11 Infant nutrition and the microbiome: Systems biology approaches to uncovering host–microbe interactions

Mei Wang, Ivan Ivanov, Laurie A. Davidson, Robert S. Chapkin, and Sharon M. Donovan

11.1 Introduction

The gastrointestinal tract (GIT) represents a vast interface between the host and the external environment. The GIT is continually exposed to compounds in the diet and microbes (commensal and pathogenic) that can be either beneficial or detrimental to the host (Takahashi and Kiyono, 1999). Recent advances in high-throughput sequencing have provided insight into the complexity of the microbial communities inhabiting the human GIT (Ji and Nielsen, 2015). Metagenomic surveys of the intestinal microbiota revealed an immense phylogenetic diversity, estimated at more than 1000 species-level phylotypes across the human population, with at least 160 prevalent species per individual (Qin *et al.*, 2010). In adults, more than 90% of the species belong to two bacterial phyla: Firmicutes and Bacteroidetes (Qin *et al.*, 2010). The human gut microbiome, the second genome within our body, encodes more than 3.3 million non-redundant genes, exceeding the number encoded by the human host genome by 150-fold (Qin *et al.*, 2010; Grice and Segre, 2012). It provides us with genetic variation and gene functionality that eukaryotic cells have not evolved on their own (Grice and Segre, 2012).

Mounting evidence indicates that gut microbiota influence the physiological function of the host in health and disease (Dave *et al.*, 2012). Indeed, the overall composition of the microbiota, in addition to specific microbial genera, have been associated with normal GIT (Gordon and Bruckner-Kardoss, 1961; Gustafsson *et al.*, 1970) and immune (Donnet-Hughes *et al.*, 2010; Hooper *et al.*, 2012) development. In addition, it has been demonstrated that gut microbiota modulate brain and cognitive development and behavior (Diaz Heijtz *et al.*, 2011; Collins *et al.*, 2012) and infant growth and body composition (White *et al.*, 2013; O'Sullivan *et al.*, 2015; Scheepers *et al.*, 2015). Dysbioses, or alterations in the normal microbiota composition, are also linked to common acute infections (Berrington *et al.*, 2014; Li *et al.*, 2014b; Yurist-Doutsch *et al.*, 2014), inflammatory states (Carlisle and Morowitz, 2013; Mai *et al.*, 2011), long-term immune-related diseases (Li *et al.*, 2014a), and metabolic diseases such as diabetes and obesity (Bäckhed *et al.*, 2004; Turnbaugh *et al.*, 2006; Greiner and Bäckhed, 2011), including the risk of childhood obesity (Karlsson *et al.*, 2012).

Nutrigenomics and Proteomics in Health and Disease: Towards a Systems-Level Understanding of Gene–Diet Interactions, Second Edition. Edited by Martin Kussmann and Patrick J. Stover.

The development of the microbiome is strongly influenced by diet and other environmental factors, such as route of delivery and antibiotic use (Tanaka *et al.*, 2009; Voreades *et al.*, 2014; Wang *et al.*, 2016). Also, host genetics affect microbial colonization (Blekhman *et al.*, 2015) and recent work has begun to provide insight into specific genes that are important in this process (Benson *et al.*, 2010; Leamy *et al.*, 2014; Blekhman *et al.*, 2015). In turn, the microbiota interacts with the host by influencing intestinal gene expression (Chowdhury *et al.*, 2007), gut growth, and digestive enzyme activity (Kozáková *et al.*, 2001) and immunology (Haverson *et al.*, 2007), which subsequently influences host homeostasis (Chow *et al.*, 2010; Thum *et al.*, 2012). Most of our understanding of the role of microbial colonization in host physiology has been obtained from germ-free (GF) and gnotobiotic (GN) animal models (Grover and Kashyap, 2014;Wang and Donovan, 2015); however, exfoliated epithelial cells present an opportunity to investigate non-invasively host–microbe interactions in the developing intestine (Donovan *et al.*, 2014). The purpose of this chapter is to review the factors that contribute to the development of the gut microbiota in the neonate, including host genetics, the impact of colonization on host health and gene expression, and host–microbe interactions.

11.2 Environmental factors influencing development of the infant gut microbiota

11.2.1 Perinatal factors

Development of the human gut microbiota is a complex process and involving stepwise succession and host–microbial interaction (Mackie *et al.*, 1999; Marques *et al.*, 2010; Rautava *et al.*, 2012a; Meropol and Edwards, 2015). In the past, it was thought that the fetal GIT was sterile and that colonization began during and after birth when the infant was exposed to the microbes from the mother and the environment (Mackie *et al.*, 1999). However, more recent studies report the presence of bacterial isolates and/or DNA in meconium, amniotic fluid, and fetal membranes, umbilical cord blood, and placenta of the healthy mother and infants, suggesting that colonization starts before birth (Li *et al.*, 2014b). Indeed, Jiménez and colleagues provided direct evidence that maternal gut microbiota are transferred to the fetus *in utero* by orally inoculating pregnant mice with a genetically labeled *Enterococcus faecium* strain (Jiménez *et al.*, 2005, 2008). The labeled strain was subsequently detected in the amniotic fluid of the dam and the meconium of pups obtained by cesarean delivery (CD). It is proposed that bacteria in the gut can be taken up by dendritic cells in Peyer's patches and translocated to other locations in the body via the lymph and blood (Jiménez *et al.*, 2005; Thum *et al.*, 2012). Bacteria may be transferred to the fetus through the paracellular pathway of the placental barrier (Thum *et al.*, 2012). A similar outcome was observed in a double-blind randomized controlled clinical trial of probiotic supplementation with *Bifidobacterium lactis* and *Lactobacillus rhamnosus* GG (LGG) in women during the final 2 weeks of pregnancy prior to elective CD (Rautava *et al.*, 2012b). Lactobacilli were present in the placenta of all subjects and *Bifidobacterium* group, *Bacteroides* group, and *Clostridium leptum* were detected in the placenta of 30–40% of subjects. Bacterial DNA was also detected in 43% of amniotic fluid samples, with bifidobacteria and lactobacilli being the most commonly detected genera. In addition, fetal exfoliated intestinal epithelial gene expression was measured in the meconium using non-invasive methodology developed by Chapkin and

