

Chapter 2

Host Genotype and the Effect on Microbial Communities

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Introduction

Microbial communities inhabit a variety of body surfaces of mammalian hosts. In the human mouth, 10^2 – 10^3 different species from nine bacterial and a single archaeal phyla have been found (Aas et al., 2005). Teeth, cheek, and tongue all have their own specific communities with anaerobic bacteria present at the gum-line and between the teeth. For each of these sites, the selective trait is based on the surface adherence capabilities of the microbes, typically resulting in multispecies-biofilm formation (Aas et al., 2005). Microbial diversity and abundance normally decrease further down the gastrointestinal (GI) tract until the stomach. In the esophagus approximately 100 species from six phyla are found, most of which are similar to the species found in the mouth (Pei et al., 2004). The stomach is generally regarded derelict for any microbial species except for *Helicobacter pylori* (Finegold, 1983). Nevertheless, 16S rRNA surveys have reported up to 128 species from eight phyla in the stomach; however, it seems likely that these findings represent remnants from ingested strains rather than true residents (Pavoine et al., 2004). After the stomach, bacterial populations increase again in the small intestine, ranging from 10^4 – 10^5 g⁻¹ in the duodenum and jejunum to 10^7 g⁻¹ in the terminal ileum. In this region, intestinal transit slows down and the microbiota composition changes, favoring the more anaerobic species (Finegold, 1983; Hayashi et al., 2005). Next, along the GI tract is the colon. In the ascending colon, polysaccharide hydrolysis and carbohydrate fermentation support rapid microbial growth, whereas in the transverse and descending colon, amino acid and host-derived glycans (mucin) fermentation occurs, coinciding with a reduction of bacterial growth rate (Cummings and Macfarlane, 1991). The fermentations along the entire colon cause a microbial population increase up to 10^{11} – 10^{12} cells g⁻¹ in feces accompanied by a strong proportional decrease of facultative anaerobes (Marteau et al., 2001). In the colon, the

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most numerous species are obligate anaerobes belonging to the phyla *Bacteroidetes* and *Firmicutes* (Backhed et al., 2005; Eckburg et al., 2005).

No other body site attains the high bacterial abundance as observed in the colon, although the mouth harbors a taxonomic richness approximating that of the colon. Furthermore, the recent expansion of the human skin ribosomal operon database indicates a diversity level close to that of the GI tract as well (Gao et al., 2008). Unfortunately, interactions between host and skin microbiota are for the most part unexplored. Next to skin and GI tract, the urinary tract is being studied on a microbial level as well. The vaginal microbiota is generally assumed to have low diversity and to be dominated by *Lactobacilli*. However, *Lactobacilli* are not dominant vaginal inhabitants in all healthy women (Fredricks et al., 2005). One can conclude that the GI microbiota is the most densely populated microbial ecosystem of the mammalian host and has been subjected to many studies. Hence the remainder of this chapter will mainly focus on the knowledge obtained from GI ecosystems.

A variety of functional metabolic activities are generally thought to derive from most, if not all, of the resident communities. Several general processes necessitate the presence of microbial inhabitants in order to function properly, such as the maturation of the immune system, resistance to pathogens, digestion of nutritional components, and the production of essential nutrients. Especially important to the host are microbiota-derived nutrient conversions and contributions that cannot be executed by the host itself, including the degradation of complex polymers in the GI tract that cannot be (completely) digested by the host's enzyme machinery (nondigestible carbohydrates, proteins, and lipids) (Cummings and Macfarlane, 1997). Besides the improvement of our nutritional access to complex nutrients, microorganisms in the GI tract provide a significant supply of essential amino acids (Metges, 2000) and other important compounds such as various vitamins, including vitamin K and several B vitamins (Ramotar et al., 1984; Albert et al., 1980). Conversion of several bio-active molecules, such as sex steroid hormones in order to promote their circulation, is another health-related function humans receive from their GI inhabitants (Begley et al., 2005; Adlercreutz et al., 1984). These types of microbial functions complement the metabolic potential of the host, as the host itself does not encode for the required proteins. In addition to the beneficial contributions, the microbial communities can introduce metabolic activities that are detrimental for their host, such as the production of hydrogen sulfide (Attene-Ramos et al., 2006; Schicho et al., 2006) and the potentially tumor-promoting secondary bile acids (Summerton et al., 1985).

It is becoming a generally accepted view that multicellular organisms, especially mammals, should not be considered as autonomously living entities. "Super-organism" is a popular term that better describes mammals for what they really are: a cohort of host cells and microbial cells. This coalition of cells from the different domains of life is striving for the common mutual cause of survival. The microbiome is a commonly used term for the genetic composition of all microbial cells belonging to a super-organism. According to the super-organism concept, metagenomics can be defined as the mammalian host genes combined with the entire microbiome (Turnbaugh et al., 2006). Currently, there are no completed metagenomes available for any super-organism. Hence, it remains difficult to establish or estimate

the importance of the host genome. It is obvious that many organisms, such as humans, contribute far less genes to their metagenome than their microbiome counterparts. In the human GI tract alone there are already ten times more microbial cells present than host cells in the entire human body (Savage, 1977). Humans are believed to contain approximately 23,000 genes in their genomes (Wei and Brent, 2006), whereas current estimations for the GI microbiota unique gene count are up to 9,000,000 (Yang et al., 2009). In other words, our human genes are outnumbered by several orders of magnitude by the GI microbiome alone! As more and more body sites are being sampled, this difference can only increase in favor of our microbiome. Such observations underline the limited human genetic input in the whole “super-organism” and pose the question: To what extent our genes matter during our life as a super-organism? Maybe, we as hosts have predominantly lost functions during evolution because our microbes provided them and could execute the corresponding functions more efficiently? Possibly, our evolutionary efficiency is increased by encoding a “limited” gene set, which could be specialized in molecular communication with microbes in order to recruit, nourish, and maintain a microbiota that is able to complement for the essential functions that are lacking in our own genomes? After all, we are still around despite our “limited” genotype.

Interactions in a Super-Organism

In mammals, a dynamic and complex relationship exists among diet, host phenotype, and the associated GI microbiota. All interactions are dependent on the host genotype, which can be seen as a matrix on which host phenotype and the resident microbiota are projected (Fig. 2.1). Diet and transient food organisms are external, yet important, components that complete the complex host–microbiota interactions.

