the activation of the immune function could be important also in protection from infections and to outcompete pathogenic bacteria. A diet poor in simple sugars and rich in fibers could in fact explain the marked reduction in potentially pathogenic phyla in the rural BF children with respect to the Europeans (De Filippo et al., 2010), a paradox if one considers the fact that Burkina children drink from polluted water and live in a much less sanitized environment.

Role of Gut Microbiota in Obesity and Metabolic Disease

Several studies focused on the ability of different microbiota to differently extract food calories. This trait has indeed been under selection during our evolution, and the ability to store energy would be a beneficial attribute for ancient humans who had variable access to food. However, in modern societies, where there is ready access to high-calorie diets, this benefit becomes detrimental. Many studies described

that one manifestation of this symbiotic relationship is microbial processing of components of the diet and deposition of the extracted energy in host fat depots.

Obesity has reached epidemic proportions in Westernized cultures, and the diseases associated with it, including insulin resistance and type 2 diabetes mellitus, hepatic steatosis and steatohepatitis, dyslipidemia, and atherosclerotic cardiovascular disease, have become major public health problems. Traditional obesity research has focused on environmental and host genetic factors, including descriptive studies in humans and mechanistic studies using genetically altered rodent models. More recently, research over the last 5 years using culture-independent methods and NGS has suggested that the pathogenesis of obesity may be influenced by our endogenous gut microbiota (Bäckhed et al., 2004, Ley et al., 2006, Turnbaugh et al., 2007). A fundamental breakthrough came from 16S rRNA-based interrogation of community structure (phylotype abundance distribution) associated to sequencing of fecal microbial communities (metagenomics) of obese and nonobese adult human twins and their mothers. Family members had considerable overlap in their gut microbial communities, with the degree of variation being similar between monozygotic and dizygotic twin pairs. Notably, however, obese individuals showed an impressive overall reduction in microbial diversity. One of the most striking results was an altered balance of the ratio between Bacteroidetes and Firmicutes, the two major phyla of commensal bacteria residing in the GI tract. An important metabolic difference between these two phyla is the efficiency of energy extraction from indigestible dietary carbohydrates. Therefore, alterations in their relative ratio can result in increased energy accumulation, which is of special concern in certain pathological conditions. For this reason, several studies have focused in recent years on the composition of human gut microbiota in obese subjects under different dietary regimens (Ley et al., 2006). The authors liken this reduced diversity to a fertilizer runoff, in which a subset of the microbial community blooms in response to abnormally high-energy input, as opposed to the rainforest- or reef-like community of the lean gut, which displays high species diversity in the face of high-energy flux. In order to selectively test the role of microbiota in obesity, animal models have been used as a controlled system to investigate the role of dietary changes on microbiota.

"Top-down" systems biology (Nicholson, 2006) comparisons of metabolic profiles of normal human and mice microbiota revealed that absorption, storage, and metabolism of dietary lipids were specifically modulated by the microbiome (Martin et al., 2007). Moreover, the induction of type 2 diabetes and obesity with a high-fat diet in rats has been shown to correlate with preexisting metabolic patterns associated with differences in gut bacterial activities, indicating causality between microbiome and host predisposition to diseases (Li et al., 2007).

Changes in the gut microbiota of humanized gnotobiotic mice have been studied after the mice are switched from a diet low in fat and rich in plant polysaccharides to a Western diet high in fat and sugar and low in plant polysaccharides (Turnbaugh et al., 2009). After just 1 day on the Western diet, mice show changes in their microbial composition, metabolic pathways, and gene expression, and within 2 weeks, they develop more adiposity. Mice on a Western diet show an increase in bacteria of the Firmicutes phylum and a decrease in those of the Bacteroidetes phylum.

These concepts represent an important paradigm shift in understanding health conditions and disease and are likely to have a significant impact on the future of the prevention and therapy of intestinal diseases (Nicholson, 2006).

Despite the advantage of selectively and precisely controlling all the variables, mice seem to be a questionable model for studies on human gut microbiota. The conclusions obtained from animal models or from a handful of human individuals can be hardly generalized to human populations.

The challenge we face now is to understand how the different environments and wide range of diets which modern humans around the world experience has affected the microbial ecology of the human gut.

Conclusions

The findings presented in this report emphasize the need to sample humans across the globe with a variety of extreme diets and life-styles, including relatively ancestral hunter–gatherer lifestyles, in order to provide new insights into the limits of variation within a host species and the possibility that our microbes, in coevolving with our bodies and our cultures, have helped shape our physiological differences and environmental adaptations. The identification of worldwide variation in the signatures of adaptations to dietary changes in the gut microbiota of human populations with different dietary habits may shed light not only on the evolutionary history of our species but also on the mechanisms that underlie many autoimmune diseases in modern human populations.

The worldwide variation in the human microbiome is a virtually untapped goldmine of tremendous importance to improve our health and have the benefits of living a modern life without losing the beneficial flora developed from thousands of years of human evolution. We expect that in the future the knowledge of the composition of the indigenous microflora, or of a microflora that retains peculiar specialization for the extraction of certain nutrients, could lead interventions in developing countries which aim at alleviating malnutrition while taking the host microbiome into account.

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Part VII
Plant Genomes

Karen E. Nelson • Barbara Jones-Nelson
Editors

Genomics Applications for the Developing World