colleagues, which will be discussed later in this chapter (Chapkin *et al.*, 2010). The presence of *Lactobacillus* DNA in amniotic fluid was associated with decreased expression of Toll-like receptor (TLR) 2 mRNA and increased expression of TLR3 mRNA in fetal exfoliated epithelial cells. It was concluded that prenatal microbial contact modulates the fetal intestinal innate immune gene expression profile. Hence, fetal immune physiology may be modulated by maternal dietary intervention using specific probiotics (Rautava *et al.*, 2012b).

11.2.2 Postnatal factors

Although prenatal bacterial exposure occurs in healthy pregnancies, the total bacterial load is relatively low and colonization of infants occurs predominantly during delivery and the first few hours of birth. The first colonizers are facultative anaerobes, such as *Staphylococcus*, *Streptococcus*, *Enterococcus*, and Enterobacteriaceae. These bacteria decrease the intraluminal oxidation potential, thereby creating anaerobic conditions favorable for the growth of obligate anaerobes, such as *Bifidobacterium*, *Bacteroides*, *Clostridium*, and *Eubacterium* (Mackie *et al.*, 1999; Meropol and Edwards, 2015). In general, the development of gut microbiota is influenced by host genetics and environmental factors, such as gestational age, mode of delivery (vaginal delivery versus cesarean section), diet (breast versus formula feeding, weaning to solid food, and pre- and probiotic use), antibiotic treatment, maternal weight, weight gain, and stress (Li *et al.*, 2014b; Collado *et al.*, 2010; Zijlmans *et al.*, 2015).

11.2.2.1 *Mode of delivery*

How an infant is born plays a key role in the content and composition of the gut microbiota. A study comparing fecal microbiota of vaginally (VD) and versus CD newborn babies infants within 24 h of delivery showed that VD infants acquired a bacterial community resembling their mother's vaginal microbiota with *Lactobacillus*, *Prevotella*, and *Atopobium* spp. predominating, whereas infants born by CD harbored a bacterial community that was more similar to their mother's skin, with an abundance of *Staphylococcus* and *Corynebacterium* (Dominguez-Bello *et al.*, 2010). In general, the microbial composition within the first year of life is typified by low species diversity and high instability (Koenig *et al.*, 2011; Meropol and Edwards, 2015). By 2–3 years of age, the microbiota of infants becomes more complex and stable, and resembles that of adults, with the phyla Firmicutes and Bacteroidetes dominating (Yatsunenko *et al.*, 2012; Lozupone *et al.*, 2012).

11.2.2.2 *Antibiotics*

Antibiotic treatment induces major changes in the composition of the gut microbiota and decreases bacterial diversity (Dethlefsen *et al.*, 2008; Tanaka *et al.*, 2009; Jakobsson *et al.*, 2010). Tanaka and co-workers studied the influence of antibiotic exposure in the early postnatal period on the intestinal microbiota of infants who were orally administered a broad-spectrum antibiotic for the first 4 days of life compared with untreated infants (Tanaka *et al.*, 2009). The intestinal microbiota of antibiotic-treated infants showed less overall diversity, exemplified by an attenuation of colonization with *Bifidobacterium* and overgrowth of enterococci in the first week of life compared with untreated infants. At 1 month of age, the

Enterobacteriaceae population remained higher in the antibiotic-treated group than in the untreated group. Azad *et al.* (2016) assessed the impact of maternal intrapartum antibiotic that was administered for Group B *Streptococcus* prophylaxis, premature rupture of membranes, or during elective or emergency CD on infant microbiota at 3 months and 1 year of age in the Canadian Healthy Infant Longitudinal Development (CHILD) study cohort. They observed that intrapartum antibiotic use was associated with reduced microbiota richness, depletion of Bacteroidetes, and enrichment of Firmicutes at 3 months. Differences persisted until 12 months of life, but was modulated by breastfeeding and CD; dysbioses were more likely to persist in infants delivered by emergency cesarean delivery who were breastfed for less than 3 months postpartum (Azad *et al.*, 2016). Thus, antibiotic exposure in the early postpartum period, through either maternal or infant treatment, influences the development of neonatal intestinal microbiota. Importantly, antibiotic-associated dysbiosis may have long-term implications for infant health as early-life antibiotic exposure has been associated with increased risk of metabolic diseases in rodents (Cox *et al.*, 2014) and humans (Yallapragada *et al.*, 2015) and potentially atopy in children (Risnes *et al.*, 2011), although some studies have cautioned against reverse causation (Penders *et al.*, 2011).

11.3 Infant nutrition and the development of gut microbiota

11.3.1 Human milk and infant formula

Human milk is the optimal diet for the health, growth, and development of infants. Exclusive breastfeeding is recommended by the American Academy of Pediatrics (AAP, 2012) for the first 4–6 months of life. The rate of initiation of breastfeeding for the total US population is 79%; however, breastfeeding typically does not continue for as long as recommended and a large proportion of babies (~81%) are fed formula or formula in combination with human milk by 6 months of age (CDC, 2014). Compared with breastfed infants, formula-fed infants have higher incidences of GIT, respiratory and urinary tract infections, otitis media, necrotizing enterocolitis (NEC), and death (AAP, 2012).