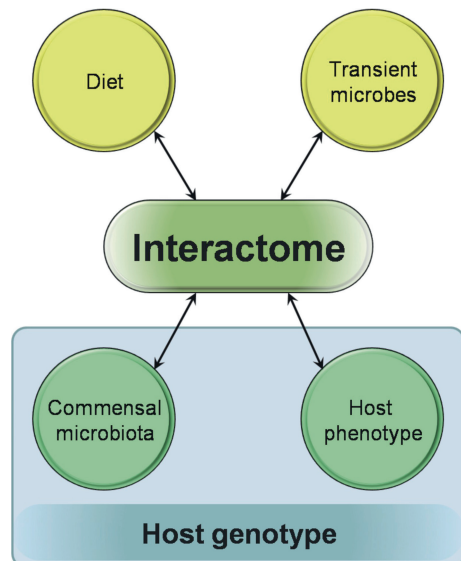


Fig. 2.1 Factors involved in host–microbiota–diet interrelations

Classical views on many disorder-associated phenotypes do not take into account all underlying factors. For example, the main focus usually lies on diet and host genotype in disorders such as obesity, diabetes, and many cardiovascular diseases. However, the GI microbiota should not be excluded in studies or treatment of these disorders, since changes in the gut communities have been associated with some of these disorders (Turnbaugh et al., 2006; Cani et al., 2007; Holmes and Nicholson, 2007; Wen et al., 2008; Zhang et al., 2010). Even though it is hard to determine the causality of observed microbiota deviations with respect to these diseases and disorders, several studies do suggest causal contributions from the gut communities. Microbiota transplants from obese mice to germ-free (GF) littermates induce the obese fat-storage phenotype (Turnbaugh et al., 2006). Furthermore, already before the onset of type-1 diabetes in genetically predisposed rat models, which are on the same diet, the gut microbiota was different in the rats that eventually developed diabetes compared to those that did not (Brugman et al., 2006). Moreover, antibiotic treatment of these rats significantly delayed and lowered the incidence of diabetes development (Brugman et al., 2006). These findings suggest a causal role of the gut microbiota in the development of diabetes.

Even in the extensively studied GI tract, the interactions between host and microbiota are not yet understood. Most studies to date are restricted to composition analyses. However, more and more insights are emerging. Especially the use of high-throughput technologies to study the diversity and functionality of the GI tract is greatly enhancing the current knowledge level. These technologies use a variety of approaches, such as the revival of culturing methods that are high-throughput (Zengler et al., 2005; Ingham et al., 2007), metabolite detection (Holmes and Nicholson, 2007), phylogenetic microarrays based on 16S rRNA sequences (Palmer et al., 2007; Rajilic-Stojanovic et al., 2009), and sequencing of 16S rRNA genes as well as sequencing of random microbial DNA (Turnbaugh et al., 2006, 2009a). Sequencing of the GI microorganisms has opened up the possibilities for functional metagenomics, which will allow further exploration of the microbial activity patterns.

It is generally accepted that the GI tract is sterile at birth and is swiftly colonized by microbes acquired from maternal and environmental sources. Recently, bacteria have been detected in the fluid in intact amniotic sacs of women in preterm labor (DiGiulio et al., 2008). This finding questions the broadly accepted view of postnatal GI tract colonization, since fetuses swallow and “inhale” amniotic fluid continuously, hence exposing their respiratory and GI tracts to everything that resides in it. However, bacteria were only found in 15% of the subjects ($n = 166$) (DiGiulio et al., 2008). Regardless of the precise moment of initial colonization, it remains without doubt that the human GI microbiota evolves over time. The development is especially drastic in the first 2 years of life, followed by stabilization of the GI community into a microbiota that resembles that of an adult (Conway, 1995). In adults the fecal microbiota is shown to be highly stable over time within one individual, as well as specific for its host (Zoetendal et al., 1998). Interestingly, despite a high variability in the GI species composition between individuals, functional capacity seems to be much more uniform between human adults (Kurokawa et al., 2007).

Moreover, a significant proportion of microbial phylotypes found in the gut are continuously present during a 10-year timeframe (Rajilic-Stojanovic et al., 2007). Therefore it seems likely that adults have, next to some transient guest organisms, a stable individual core of permanent GI tract colonizers (Rajilic-Stojanovic et al., 2007; Zoetendal et al., 2008). Tap et al. have reported 66 microbial phylotypes, which are present in more than 50% of the samples they investigated ($n = 17$) (Tap et al., 2009). Such findings suggest that besides an individual core, a limited number of microbial phylotypes are more prevalently found in people (>50% of the individuals) and appear to represent a common core of the human GI tract microbiota.

Host Genotype and Microbiota Selection

As noted before, the mechanism(s) behind GI tract colonization, succession within the community, and community structure itself are poorly understood. One hypothesis is that colonization at weaning is determined by the primary nutrient foundation supplied by the host (Hooper et al., 1999). This may be true, but there are several indications that colonization is influenced by the host genotype as well. Mouse studies have revealed that the composition of fecal microbiota is affected by the major histocompatibility complex (Toivanen et al., 2001). Furthermore, different mammalian host species develop a different make-up of their GI microbiota.

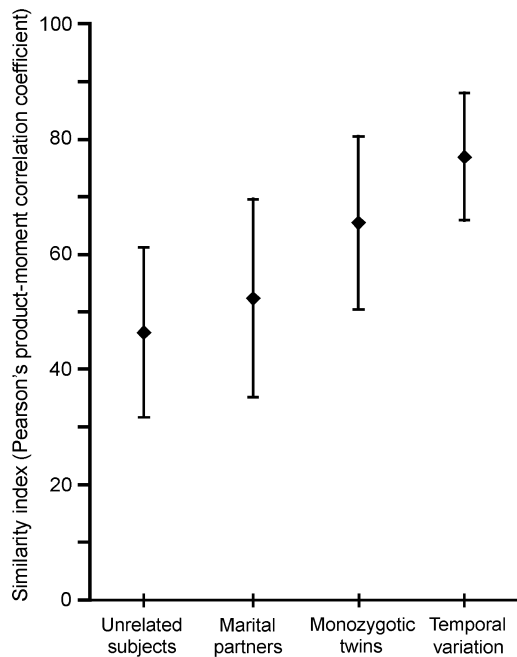
Additionally, studies with GF hosts that received inter-species GI microbiota transplants indicate that the hosts might be able to modulate their received microbial lineages toward a composition normally found in their non-GF, conventional status (Rawls et al., 2006). This can be attributed to obvious variables such as the type of food, the environment the host lives in, the nutritional requirements of the host, or more physiological aspects like host intestinal tract anatomy, body temperature, intestinal peristalsis, and residence time, etc. Intriguing results were obtained from the reciprocal GI microbiota-transplantation of zebra fish and mice, raised under GF conditions (Rawls et al., 2006). The GF hosts did not have any community legacy, and appeared to retain all intestinal species that were “given” to them during transplantation. Yet they reconstructed, in terms of relative abundance, the gut communities normally associated with conventionally raised animals (Rawls et al., 2006), indicating that a powerful and poorly understood host-mediated mechanism must be in place to coordinate microbial community composition. It seems that zebra fish and mouse host genetics play a prominent role in the natural selection of gut inhabitants, although there are many confounding factors in the differences between these hosts, such as differences in body and environment temperatures, host habitat and activity, bowel anatomy and dimensions, residence time, and dietary intake.

Last but not least, in adult humans, the extent of the variation of the dominant bacteria is associated with the degrees of relatedness between the subjects (Zoetendal et al., 2001; Van de Merwe et al., 1983). This family relatedness, especially with respect to twins, provides perhaps the most profound evidence of

host genotypic influences on microbial communities, and will be discussed in more detail in the next sections. In conclusion, the GI microbiota composition must be dependent on the host genotype, but the exact degree of this dependence remains to be elucidated.