Differences in bacterial composition between breastfed and formula-fed infants have been reported in several studies (Penders *et al.*, 2005, 2006; Wang *et al.*, 2015). In general, the microbiota of breastfed infants is predominated by *Bifidobacterium*, whereas formula-fed infants are often colonized by a more diverse microbiota with a lower abundance of *Bifidobacterium* and higher abundance of potential pathogens (Harmsen *et al.*, 2000; Yatsunenko *et al.*, 2012). For example, at 1 month of age, formula-fed infants were more often colonized with *Escherichia coli*, *Clostridium difficile*, *Bacteroides*, and *Lactobacillus* than breastfed infants. Our laboratory confirmed that 3-month-old formula-fed infants exhibit higher abundances of *Clostridium* cluster XVIII, *Lachnospiracea incertae sedis*, *Streptococcus*, *Enterococcus*, and *Veillonella* than breastfed infants (Wang *et al.*, 2015).

Dissimilarity in gut microbiota composition between breastfed and formula-fed infants may arise in part due to the presence of pre- and probiotics in human milk. Human milk contains more than 200 species of bacteria, including members of *Staphylococcus*, *Streptococcus*, *Propionibacterium*, *Bacteroides*, *Veillonella*, *Faecalibacterium*, *Roseburia*, *Lactobacillus*, and *Bifidobacterium* (Martín *et al.*, 2003; Hunt *et al.*, 2011, Jost *et al.*, 2013).

Infants consuming 800 ml per day of human milk will ingest 1×10^5–1×10^7 bacteria each day (Heikkilä and Saris, 2003). Consistent with a role of human milk bacteria in infant colonization is the report of the same specific bacterial strains of *Bifidobacterium*, *Lactobacillus*, and *Staphylococcus* in human milk and infant fecal samples (Makino *et al.*, 2011; Martín *et al.*, 2012).

From a prebiotic perspective, the third most abundant component of human milk is a group of structurally diverse oligosaccharides (HMOs) (Kunz *et al.*, 2000; Ninonuevo *et al.*, 2006). In contrast, bovine milk and bovine milk-based infant formulas contain only trace amounts of less complex oligosaccharides (Bode, 2006). HMOs are resistant to enzymatic hydrolysis and the majority of them reach the colon (Gnoth *et al.*, 2000), where they serve as a prebiotic for specific subsets of bacteria. HMO utilization by gut bacteria has been primarily studied *in vitro* by measuring the growth of bacterial isolates in culture (Marcobal *et al.*, 2010) or through *ex vivo* fermentation of HMOs using fecal/intestinal microbiota from infants or animals (Li, M. *et al.*, 2012; Vester Boler *et al.*, 2013). Two studies have investigated associations between gut microbiota composition and HMO profiles in human infants. De Leoz *et al.* (2015) related the abundance of infant fecal microbiota with the profile of HMOs excreted in the feces of two infants at birth and at 1, 2, and 13–14 weeks of age. When infants were colonized with non-HMO-consuming species, high concentrations of HMO were present in the infant stool. Colonization with Bacteroidaceae and Bifidobacteriaceae, which are HMO utilizers, was accompanied by a decrease in HMO excretion. Recently, the correlation between the composition of infant gut microbiota and HMO profiles of their mothers' milk was studied. Aspects of the bacterial genera present in infant feces, including *Bifidobacterium*, *Bacteroides*, *Enterococcus*, *Veillonella*, and *Rothia*, were correlated with their mothers' HMO profiles, whereas the relative abundances of other fecal genera, such as *Escherichia/Shigella*, *Streptococcus*, and *Staphylococcus*, in infants' feces were not associated with the HMO consumed (Wang *et al.*, 2015). These findings suggest that human milk provides both pre- and probiotic components, which present opportunities for the improvement of infant formula composition (Martín *et al.*, 2004; Chichlowski *et al.*, 2011).

11.3.2 Weaning

Weaning is typically a gradual process of switching an infant's diet from human milk or formula to other foods and fluids. Although the WHO recommends exclusive breastfeeding as the sole source of nutrition for infants for the first 6 months of life, mothers in developed counties tend to wean earlier than recommended, starting at around 4 months of age (AAP, 2012). Several studies have shown that the introduction of solid food significantly shifts the composition of infant gut microbiota and increases the microbial diversity. For example, comparison of the fecal microbial composition of infants before (at 6 weeks of age) and 4 weeks after the introduction of their first solid foods showed that weaning reduced the proportions of bifidobacteria, enterobacteria and *Costridium difficile* + *Clostridium perfringens* species and increased the proportions of the *Clostridium coccoides* and *Clostridium leptum* groups (Fallani *et al.*, 2011). In addition, introduction of solid food also induced a complement of genes associated with the adult microbiome's core metabolic functions, including enrichment of genes associated with polysaccharide breakdown, vitamin biosynthesis, and xenobiotic degradation (Koenig *et al.*, 2011).

11.3.3 Pre- and probiotics

As noted above, the composition of infant gut microbiota can be influenced by consumption of pre- or probiotics. Prebiotics are defined as "a selectively fermented ingredient that results in specific changes, in the composition and/or activity of the GIT microbiota, thus, conferring benefit(s) upon host health" (Roberfroid *et al.*, 2010). Recently, Bindels *et al.* (2015) proposed a new definition for prebiotics: "a prebiotic is a nondigestible compound that, through its metabolization by microorganisms in the gut, modulates composition and/or activity of the gut microbiota, thus conferring a beneficial physiological effect on the host." This new definition places more emphasis on the causal link between the microbial metabolism of the compound, the resulting modulation of the gut microbe, and the beneficial physiology effects (Bindels *et al.*, 2015).

Infants fed a formula containing a mixture of short-chain galactooligosaccharides (scGOS) and long-chain fructooligosaccharides (lcFOS) in a ratio of 9:1 had fecal bifidobacteria and lactobacilli counts and short-chain fatty acid (SCFA) profiles closer to those of breastfed infants (Haarman and Knol, 2005; Salvini *et al.*, 2011). Similarly, infants fed formula with polydextrose (PDX) and GOS (4 g/l, in a ratio of 1:1) had softer stools and higher *Bifidobacterium* counts than observed in infants fed control formula after 60 days of feeding (Scalabrin *et al.*, 2012). Although these prebiotics differ from HMO, they are able to recapitulate some of the beneficial aspects of breastfeeding in the formula-fed infant.

Probiotics are "live microorganisms which, when consumed in adequate amounts, confer a health benefit on the host" (FAO/WHO, 2001). Administration of specific probiotic bacteria to infants shortened the duration of rotavirus diarrhea (Szymański *et al.*, 2006), prevented antibiotic-associated diarrhea (McFarland, 2014), reduced the incidence of eczema in high-risk children (Kalliomäki *et al.*, 2001), and decreased the risk of NEC in very low birth weight infants (Deshpande *et al.*, 2010).

Furthermore, as discussed previously, human milk contains a large number of potential probiotic bacteria, such as *Bifidobacterium*, *Lactobacillus*, and *Streptococcus*, and these bacteria serve as a source of continuous inoculum to the breastfed infant gut. Hence supplementation of probiotics to infant formula has been used as an approach to improve infant health and to mimic the probiotic property of human milk. Several studies have investigated the significance of the ability of a probiotic to alter the composition of the gut microbiota; however, the outcomes are contradictory. Kitajima *et al.* (1997) examined the effects of administration of *Bifidobacterium breve* YIT4010 for the first 28 days of life on the fecal microbial colonization patterns in very low birth weight infants. They found the colonization patterns differed between the administered and placebo groups. The counts of *Enterococcus* were lower in the infants in the *B. breve* group compared with placebo for the first 4 weeks. Furthermore, a rapid increase in *Lactobacillus* counts from 7 weeks of age was observed in the *B. breve* group, but not in the placebo group. A double-blind placebo-controlled trial investigating the impact of addition of *L. rhamnosus* GG and *B. longum* BB536 to a milk formula on the fecal microbiota of infants at risk of atopic disease reported that the overall bacterial colonization pattern was unaffected by probiotic administration (Mah *et al.*, 2007). Similarly, when *L. rhamnosus* LPR and *B. longum* BL999 were administered to formula-fed German infants for 4 months, the effect of the probiotics on the microbiota composition was minor (Grześkowiak *et al.*, 2012). These inconsistencies may relate to the different probiotic strains used, daily doses administered, timing and period of administration, the geographically distinct infant groups studied, and pre-existing microbiota.

11.3.4 Dietary patterns

Relatively few studies have investigated the impact of dietary pattern on the microbiota of infants and children (De Filippo *et al.*, 2010; Yatsunenko *et al.*, 2012). The diets of people living in Western countries contain higher fat and protein and lower fiber, fruit, and vegetables compared with those of people living in developing countries. Yatsunenko *et al.* (2012) compared the fecal microbiota composition of subjects aged 0–80 years in Venezuela, Malawi, and the United States. The microbial composition of Americans differed from those of Venezuelans and Malawians regardless of age, whereas Venezuelan and Malawian samples were more similar to each other, especially at early ages. Another study compared the fecal microbiota of 1–6-year-old children living in rural Africa and Italy (De Filippo *et al.*, 2010). When infants were breastfed, the microbiota across both populations was similar and was dominated by *Bifidobacterium*. However, once the children were weaned onto their respective African or Western diets, the African children showed a significant enrichment in Bacteroidetes, especially *Prevotella* and *Xylanibacter*. African children consumed more fiber than Italian children and *Prevotella* and *Xylanibacter* are known to contain a set of bacterial genes for cellulose and xylan hydrolysis. In contrast, Italian children had higher proportions of *Bacteroides* and Firmicutes than African children, together with a higher intake of protein, fat, and carbohydrates. These findings are consistent with those of Wu *et al.* (2011), who found a strong association between long-term dietary patterns and microbial "enterotypes" in adults. The *Prevotella* enterotype was associated with higher intakes of carbohydrates and simple sugars (prevalent in agrarian societies), whereas the *Bacteroides* enterotype was associated with a diet rich in animal protein, a variety of amino acids, and saturated fats. Although comparative studies between populations with different diets provide useful information on how dietary patterns shape the gut microbiota, most of the studies involved international cohorts that introduce confounding factors as they live in different geographic regions and have differences in culture, socioeconomic status, genetics, and sanitary conditions (Voreades *et al.*, 2014). More studies are needed to examine the impact of dietary patterns in homogenous populations.

11.4 Host genetics and the development of gut microbiota

Some of the first evidence for the importance of the host genetic background on gut microbiota composition arose from observations in twin studies. Goodrich *et al.* (2014) assessed the role of genetics on gut microbiome composition by comparing the fecal microbiota of 416 twin pairs and found that the microbiota were more similar within twin pairs compared with unrelated individuals. The abundances of many microbial taxa were influenced by host genetics, with the family Christensenellaceae being the most heritable taxon. In a more recent study, the fecal microbiota of a dichorionic triplet set (a pair of monozygotic twins and a fraternal sibling) were compared. Principal coordinate analysis revealed clustering of the monozygotic pair at month 1 and a separation of the fraternal infant. By 12 months, the monozygotic pair no longer clustered separately from the fraternal infant. These results suggest that host genetics shape the composition of an individual's gut microbiota (Murphy *et al.*, 2015).