The effect of host genetics on the gut microbiota is most profoundly observed in studies conducted on related individuals. Especially revealing were studies conducted with samples obtained from identical (monozygotic) and/or fraternal (dizygotic) twins. Already in 1983, indications were found that monozygotic twins have more similar fecal microbiota than dizygotic twins (Van de Merwe et al., 1983). Although these findings were based on cultivation-dependent techniques, which gives an incomplete picture of the GI microbiota (Zoetendal et al., 2008), this study provided a clear indication of host genetics and its influence on the fecal communities. Two decades later, Zoetendal et al. confirmed the significantly higher bacterial profile similarity in monozygotic twins with a culture-independent technique (Zoetendal et al., 2001). This study was performed 10 years ago using denaturing gradient gel electrophoresis (DGGE) on fecal bacterial 16S rRNA gene amplicons to assess the bacterial composition similarity. Samples in this study originated from human adults with varying degrees of genetic relatedness (ranging from parents and children, non-twin siblings to twin siblings). This study revealed a positive relationship between the DGGE profile similarity and the genetic relatedness of the subjects (Fig. 2.2). Marital partners showed slightly higher similarities than unrelated individuals, but this was not found to be significant (Zoetendal et al., 2001). The latter is quite remarkable as marital partners essentially live in the same

Fig. 2.2 Plot of the similarity indices (Pearson's product-moment correlation coefficient) from unrelated subjects, marital partners, monozygotic twins, and temporal variation comparisons. The mean (*diamonds*) and standard deviation (*black bars*) are plotted. DGGE profile of the total bacterial community was used to calculate the similarities. Adapted from Zoetendal et al. (2001)



environment and generally have similar dietary habits. Overall, these results indicate that host-genotype factors indeed have a strong impact on the bacterial community in the adult GI tract.

In a later study, a slightly different cultivation-independent technique, temporal temperature gradient gel electrophoresis (TTGE), was used to assess the influence of host genetics on the fecal microbiota composition in children (Stewart et al., 2005). TTGE profile similarity was again the lowest among unrelated children, higher between dizygotic twins, and clearly the highest between monozygotic twins (Stewart et al., 2005).

The high-throughput cultivation-independent Human Intestinal Tract Chip (HITChip), a phylogenetic microarray developed by Rajilic et al. (2009), was used to re-analyze the five monozygotic twin pair samples from Zoetendal et al. (2001). Average similarities between the twins was notably higher than the similarity between random unrelated individuals (Rajilic-Stojanovic et al., 2007). Even though only five twin pairs and five unrelated individuals were compared, the observed similarity difference was already borderline significant ($p = 0.067$) (Rajilic-Stojanovic et al., 2007). Thus the previous results obtained by DGGE were confirmed by phylogenetic microarray analysis. A recently conducted study on 40 monozygotic twin pairs showed that HITChip profiles were significantly ($p < 0.001$) more similar between the twins than between random unrelated subjects within this cohort (Fig. 2.3, Tims et al., unpublished observations). Palmer et al. also used

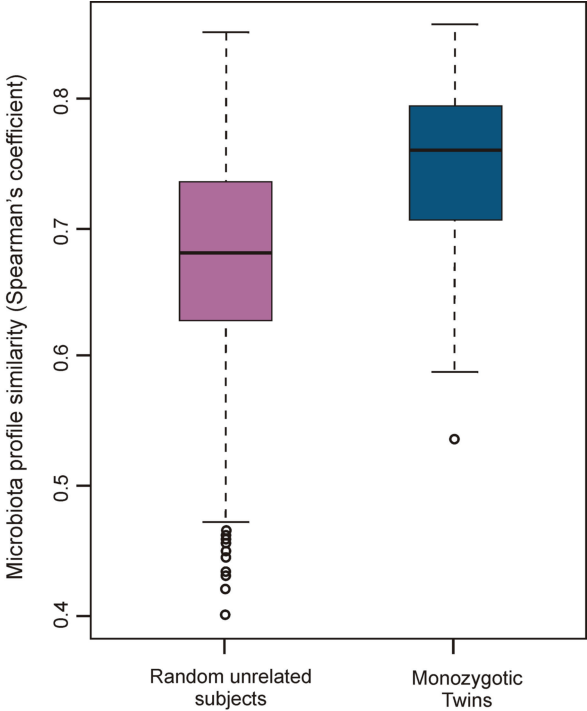


Fig. 2.3 Box-whisker plots of the similarity between the total microbiota profiles expressed as Spearman's correlation coefficient. *Purple box* represents similarities between random unrelated subjects and the *blue box* represents similarities between monozygotic twins. Similarities were calculated with total microbiota HITChip profiles (Tims et al., unpublished observations)

a phylogenetic microarray (different from the HITChip) to study GI microbiota development in human infants (Palmer et al., 2007). They included one dizygotic twin pair and this pair showed a more similar microbiota profile, at any stage of development, compared to the other 12 unrelated children (Palmer et al., 2007).

Recent developments in sequencing technologies and corresponding reductions of sequencing costs have been of great importance for GI microbiota research. Turnbaugh et al. performed pyrosequencing on variable regions of the 16S rDNA on fecal microbial DNA extracts in a cohort of 154 subjects (Turnbaugh et al., 2009a). On average, nearly 4,000 16S V2 region sequences were obtained for all subjects and additionally nearly 25,000 sequences per sample were acquired for 33 subjects. The studied group was composed of 31 monozygotic twin pairs, 23 dizygotic twin pairs, and the mothers of 46 of the twins, and included two samples per individual collected with a 57-day interval. Subjects included in this cohort were differentiated based on concordant leanness or obesity among the twin pairs. Most of the twins (71%) did not live together anymore. Pyrosequencing confirmed the previous observation that each individual has a unique GI microbiota composition and short-term changes are inferior to the inter-subject variations (Turnbaugh et al., 2009a; Zoetendal et al., 1998). Among all of the 154 individuals, no shared 16S rRNA gene-based phylotypes could be identified with an abundance of 0.5% or more. These results are in apparent contradiction with previous suggestions concerning the existence of a shared common core between humans, but different criteria were employed to define such a core community (>50% phylotype prevalence (Tap et al., 2009) versus 100% prevalence of phylotypes (Turnbaugh et al., 2009a)). Nevertheless, subjects from the same family had more similar microbial community structures and shared significantly more phylotypes (Turnbaugh et al., 2009a). Neither the obesity status per individual nor distance between the family members' homes confounded the observed higher similarities for the families (Turnbaugh et al., 2009a). In contrast to earlier findings (Stewart et al., 2005), the similarity between dizygotic twins were not lower than the similarities between the monozygotic twins (Turnbaugh et al., 2009a). Noteworthy is the fact that pyrosequencing still suffers from artifacts such as the formation of chimeric sequences during amplification and/or sequencing errors that are interpreted as distinct phylotypes. When such errors are not effectively removed they obviously influence the results obtained from sequencing-based studies. Perhaps future improvement of sequencing technology (in terms of reduced sequence error rates, advanced data analysis software suites, and/or extended sequence length) combined with increased sequencing depths may resolve the apparent contradictions in the current conclusions. Recently, Claesson et al. showed that pyrosequencing at the deepest sequencing-depth currently feasible, i.e., at approximately 400,000 sequences per sample, still does not capture the full microbial richness of GI tract samples (Claesson et al., 2009).

A common approach in the GI tract studies is inferring the possible microbiota functionalities from the microbial lineages present as detected by 16S rRNA gene sequences. Current functional metagenomic study designs are moving toward random sequencing of as much microbial DNA as possible (Zoetendal et al., 2008). Through these random sequences, a more direct view on the functional repertoire

of the microbiota can be obtained. Therefore, in addition to the 16S rRNA gene sequencing, Turnbaugh et al. also analyzed the samples of six families ($n = 18$) by random shotgun pyrosequencing (Turnbaugh et al., 2009a). In line with the 16S rRNA-based study, the profiles of the functional categories present in the gut communities were more similar between relatives. Interestingly, GI microbiota seemed to have even more similar functional profiles among all subjects despite their sometimes highly distinct microbiota composition profiles (Turnbaugh et al., 2009a). This raises the hypothesis that a core microbiome exists at a functional (and metabolic) level rather than at the level of microbial composition. In line with this hypothesis are results of earlier studies that determined in situ concentrations of short-chain fatty acids (SCFAs) in fecal material of different individuals. Different population survey data show the same fecal composition with respect to the ratios of the three main SCFAs acetate, propionate, and butyrate (Topping et al., 2001). Dietary changes have been shown to modulate SCFA production and absorption somewhat, but the SCFA ratios are not drastically altered (Cummings, 1981). As an individual has its own unique GI microbiota composition, the fairly constant SCFA ratios are quite remarkable and indicate that the gut microbes perform similar overall functions. Even though a core GI microbiota is likely to exist more on a functional level it must be noted that the species composition, although hypervariable and seemingly chaotic, is not random. Grouping of mammals based on their gut community composition is associated with their dietary needs, i.e., whether a mammal is a herbivore, carnivore, or omnivore (Ley et al., 2008). This could indicate that when roughly similar digestive tasks are required, the GI community tends to be more similar at higher taxonomic levels (Ley et al., 2008). However, the latter statement needs to be verified as the GI tract anatomy differs between individual mammalian species belonging to the carnivores, herbivores, and omnivores.

More comprehensive studies following both monozygotic and dizygotic twins from birth to adulthood can provide valuable information on interactions between host genotype and its inhabiting microbiota. In such studies diet should be taken into account, due to its drastic influences.

Dietary Influences

Diet is a strongly confounding factor in the ambition to obtain insight in the exact roles of the host genotype and GI microbiome in a super-organism. Not only the GI community is influenced by the dietary influx of microbes and nutrients but also the host phenotype itself, mainly through regulation of gene expression and physiological adaptation. Several genes from a group of nuclear hormone receptors called peroxisome proliferator-activated receptors (PPARs) can be used to illustrate host adaptation to its diet on a transcriptional level. Unsaturated fatty acids are, among others, activating ligands for PPAR- γ (Kliewer et al., 1997), and thereby PPAR- γ activities are directly influenced by the diet. PPAR- γ acts as a regulator of a variety of relevant physiological processes, including transcription control of many genes involved in fat-cell differentiation, insulin sensitivity, and lipid homeostasis

(Debril et al., 2001). The host genotype, in terms of PPAR- γ gene variants, affects the extent to which the host reacts to its diet. For example, a PPAR- γ 2 gene polymorphism (Yen et al., 1997) correlates with the inter-individual variability in serum triacyl-glycerol levels after administration of $n-3$ fatty acids (Lindi et al., 2003). This example is just one of the many available in literature, which indicates that although the host contributes with a marginal amount of genes to the metagenome, variations in the host genotype can still have drastic effects for the super-organism. Furthermore, this example indicates the major role of the host's diet as well. This raises the question of how important the diet is exactly.

Obesity in Animal Models

Obesity has gained popularity with respect to study of the importance of dietary influences on the gut microbiota and the host. The prime cause of obesity is an excessive caloric intake. Such a surplus intake disturbs the normal balance between the amount of energy harvested from the diet and the amount used by the host. This balance, or energy homeostasis, is at least partly defined by the GI microbiota. Studies with GF mice clearly show the microbial impact on host energy homeostasis. GF mice are resistant to obesity development when fed a "Western" (high-fat/high-sugar) diet (Backhed et al., 2007), but GI tract colonization was stimulating weight gain in these mice (Samuel et al., 2008). Colonization of GF mice leads to an increased release of monosaccharides and SCFAs from complex dietary polysaccharides, enhanced conversion rates of fatty acids toward complex lipids in the liver, and by regulating host genes involved in storage of the converted lipids into adipocytes (Backhed et al., 2004).

However, next to the GI microbiota, host-dependent factors are also required to develop obesity. For instance, mice lacking a functional copy of the *Gpr41* gene, a G-protein-coupled receptor that binds SCFAs, do not readily develop obesity (Samuel et al., 2008). Without *Gpr41*, the mice displayed an increased intestinal motility and decreased SCFA absorption (Samuel et al., 2008). On a side note, another G-protein-coupled receptor called *Gpr43* displays a different role in mice (Maslowski et al., 2009). Mouse models of colitis, arthritis, and asthma required stimulation of *Gpr43* by SCFAs to counteract their inflammation (Maslowski et al., 2009). Thus, besides metabolic regulation, immune and inflammatory responses are affected by the bacterial SCFA production as well.

Simply having a gut microbiota is not the only microbial factor affecting the energy balance in mice. The composition of the microbial community determines to what extent the microbiota improves energy harvest from food. Both GF mice inoculated with distal gut microbiota from conventionalized obese and lean animals resulted in an increase of bodyweight and total body fat (Turnbaugh et al., 2006). Yet this increase was found to be significantly larger in the GF mice that had received the microbiota from the obese animals (Turnbaugh et al., 2006). Correlations between phenotypic variations and attributes of the microbial gut community were reported after studies conducted with lean and genetically obese (*ob/ob*) mice (Turnbaugh

et al., 2006; Ley et al., 2005) as well as with lean and genetically obese rats (Waldram et al., 2009). Variations of the members belonging to the bacterial phyla Bacteroidetes, Firmicutes, and Acintobacteria appeared to be associated with leanness or obesity (Turnbaugh et al., 2006; Ley et al., 2005; Waldram et al., 2009). Especially the Bacteroidetes to Firmicutes ratio was found to be lower in the obese rodents.

Most animal models used to study obesity consist of specific gene knockout mutants, such as those focusing on the role of the *ob* gene in mice that predisposes them to develop obesity. The *ob* gene encodes for the protein hormone leptin, which regulates body weight, metabolism, and reproduction in mammals (Friedman et al., 1998). Both inactivating mutations in the leptin (*ob*) gene and in its receptor (*db*) gene lead to genetically obese mice (Friedman et al., 1998). Therefore, the host genotype is important in the development of obesity as well. Another study involving wild-type and *Apoa-I* knockout mice indicates that all three aspects are implicated in the development of obesity and metabolic syndrome (MS) (Zhang et al., 2010). *Apoa-I* knockout mice have an impaired glucose tolerance and high body-fat levels. Groups of wild-type and of *Apoa-I* knockouts were fed a high-fat diet and normal chow diet for 25 weeks. Diet as well as genetic mutation could explain 57 and 12% of the observed variation found in the GI microbiota communities, respectively (Zhang et al., 2010). The results of this study indicate a stronger, possibly dominating, role of the diet compared with the genetic variations of the host. Nevertheless, the influence of host genotype is not negligible and should therefore be taken into account in MS studies.