In addition, some associations between genetic polymorphisms and the gut microbiota have been made in specific disease states in humans. Single nucleotide polymorphisms

(SNPs) in the MEFV (Mediterranean fever) gene are associated with changes in overall gut bacterial community structure (Khachatryan *et al.*, 2008), and inflammatory bowel disease (IBD)-risk loci are associated with changes in gut microbiome composition (Li, E. *et al.*, 2012). The protein product of the MEFV gene is pyrin, which is produced by neutrophils, eosinophils, and monocytes, and plays a role in inflammation and in fighting infection. In addition, a loss-of-function polymorphism in the fucosyltransferase-2 (FUT2) gene, which is a known risk factor for Crohn's disease, may modulate the energy metabolism of the gut microbiome (Tong *et al.*, 2014). Lastly, the number of risk alleles of the nucleotide-binding oligomerization domain-containing protein 2 (NOD2) gene was correlated with a greater relative abundance of fecal Enterobacteriaceae in patients with IBD (Knights *et al.*, 2014).

To gain a fuller understanding of genes or genetic variants in the human genome that are responsible for regulating gut microbiota composition, Blekhman *et al.* (2015) analyzed genome-wide associations between host genetic variation and microbial populations across 15 body sites. A total of 83 associations between genetic variation in host genetic sequence and the abundance of specific bacterial taxa were observed in 10 of the 15 body sites. Among these, eight associations were related to microbiota composition in the GIT (Table 11.1). For example, host genetic variation in two SNPs in the lactase-phlorizin hydrolase (*LCT*) gene was correlated with the relative abundance of *Bifidobacterium* in stool. Furthermore, the host genetic loci that were correlated with microbial composition were also linked to loci for disease susceptibility (e.g. obesity and IBD) and for innate and adaptive immune function. Lastly, they found that the host genomic regions that were associated with the microbiome had high levels of genetic differentiation among human populations, implying host genomic adaptation to environment-specific microbiomes. Using a similar approach, Davenport *et al.* (2015) examined the association of host genotypes with the relative abundances of bacterial taxa in a founder population, the Hutterites, during winter and summer. Members of this population live and eat communally, limiting the inter-individual variation in environmental exposure, including diet, which could potentially mask small genetic effects. They identified at least eight bacterial taxa in each season (15 in winter, 14 in summer, and eight when summer and winter were combined) whose abundances were significantly associated with at least one nucleotide polymorphism (SNP) in the host genome.

The impact of host genetics on the composition of the gut bacterial community has also been shown in several animal model systems, and the quantitative trait loci (QTL) that influence the gut microbiota composition in mice are summarized in Table 11.2. Using the QTL mapping method, Benson *et al.* (2010) tested for co-segregation of the abundances of 64 conserved taxonomic groups with 530 fully informative SNP markers in a large mouse advanced intercross population. They identified 12 host QTLs that significantly controlled the variability in the relative abundances of specific bacterial taxa. They found that these QTL influenced the microbial composition in three ways: first, some of the loci controlled individual microbial species, while second, several controlled groups of related taxa, and, lastly, others had putative pleiotropic effects on groups of distantly related organisms.

Using a similar approach, a more recent study from the same laboratory revealed five QTL regions at a genome-wide level for six taxonomic groups in the BXD mouse reference population (McKnite *et al.*, 2012). The identified QTLs were either restricted to a particular taxon or a branch or influenced the variation of taxa across phyla (Table 11.2). For instance, a QTL region on chromosome 15 was associated with the abundance of Rikenellaceae, whereas a QTL on chromosome 17 influenced the variation of the Bacillales/Staphylococcaceae/*Staphylococcus* branch (McKnite *et al.*, 2012). In addition, gene expression of the GIT and

Table 11.1 Human genes that influence the gut microbial composition.

Chromosome	Position	Gene name; (accession number)	Function	Bacterial taxon
2	136511817	UBX Domain-Containing Protein, *UBXN4*; (NM_014607)	Involved in endoplasmic reticulum-associated protein degradation	*Bifidobacterium*
2	136561557 136590746	Lactase-Phlorizin Hydrolase, *LCT*; (NM_002299)	Small intestinal brush border enzyme that cleaves lactose into glucose and galactose	*Bifidobacterium*
3	129297223	Plexin D1, *PLXND1*; (NM_015103)	Important role in cell-cell signaling, regulating the migration of a wide range of cell types and synapse formation	*Prevotella*
7	37961001	Ependymin Related 1, *EPDR1*; (NM_001242948)	Type II transmembrane protein that is similar to the cell adhesion molecules, protocadherins, and ependymins. May play a role in calcium-dependent cell adhesion	*Lachnobacterium*
11	57982620	Olfactory Receptor, Family 1, Subfamily S, Member 1, *OR1S1*; (NM_001004458)	Olfactory receptor; members of a large family of G-protein-coupled receptors	*Prevotella*
14	55907172	TATA Box Binding Protein Like 2 m, *TBPL2*; (NM_199047)	Transcription factor required in complex with TAF3 for the differentiation of myoblasts into myocytes	*Prevotella*
20	2816821	Family With Sequence Similarity 113, Member A, *FAM113A*; (NM_022760)	Similar to PCED1A; member of the GDSL/SGNH superfamily, which are hydrolytic enzymes with esterase and lipase activity and broad substrate specificity	*Alistipes*

Abbreviations: PCED1A, PC-esterase domain containing 1A; TAF3, TATA-box binding protein associated factor 3.
Source: Data extracted from Blekhman *et al.* (2015).

Table 11.2 Quantitative trait loci (QTL) that are associated with gut microbial composition in mice.

Chromosome	Peak position (Mb)	Confidence interval (Mb)	Bacterial taxon	Ref.
1	49.1[a]	43.3–50.7	*Blautia*	Leamy *et al.* (2014)
1	80	63–139	*Barnesiella*	Benson *et al.* (2010)
1	127.2[a]	123.3–140.7	*Butyricicoccous*	Leamy *et al.* (2014)
1	173.3	167.0–182.1	*Prevotella*	Leamy *et al.* (2014)
2	59.5	57.5–62.5	*Parabacteroides*	Leamy *et al.* (2014)
2	60.2	57.5–62.5	*Alistipes*	Leamy *et al.* (2014)
2	172.5	170.3–173.6	*Hydrogenoanaerobacterium*	Leamy *et al.* (2014)
3	16.9	11.8–22.6	*Clostridium*	Leamy *et al.* (2014)
3	20.4	11.8–22.6	*Blautia*	Leamy *et al.* (2014)
4	12.5[a]	8.3–19.7	*Roseburia*	Leamy *et al.* (2014)
4	87.70–88.04	87.58–95.23	*Bacteroides*	McKnite *et al.* (2012)
5	115.2	112.9–118.1	*Desulfotomaculum*	Leamy *et al.* (2014)
6	13	13–39	*Helicobacter*	Benson *et al.* (2010)
6	28	28–40	Proteobacteria	Benson *et al.* (2010)
6	54.0	53.1–59.2	*Eubacterium*	Leamy *et al.* (2014)
6	115.6	112.2–120.0	*Desulfocurvus*	Leamy *et al.* (2014)
6	126.9	122.4–131.6	*Lactobacillus johnsonii*	Leamy *et al.* (2014)
7	19	14–28	*Variovorax*	Benson *et al.* (2010)
7	66	47–71	*L. johnsonii/L. gasseri*	Benson *et al.* (2010)
7	73	71–88	*Turicibacter*	Benson *et al.* (2010)
8	43	33–63	Proteobacteria	Benson *et al.* (2010)
8	71.6	64.4–79.1	*Butyricimonas*	Leamy *et al.* (2014)
8	74.9	64.4–79.1	*Alistipes*	Leamy *et al.* (2014)
8	77.5	66.5–79.1	*Alistipes*	Leamy *et al.* (2014)
9	29.8	22.0–33.4	*Hydrogenoanaerobacterium*	Leamy *et al.* (2014)
9	37.3	34.6–40.9	*Alistipes*	Leamy *et al.* (2014)
9	40.2	33.5–42.3	*Clostridium*	Leamy *et al.* (2014)
9	40.7[a]	33.5–40.9	*Prevotella*	Leamy *et al.* (2014)
9	40.7	33.5–40.9	*Odoribacter*[a]	Leamy *et al.* (2014)
9	40.7	40.5–42.4	*Bacteroides*[a]	Leamy *et al.* (2014)
9	40.7	33.5–40.9	*Bacteroides*[a]	Leamy *et al.* (2014)
9	113.3	112.3–115.0	*Lactococcus lactis*	Leamy *et al.* (2014)
10	6.4	4.7–9.07	*Roseburia*	Leamy *et al.* (2014)
10	107	100–111	*Lactococcus*	Benson *et al.* (2010)
10	119	106–122	*Coriobacteriaceae*	Benson *et al.* (2010)
11	41.8	35.1–44.5	*Oscillibacter*	Leamy *et al.* (2014)
11	97.8	93.4–114.0	*Alistipes*	Leamy *et al.* (2014)
12	17	17–26	*Ruminococcaceae*	Benson *et al.* (2010)
12	90.15	78.36–95.80	*Prevotellacea*	McKnite *et al.* (2012)
14	17.1	10.5–20.3	*Odoribacter*	Leamy *et al.* (2014)
14	71.1	67.6–87.5	*Clostridium*	Leamy *et al.* (2014)
14	88.7	79.7–87.5	*Hydrogenoanaerobacterium*	Leamy *et al.* (2014)
14	93	86–103	*L. johnsonii/L. gasseri*	Benson *et al.* (2010)
15	95.15–95.78	92.73–97.39	*Rikenellaceae*	McKnite *et al.* (2012)
16	6.9	3.98–9.92	*Bacteroides*	Leamy *et al.* (2014)
16	44.8	42.7–48.1	*Alistipes*	Leamy *et al.* (2014)
16	45.7	42.7–57.8	*Mucispirllum*	Leamy *et al.* (2014)
16	63.3	51.6–70.4	*Lactobacillus*	Leamy *et al.* (2014)
17	7.75–10.17	7.37–10.44	Bacilales	McKnite *et al.* (2012)
17	7.75–10.17	7.39–10.44	*Staphylococcaceae*	McKnite *et al.* (2012)
17	7.75–10.17	5.26–10.43	*Staphylococcus*	McKnite *et al.* (2012)

(*Continued*)

Table 11.2 (Continued)