Obesity in Humans

Extrapolation of the relations among diet, host genotype, and GI microbiota discovered in animal studies to human obesity seems sensible but has proven to be difficult. Nevertheless, deviating leptin concentrations are associated with obesity in humans as well (Considine et al., 1996). Leptin normally suppresses hunger and increases metabolism, and it has been suggested that obese humans are insensitive to leptin. However, although several cases have been described (Clement et al., 1998), mutations in the human *db* gene are only rarely seen in obese people. However, to accurately assess the importance of *db* gene variations with respect to obesity development risks, the *db* mutation frequencies in lean individuals should also be investigated.

In contrast to mice studies, research with human subjects provide conflicting results regarding the association of obesity with relative abundances or abundance ratios of specific bacterial phyla (Ley et al., 2006; Schwiertz et al., 2009; Duncan et al., 2008). However, human studies reported to date employed different molecular techniques and targeted populations of different geographic origin. Duncan et al. found no differences in bacterial phyla abundances or abundance ratios, but identified a significantly higher proportion of butyrate producers in the obese subjects (Duncan et al., 2008). This observation is in agreement with the finding of

increased butyrate concentrations in the cecum of *ob/ob* mice as compared to their lean littermates (Turnbaugh et al., 2006). The SCFA butyrate is mainly produced during carbohydrate fermentation in the colonic lumen, mainly by members of the *Firmicutes* phylum, especially those belonging to *Clostridium* cluster IV. Luminal butyrate is quickly absorbed by the colon mucosa where it serves as the main energy source for the colonocytes (colonic epithelial cells) (Cummings, 1981; Roediger, 1980). However, the exact physiological effects of butyrate are not fully understood. Different, but related cell-line models can yield direct opposite results regarding the role of butyrate in the modulation of cell proliferation, differentiation, and apoptosis (Hague et al., 1997; Medina et al., 1998). These conflicting results, commonly referred to as the “butyrate paradox,” are extensively reviewed elsewhere (Hamer et al., 2008). In short, in vivo human data are insufficient but most studies support beneficial roles for butyrate, including the restraining of inflammation and carcinogenesis, reinforcement of various components of the mucosal barrier, lowering of colonic oxidative stress, and promotion of satiation (Hamer et al., 2008). Overall, it is obvious that the production of butyrate by the GI microbiota has a major influence on colonic mucosa. Thereby the differential abundance levels of butyrate-producing microbes, as reported by Duncan et al., seem relevant with respect to human health and may be associated with energy homeostasis and obesity risk.

Diet: Transient or Permanent Effects?

One may conclude from the animal experiments and the observations in human volunteers that the interactive factors, constituted by dietary intake and gut microbial ecology, are of major importance for the host's well-being. This raises the question whether dietary effects should be seen as transient or can also generate permanent effects. The microbiota transplant approach in mice revealed that efficient energy-harvesting traits are transferable by the GI microbiota (Turnbaugh et al., 2006). In continuation of this approach, C57BL/6 J mice were conventionalized in such a way that all animals inherited similar gut microbiota (Turnbaugh et al., 2008). Also in these mice, the change from a chow diet (low-fat/high-fiber) to the “Western” diet (high-fat/high-sugar) resulted in an increased weight gain (Turnbaugh et al., 2008). In the diet-induced obese mice the relative abundances of the *Firmicutes* were higher, whereas those of the *Bacteroidetes* were lower compared with their lean status (Turnbaugh et al., 2008). These findings are in agreement with previous results obtained with genetically obese (*ob/ob*) mice (Ley et al., 2005). However, the changes in the *Firmicutes* phylum were not division wide but appeared to be mainly restricted to an increased abundance of the Mollicutes class (Turnbaugh et al., 2008). Apparently, these diet-induced changes invoked an adaptation of the microbiota to the quality and quantity of the available nutrients, and these diet-induced microbiota adaptations are apparently reversible (Turnbaugh et al., 2008). Follow-up experiments in mice receiving human gut microbiota transplants confirmed that the diet-induced changes on the GI communities in these so-called humanized mouse models are reversible as well (Turnbaugh et al., 2009b). Interestingly, obese and lean

associated microbial communities could be maintained by diet alone. Maintenance of community structure and diversity was even achieved across several generations of mice following initial transplantation (Turnbaugh et al., 2009b). This again illustrates the prominent influence of the diet. Whether the diet is able to overrule the host genotype by permanent alterations of the GI community remains to be explored. In rats and humans, dietary “metabolic imprinting” through epigenetic modifications on the host genotype seems likely (Bateson et al., 2004; Godfrey and Barker, 2006; Lillycrop et al., 2005). However, the reversibility of the gut ecology in the inter-host as well as intra-host species microbiota transplants described above seem to rule out the possibility of imprinting through the GI community (Turnbaugh et al., 2008, 2009b). Although not permanent, it remains a fact that diet has a major and rapid impact on the microbiota.

Host–Microbiota Co-evolution: Selective Geographic Pressure?

Environment can be easily overlooked while studying GI tract microbiota, but is quite important as it determines physiological as well as microbial influences, e.g., through availability of food, food consumption habits, temperature, and humidity. Many environmental aspects are geographically confined, which raises the question if geography and its associated factors, such as climate, availability of food, and composition of the diet, could be an important aspect in host–microbe interactions. Several studies indicate an intimate co-evolution of humans and the gastric pathogen *H. pylori* (Linz et al., 2007; Moodley et al., 2009). *Helicobacter pylori* appears to have spread from east Africa 58,000 years ago along with its human host. This finding implies that geography can also influence the microbial community, although modern commuting could blur the extent of such geographical impact. Naturally, the exact course and speed of this “blurring” depends on the magnitude of the evolutionary developed differences between the ethnic groups involved.

Many studies try to minimize the drastic effects of diet on the microbiota. Dietary habits usually depend on the geographical location of the subjects. Hence, not many studies have been performed on GI microbiota composition across country boundaries. One of the first studies across several countries was performed by Lay et al. and conducted on 91 subjects from five Northern European countries (France, Denmark, Germany, the Netherlands, and the United Kingdom) who consumed a nonrestricted Western European diet (Lay et al., 2005). However, the identified bacterial proportion of the gut microbiota did not significantly differ in composition when grouping the samples according origin, gender, or age (Lay et al., 2005). Mueller et al. conducted a study that included as many as 230 healthy subjects from the more distant European countries such as France, Germany, Italy, and Sweden (Mueller et al., 2006). Several differences were found regarding country, age, gender, and combinations thereof (Mueller et al., 2006). Without much effort, dietary justifications can be found for most of the observed phylogenetic differences. An interesting example is the relative abundance of *Faecalibacterium*

F. prausnitzii. Strict vegetarians appear to have no detectable amounts of *F. prausnitzii* (Hayashi et al., 2002). The authors suggest that the highest levels of *F. prausnitzii* and related species was in the Swedish subjects and may be related to a high level of fish and meat consumption, which is a known dietary tradition of the Swedish population (Mueller et al., 2006). However, at the time of this study the Swedes, Italians and French consumed the same amount of animal products (World Resources Institute - EarthTrends Environmental, <http://earthtrends.wri.org/>), hence other dietary influences are probably implicated as well.