Chromosome	Peak position (Mb)	Confidence interval (Mb)	Bacterial taxon	Ref.
17	21.83–23.00	21.71–26.63	Bacilales	McKnite *et al.* (2012)
17	21.83–23.00	21.71–26.70	*Staphylococcus*	McKnite *et al.* (2012)
17	48.7	41.9–58.2	*Odoribacter*	Leamy *et al.* (2014)
18	68.4	65.4–70.2	*Clostridium*	Leamy *et al.* (2014)
18	83.1	83.1	*Bacteroides*	Leamy *et al.* (2014)
19	24.3	22.6–24.7	*Clostridium*	Leamy *et al.* (2014)
19	56	54–56	Deltaproteobacteria	Benson *et al.* (2010)
X	66.1	54.5–74.6	*Prevotella*	Leamy *et al.* (2014)

[a] Indicates the QTLs that interacted significantly with diet.

sequence analysis of parental genomes in the QTL regions uncovered several candidate genes that have the potential to alter the gut immune profile and subsequently impact gut microbiota composition. A QTL region on chromosome 4 that overlapped several interferon genes modulated the population of *Bacteroides* and *Tgfb*3, a cytokine modulating the barrier function of the intestine and tolerance to commensal bacteria, overlapped a QTL on chromosome 12 that influenced the variation of Prevotellaceae. Recently, Leamy *et al.* (2014) studied the convergence of host and diet in a G10 mouse population to search for the QTLs that affect gut microbiota composition in mice fed normal or high-fat diets. They observed 42 QTLs in 27 genomic regions that influenced the relative abundances of 39 bacterial taxa (Table 11.2). Some of the QTLs showed apparent pleiotropy. Several of these QTLs also exhibited a significant interaction with diet, suggesting that diet can modify the effect of some host loci on gut microbial composition. Taken together, these findings provide clear evidence for the importance of host genetics in shaping microbiome composition and contribute to our understanding of the host factors that govern the assembly of gut microbiota associated with diet and complex disease phenotypes. However, future research is needed to establish mechanisms of action.

11.5 Host–microbe interactions regulating host phenotype and gene expression

The relationship between the host and its colonizing bacteria is one of commensalism, where under ideal conditions, both thrive. Thus far in this chapter, we have focused on how gut microbiota are modulated by host genetics and external factors to the host. In the next two sections, we review how the microbiota influences several aspects of host phenotype and intestinal gene expression with a focus on infancy. Readers are referred to recent reviews that describe the relationships between the gut microbiota and central nervous system disorders (Wang and Kasper, 2014; Ghaisas *et al.*, 2016), cardiometabolic diseases (Aron-Wisnewsky and Clément, 2016), obesity and diabetes (Greiner and Bäckhed, 2011; Chakraborti, 2015), liver disease (Gkolfakis *et al.*, 2015), inflammatory bowel diseases (Øyri *et al.*, 2015; Satokari, 2015; Ghaisas *et al.*, 2016), colon cancer (Ou *et al.*, 2013), and immune-related disorders (Li *et al.*, 2014b).

11.5.1 Host phenotype

The microbiota acquired in early infancy have short- and long-term effects on host metabolism, GIT, and immune and neural system development (Abrams *et al.*, 1963; Sudo *et al.*, 2004; Bäckhed *et al.*, 2007, Round and Mazmanian, 2009). Reduced microbial diversity and dysbiosis are associated with a number of GIT and systemic disorders in childhood and later in life, including necrotizing enterocolitis (NEC) (Mai *et al.*, 2011), eczema (Gore *et al.*, 2008; Wang *et al.*, 2008), asthma (Vael *et al.*, 2011), IBD (Aomatsu *et al.*, 2012), obesity (Ley *et al.*, 2006; Karlsson *et al.*, 2012), and autism (Kang *et al.*, 2013; Wang *et al.*, 2013).

Elucidating the underlying mechanism by which the intestinal microbiota influences human health and disease has been hampered by individual variations in the host genetics and microbiota, and also ethical concerns of using invasive procedures in health subjects. Accordingly, most of our knowledge of host–microbe interaction comes from animal models, especially GF and GN rodents (Smith *et al.*, 2007; Chow *et al.*, 2010).

11.5.1.1 *Host metabolism*

Data accumulated over the last decade indicate that gut microbiota have profound effects on energy harvest and fat storage. Colonization of adult GF mice with a gut microbiota from conventionalized (CV) mice for 14 days resulted in a 57% increase in body fat, despite a 29% reduction in chow consumption and a 27% increase in activity compared with GF mice (Bäckhed *et al.*, 2004). In addition, CV mice on a high-fat, high simple carbohydrate diet gained significantly more weight than their GF counterparts (Bäckhed *et al.*, 2007). Furthermore, GF mice colonized with feces from obese mice gained twice as much fat as those colonized with microbiota from lean mice (Turnbaugh *et al.*, 2006). Moreover, metagenomic sequencing showed that the microbiota of obese mice was enriched in genes regulating the initial steps in catabolism of indigestible dietary polysaccharides, including KEGG pathways for starch/sucrose metabolism, galactose metabolism, and butanoate metabolism (Turnbaugh *et al.*, 2006).