The European studies seem to indicate that differences in GI microbiota composition increase with the distance between the geographic origins of the subjects. At a larger intercontinental distance, comparable studies were performed by American (Eckburg et al., 2005; Gill et al., 2006) and Chinese (Li et al., 2008) research groups. Although at phylum level, the Chinese and American subjects exhibited comparable phylogenetic GI tract compositions, principal coordinate analysis showed clear differences at species-level composition (Fig. 2.4) (Li et al., 2008). Importantly, these findings reflect the differences in nuclear magnetic resonance based metabolic urine phenotypes found between large groups of Chinese and American subjects. Many of the differential urine metabolites do not have a mammalian origin but are derived from microbial sources (Dumas et al., 2006). Thus, it can be hypothesized that there is a co-variation between gut microbiota structure and the host metabolic

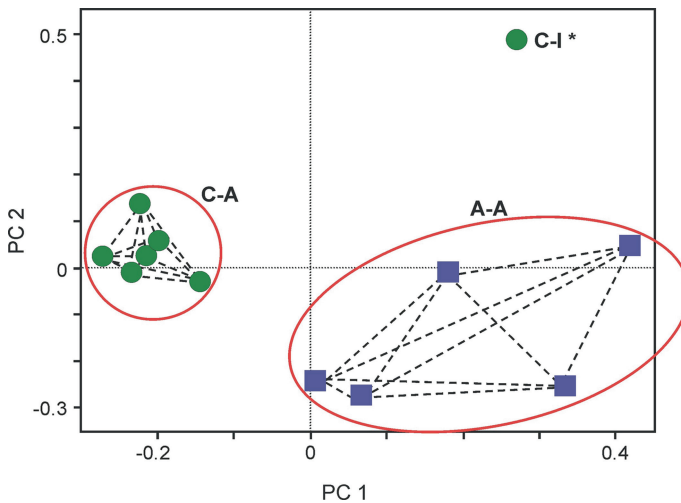


Fig. 2.4 Principal component (PC) analysis on the species-level composition of the gut microbiota of Chinese family members (green circles) and American volunteers (blue squares). UniFrac metrics were used to generate the principal coordinate scores plot. The percentage of variation described by PC 1 is 19.8% and by PC 2 is 13.5%. Red circles are drawn around the group of adult Chinese (C-A) and around the American subjects (A-A). Intersample differences within the adult groups are visualized with dashed lines. C-I* indicates the Chinese baby 1.5 years of age, which was left out the C-A group due to its still developing gut microbiota. Figure adapted from Li et al. (2008) (American microbiota data: (Eckburg et al., 2005; Gill et al., 2006))

phenotype. Urine metabolite profiles associated with microbial products are able to discriminate Japanese living in Japan and Japanese living in America (Holmes et al., 2008). Therefore, the differences found between the Americans and Chinese could be more dependent on diet and/or geography than on host genotype. Interestingly, the Chinese volunteers (Fig. 2.4) all belonged to the same family and had more similar GI communities when compared among each other than the unrelated subjects within the American cohort. Hence, this observation does associate genetic relationship with GI microbiota composition and therefore points at a role for the host genotype in gut community development.

Whether geographic pressure has left its mark on the co-evolution of men and GI microbes is impossible to tell from the studies described in the previous paragraphs, without the knowledge on the influence of the diet and host genetics. Geography could be important with respect to the availability or prevalence of environmental or dietary microbial lineages for host colonization. Although the host (genotype) is dependent on the microbes it receives, it might be able to put selective pressure on sub-populations (Rawls et al., 2006). However, currently, no colonization restrictions have been observed in people who migrated from their traditional home countries into other geographic regions.

Intriguingly, the variation in colon cancer prevalence among Afro-American and native African populations appears to be in agreement with co-evolutionary relationships between human and GI microbiota caused by geographic and environmental conditions. Americans of African origin have the highest risk of all American sub-populations to develop colon cancer, whereas native Africans rarely suffer from this type of cancer (O'Keefe et al., 2007). The biggest difference between these two populations is probably their dietary habits, which prominently influences their GI microbiota communities (Ley et al., 2006; Turnbaugh et al., 2008, 2009b). Initial cultivation-dependent analyses confirmed differences in GI communities between native Africans and Afro-Americans (O'Keefe et al., 2007), although these methods provide an incomplete impression of the microbiota. Newborn Afro-Americans are not exposed to the (relative) abundant levels of many microorganisms as they would in the native African region. Thus, no major colonization occurs of microbes that normally dominantly reside among the child's kin group, which essentially is a settled population established through generations-long consistent geographic and lifestyle factors. This means that the emigration process that commonly coincides with changes in dietary habits seems to disrupt the co-evolved mutualism between the host genotype and its GI microbiota, resulting in the increased colon cancer risk in African-Americans. In conclusion, the functioning of the human super-organism appears to be affected by currently fading barriers in human and environmental ecology caused by urbanization, global traveling, and emigration. These fading barriers probably coincide with a reduction in the number of microbe encounters, which could lead in humans to an underdeveloped immune system according to the hygiene hypothesis (Guarner et al., 2006). Such disruptions of long-term co-evolved interactions between man and microbe might partially explain the observed increase of chronic and degenerative disease frequencies in industrialized countries (O'Keefe et al., 2007; Guarner et al., 2006).

Host-Genotype Polymorphisms

The genetic make-up of humans is very similar, and small genetic polymorphisms form an important aspect of genetic variation among human beings. Evidence is accumulating that these genetic variations are important determinants for the interactions with host-associated microbiota. Intuitively, the highly polymorphic immune-related genes are eminent candidates to define the interaction between host and microbe. The mucosal epithelial barrier has traditionally been considered to prevent contact between microbiota and underlying cells, including immune cells. Any contact was thought to provoke immune reactions that would even eradicate the commensal organisms from the host. However, current knowledge clearly establishes frequent and essential communications between immune system and microbiota (Rakoff-Nahoum et al., 2004; Macdonald and Monteleone, 2005). Immune tolerance is promoted by local immune modulations that only inhibit further host tissue penetration of the commensal microbes (Macdonald and Monteleone, 2005; Cebra, 1999; Macpherson, 2005). This “peaceful situation” is normally maintained despite the massive presence of bacterial molecules that are capable of activating the host’s bacterial molecular pattern recognition receptor and their cognate immune regulation cascades (Rakoff-Nahoum et al., 2004). Polymorphisms in any of the immune-related genes involved in immune tolerance are prominent candidate determinants of the bacterial selection by the host.

Host–Microbiota Communication: Innate Immune System

Important bacterial molecular pattern recognition receptors capable of initiating innate immune responses are the toll-like receptors. Toll-like receptor-4 (TLR-4) can recognize lipopolysaccharide (LPS), a cell wall component of Gram-negative bacteria (Hoshino et al., 1999; Poltorak et al., 1998). Mutations of the TLR-4 gene result in a weakened immune response to LPS in mice (Poltorak et al., 1998). Moreover, TLR-4 sequence variants in humans correlated with a reduced response to inhaled endotoxins (Arbour et al., 2000). For Gram-positive-produced lipoproteins and lipoteichoic acids TLR-2 seems to be the main mammalian receptor (Lorenz et al., 2000). Moreover, TLR-2 either alone or in heterodimer form with other TLRs can recognize more than a single ligand (Opitz et al., 2009). Naturally occurring mutations in the human TLR-2 gene were shown to have diminished response to lipoproteins harvested from the Gram-positive bacteria *Borrelia burgdorferi* and *Treponema pallidum* (Lorenz et al., 2000). Although the polymorphisms in TLR-4 and TLR-2 are medically relevant, no studies have been reported, which determine their impact on human (or other mammalian organisms) associated microbiota. However, it is reasonable to suspect gene variants of important components of the innate immune system to be involved in the host–microbiota interactions. From mice studies, it is known that a genetically disabled innate immune system is associated with a collitogenic murine gut community (Garrett et al., 2007). Unfortunately, in these types of studies it is unclear whether the colitis

is caused by a change in community structure or by the defective defense system of the murine hosts.

Other immune system components have been shown to be involved with the host-microbiota crosstalk. Besides TLRs, the innate immune system also depends heavily on the nucleotide-binding oligomerization domain (NOD) receptors (Inohara et al., 2001). An overview on the current knowledge of TLRs, NOD receptors, and other innate immune system receptors is given elsewhere (Opitz et al., 2009). NOD2 variants clearly show a relation between host genotype and gut microbiota composition, which is associated with increased risk for Crohn's disease (CD) (Hugot et al., 2001; Ogura, Y., et al., 2001). Normally, NOD2 binds the muramyl dipeptides of bacterial peptidoglycan (Girardin et al., 2003), but certain polymorphisms in NOD2 can result in failure of muramyl dipeptide detection. Such a failure results in a lack of tolerance development for commensal bacteria and dietary antigens and consequently leads to "inappropriate" immune response against them (Landers et al., 2002). Considering these results, it is not surprising that CD patients were found to have lower diversity and diminished levels of normally abundant bacteria (Manichanh et al., 2006). Especially the phylogenetic group *Clostridium leptum* in the Firmicutes phylum seems to be reduced in patients with CD. Several molecular techniques have indicated that *F. prausnitzii*, which is a member of the *C. leptum* group, is depleted in the mucosa-associated communities (Manichanh et al., 2006; Frank et al., 2007; Martinez-Medina et al., 2006). Importantly, secreted metabolites of *F. prausnitzii* have been shown to exert anti-inflammatory effects in vitro (Sokol et al., 2008). In addition, *F. prausnitzii* is associated with an in vivo reduction of pro-inflammatory cytokine synthesis and increase of anti-inflammatory cytokine production in the colon. The observations concerning *F. prausnitzii* combined with the association of CD and NOD2 mutations illustrate a potential link among host genotype, phenotype, and microbiota composition. NOD2 mutant genotypes are likely to promote a negative selection of *F. prausnitzii* in the colon, which in turn can lead to the development of CD. Nevertheless, it is possible that other variations in the host genotype can allow the selection of other microbes similar to anti-inflammatory influences as *F. prausnitzii*.

Host-microbe interactions are not restricted to direct interactions between bacterial ligands and receptors of the innate immune system. Other proteins that should be considered are, for instance, further down the signaling pathway of TLRs or are involved in other cellular processes supporting immune responses, such as cell movement and restructuring. The gene MEFV encodes the protein pyrin, which is involved in innate immune response regulation, but has currently no definite function assignment (Ting et al., 2006). This gene has no direct contact with microbes as it has been found in the cytoskeleton, but mutations of MEFV can lead to Mediterranean fever, a hereditary auto-inflammatory disorder (Khachatryan et al., 2008). In addition, patients with this disease have lower bacterial diversity and prominent population shifts in the Bacteroidetes, Firmicutes and Proteobacterium phyla during periods of active disease, whereas when the disease is in remission the bacterial gut community is more similar to normal microbial composition but still atypical (Khachatryan et al., 2008). Therefore, even polymorphisms in genes

encoding proteins that are not supposed to be in direct contact with the gut microbes can influence host–microbe interactions. Recently, in healthy human mucosa, gene expression patterns have been found, which correlate with the development of immune tolerance for the organism *Lactobacillus plantarum* (van Baarlen et al., 2009). Even for one organism, these patterns involve many genes and therefore may be affected by many potential polymorphisms, which again exemplifies the complex nature of host–microbe interactions.

Host–Microbiota Communication: Non-immune-Related Mechanisms

Variations in genes not directly involved in immune system pathways can exert influence on the microbiota composition and functioning as well. Changing the conditions for the GI microbiota through modulation of host-derived resources or available attachment site in the mucosal layer seems of considerable importance. A nice illustration can be given by the abundant commensal species in the human and murine gut *Bacteroides thetaiotaomicron*. This organism matches its demand for fucose, a growth substrate, by upregulating fucosylated glycan production of epithelial cells in mice whenever pentose sugars are scarce (Hooper et al., 1999). Other bacteria might (ab)use this epithelial fucose synthesis regulation of *B. thetaiotaomicron*, as such this mechanism is important for multiple species in the GI microbiota (Hooper and Gordon, 2001a). Extrapolating this finding to the human situation, one can imagine the involvement of the fucosyltransferase enzyme polymorphisms, which are determinant for human blood types (Becker and Lowe, 2003). This type of gene has many variants since human glycoproteins are likely to be continuously evolving through natural selection provided by commensal and pathogenic microorganisms (Hooper and Gordon, 2001b). Notably, some correlations between blood type and gut community variations indeed have been reported in the past (Van de Merwe et al., 1983; Hoskins, 1993).

Another example of potential host-genotype-dependent interactions with gut microbes is mucin encoding (MUC) genes. These MUCs encode protein backbones for mature mucin molecules, which are heavily glycosylated by O-linked oligosaccharides on the threonine, proline, and/or serine repeats (Herrmann et al., 1999). The terminal oligosaccharides of mucin molecules contain sulfate and/or O-acetyl-substituted sialic acids. Mucins can both promote and prevent bacterial cell adhesion, depending on the exact structures of their O-glycan chains (Hollingsworth and Swanson, 2004). Changes in mucin composition have been associated with inflammatory bowel diseases (Morita et al., 1993). Next to microbial mucin degradation, these changes could be due to genotype-related issues, such as polymorphisms in MUC genes, variations in MUC mRNA or protein levels, and variable post-translational modification changes (i.e., the extent of glycosylation and sulfation) (Morita et al., 1993).

Many more host genes can be found that are involved in the functioning of the GI tract and thereby polymorphisms in such genes could have an impact on the microbial communities. Mutants in host enzymes responsible for nutrient breakdown

and/or absorption could potentially influence the microbiota by altered nutrient composition and availability in the different regions of the intestine. For example, enterocytes in the small intestine can absorb glucose through active and passive glucose transporters (Leonie Los et al., 2007). Variants of these host-encoded transporters could cause alterations in the rate of carbohydrate absorption and thereby modulate the carbon source availability for the resident microbes, which may favor different microbial communities. More complex host–microbiota interactions are mediated by bile acids, which, next to their digestive functions (i.e., solubilization of lipids and lipid-soluble vitamins to enhance their absorption), have a role in maintaining the intestinal barrier (Martin et al., 2007). Mammalian-microbiota co-metabolism result in the so-called secondary bile acids, which exert biologically important effects on both host and microbiota constituents (Martin et al., 2007). Variations in level or composition of bile may have prominent effects on microbial communities and may correspond with specific consequences in mucosal cell biology. Primary bile acids are synthesized in the liver by a cascade of enzymes, providing many possibilities for gene variants that influence bile composition and corresponding host–microbe interactions.

Direct Interactions of Host Genome and Microbiome

Of all the possible interactions taking place in or around a host (Fig. 2.1), modulation of the host genotype by the microbiota seems extraordinary. Nevertheless, when 223 genes in the rough draft of the human genome were found to potentially have a bacterial origin, horizontal gene transfer (HGT) from bacteria to humans has been suggested (Lander et al., 2001). This could indicate that bacteria can manipulate their host, likely for their own benefit. However, HGT between human and bacteria is a difficult process because the genes should be stably integrated into the host DNA of germ line cells, to which bacteria normally do not have physical access. Furthermore, in 2001, Salzberg et al. carefully reexamined protein sequences of human, four other eukaryotes, and all completed prokaryote genomes at that time (Salzberg et al., 2001). They only found about 40 human genes to be possible candidates for HGT from bacteria to humans. Therefore, HGT between bacteria and humans remains doubtful since alternative, more plausible biological and technical explanations may be responsible for the few shared genes that are observed. One such biological factor is the high probability that the analyzed species had lost several genes from the common eukaryotic ancestor gene pool. Furthermore, nucleotide substitution rates can vary between genes within one genome as well as between similar genes in different organisms (i.e., evolutionary rate variation (Li, 1997)). Therefore, in HGT analyses, evolutionary relatedness cannot be based on sequence similarity alone, indicating an important technical limitation in the currently available studies on this topic (Eisen, 1998). Furthermore, only five eukaryotic genomes were available at the time of analysis, three of which belong to the animal lineage (*Caenorhabditis elegans*, *Drosophila melanogaster*, and *Homo sapiens*) (Lander et al., 2001; Salzberg et al., 2001). Hence, the total eukaryotic diversity was poorly represented. By contrast, the available prokaryotic genomes at that time embody a

much broader evolutionary diversity (Nelson et al., 2000). This limited sample size of eukaryotic genomes is yet another technical problem confounding the HGT from bacteria to human. Concluding, it seems unlikely that bacteria have permanently manipulated their human hosts through HGT.

It seems more feasible that microbes sometimes pick genes up from their hosts. For example, the possible transfer of genes encoding serpins, which are protease inhibitors involved in the regulation of many physiological processes (Ivanov et al., 2006). Although serpins are found in all three domains of life, which indicate that they could originate from a common ancestor, serpins are found in relatively few prokaryotes. The latter would imply that serpins are not essential for survival or that they may have been acquired by prokaryotes as the result of HGT. Even though the serpin of *Bifidobacterium longum* is distantly related to eukaryotic serpins, Ivanov et al. showed that it exerts inhibitory functions through an identical mechanism. Another clearer example is the presence of *nptA* gene, a sodium/phosphate co-transporter, in *Vibrio cholerae*. This gene is also present in animals but seems to be absent in any other bacterial species (Lebens et al., 2002). Furthermore, *V. cholerae* has been shown to exhibit activity similar to that of its animal homologs (Lebens et al., 2002). It is likely that this transporter facilitates *V. cholerae* in the GI tract and consequently could be involved in the pathogenicity of this microbe. However, in both the serpin and the *nptA* case, it remains difficult to prove that the genes are not derived from an ancestral gene instead of being transferred from a (mammalian) host species.

Mitochondria provide off course evidence that indeed bacterial DNA resides in the mammalian genotype. Although these eukaryotic organelles originate from the endosymbiosis of an alpha-*Proteobacterium* ancestor, they show no indications that they were introduced in order to manipulate the host for their own benefit. By contrast, recent findings show that it was the eukaryotic host that took control and manipulated the bacterial endosymbiont for its own benefit (Gabaldón and Huynen, 2007). During the transformation of bacterium to organelle, many bacterial genes not involved in energy conversion were lost or replaced by genes originating from the eukaryotic host (Gabaldón and Huynen, 2007).

Conclusions

Humans and other mammalian hosts provide only a minor quantity of genes to their super-organism metagenome. Yet these genes are essential and decisive in defining the final host–microbiota interactions. Disturbances in the host genotype can lead to malfunctioning of the super-organism, i.e., all kinds of metabolic disorders, immune diseases, and other disorders.

Human genotypes consist of a huge amount of variables, many of which could be of importance for host–microbiota interactions. Not only genes directly related to the immune system should be considered in future studies. For instance, *Escherichia coli* has been implicated to, via quorum sensing, cross-communicate with the host epinephrine signaling pathway (Sperandio et al., 2003). Although

this is coming from a pathogenic species, one cannot exclude this type of non-immune-system-related communication between the host and its commensals. Another form of communication is through metabolites, such as SCFAs or (secondary) bile acids (Maslowski et al., 2009; Martin et al., 2007). Hence, many different mechanisms constitute the overall host–microbe interactions pallet. Basically, the hierarchy in importance of human genotypes in relation to host–microbe interactions is unknown. Currently, the majority of predictions hint at immune-related factors, i.e., gene polymorphisms. However, genes involved in metabolic functions and their control or those involved in biosynthetic pathways, such as mucus production and modification, or bile metabolism are likely to be important modulators as well.

New human genotyping efforts using extensive volunteer cohorts, combined with in-depth microbiota profiling, provide a possibility to mine for all factors underlying the relation between human host and its microbial communities. Furthermore, comprehensive studies following both monozygotic and dizygotic twins from birth to adulthood will provide vital information to assess the relative contribution of host genotype to the GI microbiota composition. These studies will be most successful when they acquire additional metadata, such as dietary habits, and actual short-term nutrient intake, and preferably also include intergenerational analysis of the subjects' families. Such multivariant analyses will be essential to dissect influences of dietary, environmental, and host-genotype factors.

Nevertheless, diet will probably always be an obscuring factor due to its dramatic, but apparently reversible effects on the microbiota. Future studies could benefit from the consistent use of family members, different ethnic groups, or both. Difficult studies, from an ethical point of view, in which the subjects are isolated for longer periods of time and under strict dietary regimes, might provide better insight in the human–diet–microbiota relationship. Regardless of the chosen study types, it will be a “life-changing” experience to finally fully understand both dietary and host genotype influences involved in shaping and interacting with the intestinal microbiota. Such knowledge may enable the definition of dietary regimes that provide prophylactic and therapeutic possibilities for a variety of disorders and/or diseases, provided that a causal relationship underlies the observed diet and microbiota correlation with these disorders or diseases. Specific dietary design might be attempted to correct deviating microbiota compositions and/or activity associated with specific diseases toward a more “healthy microbiota.” The rapidly developing field of (functional) metagenomics may allow us in the near future to actually come to the accurate description of what can be considered a “healthy microbiota,” which could then be employed as a biomarker in diagnosis and treatment of diseases and/or disorders.

“He who does not know food, how can he understand the diseases of men?” – Hippocrates (460–357 B.C.)

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