Several mechanisms have been proposed to explain the role of microbiota in energy extraction and fat storage (Cani *et al.*, 2009; Greiner and Bäckhed, 2011). First, the presence of a gut microbiota increases glucose uptake and also SCFA production, particularly acetate, propionate, and butyrate, from carbohydrate fermentation in their colon and these are absent in GF mice (Høverstad and Midvedt, 1986; Bäckhed *et al.*, 2004). The SCFAs are rapidly absorbed in the cecum and colon. Butyrate provides energy for the colonic epithelium, whereas acetate and propionate enter the circulation and are taken by the liver and other organs, where they can be used as substrates for gluconeogenesis and lipogenesis (Smith and Crouse, 1984; Wong *et al.*, 2006). In addition, SCFAs can induce expression of peptide YY (PYY) through its receptor Gpr41/FFAR 3 on enteroendocrine cells (Samuel *et al.*, 2008). Increased PYY expression decreases intestinal transit and increases energy harvest and hepatic lipogenesis (Samuel *et al.*, 2008). Furthermore, the gut microbiota regulates fasting-induced adipose factor (Fiaf), a circulating lipoprotein lipase (LPL) inhibitor that regulates fatty acid oxidation in both muscle and adipose tissues (Bäckhed *et al.*, 2004). Colonization of GF mice with gut microbiota suppressed Fiaf expression in the intestine, resulting in an increase in lipoprotein lipase (LPL) activity and fat storage in adipose tissue. Moreover, colonization of GF mice with microbiota has been shown to suppress liver and skeletal muscle AMP-activated protein kinase (AMPK) and reduce AMPK-dependent fatty

acid oxidation (Bäckhed *et al.*, 2007). Lastly, a high-fat diet increased the level of plasma lipopolysaccharide (LPS) (a constituent of the cell wall of Gram-negative bacteria) and metabolic inflammation, leading to increased adiposity and insulin resistance (Greiner and Bäckhed, 2011).

11.5.1.2 *Postnatal GIT structural and functional development*

A link between gut microbiota and GIT development has been demonstrated in GF animal studies conducted over the past 50 years. First, intestinal morphology is abnormal in GF animals. Compared with CF animals, GF mice have an enlarged cecum and reduced small intestine surface area (Gordon and Bruckner-Kardoss, 1961; Gustafsson *et al.*, 1970). Ileal villi are shorter, duodenal villi are longer, and small intestinal crypts are shorter in GF mice (Abrams *et al.*, 1963; Lesher *et al.*, 1964). Relative to CV mice, GF mice have slower epithelial cell turnover rate and reduced crypt cell proliferation (Abrams *et al.*, 1963; Lesher *et al.*, 1964). In addition, the lamina propria of the small intestine, a primary site for immune cells in the gut, is thinner and less cellular in GF mice than CV mice (Abrams *et al.*, 1963; Glaister, 1973).

The gut microbiota also regulate intestinal function. For example, GF mice have greater lactase, alkaline phosphatase, and γ-glutamyltranspeptidase activities and lower sucrase and glucoamylase activities than CV mice (Kozáková *et al.*, 2001). Lastly, the intestinal microbiota impact gut motility with gastric emptying and intestinal transit times that are slower in GF than CV mice (Abrams and Bishop, 1967).

Several studies have investigated the molecular mechanisms by which microbiota trigger these effects. For example, enlargement of the cecum in GF mice may be due in large part to the accumulation of mucus, as the enlargement was rapidly reversed when GF mice were monoassociated with the mucin-degrading bacterium *Peptostreptococcus micros* (Gustafsson *et al.*, 1970; Carlstedt-Duke *et al.*, 1986). Another study found that the microbiota affect epithelial cell differentiation via their capacity to modify host glycoconjugate production (Bry *et al.*, 1996). In addition, the microbiota maintain the mucosal epithelial barrier, which is necessary for gut function and immune function and is described in the next section. Intestinal mucosal surfaces are lined by epithelial cells that constitute a vital physical barrier separating the internal milieu of mammals from food and microbes in the lumen. Moreover, specialized secretory intestinal epithelial cells, including goblet cells and Paneth cells, contribute to barrier function and host defense against microbes (Peterson and Artis, 2014).

Goblet cells secrete a protective mucus gel composed of highly glycosylated mucins that constitute the first line of defense against microbial invasion (Peterson and Artis, 2014). *In vitro* cell culture and animal studies have shown that the production of intestinal mucus is regulated by the gut microbiota. GF mice have fewer and smaller goblet cells than CV mice (Kandori *et al.*, 1996) and the mucus layer was thicker in CV than in GF rodents, indicating increased mucus production (Szentkuti *et al.*, 1990). Exposure of HT29 colon cells to probiotics, such as *Lactobacillus plantarum* 299v and *Lactobacillus rhamnosus* GG, increased the expression of both MUC2 and MUC3 mucin-producing genes *in vitro* (Mack *et al.*, 1999).

Paneth cells located at the base of the crypts of Lieberkühn are specialized cells that produce most of the antimicrobial peptides (AMPs) in the small intestine (Hooper and Macpherson, 2010). Angiogenin-4 (Ang4) is an antimicrobial peptide produced by Paneth cells that exhibits antibacterial activity against several gut microbes *in vitro*, suggesting a role in epithelia host defense (Hooper *et al.*, 2003). The intestinal microbiota regulates Ang4

expression (Hooper *et al.*, 2003). For example, colonization of GF mice with a complex microbiota harvested from CV mice or a single bacterium, such as *Bacteroides thetaiotaomicron*, increased Ang4 mRNA expression in the small intestine. In addition, Ang4 expression increased in the small intestine of CV mice, but not GF mice, during the weaning period, which is a time of rapid expansion of microbial diversity in CV animals (Hooper *et al.*, 2003).

11.5.1.3 *Host immune system*

The mammalian immune system is perhaps the most elaborate example of the complex symbiotic relationship that has resulted from the co-evolution of the host and its microbiota. The immune system controls the composition of the gut microbiota, while at the same time the resident microbiota profoundly shapes mammalian immunity (Round and Mazmanian, 2009; Hooper *et al.*, 2012).

Comparative studies on GF and CV animals have established the essentiality of the intestinal microbiota for the development and function of the mucosal and systemic immune systems, particularly in early life. Within the intestine, GF mice have fewer and smaller Peyer's patches and mesenteric lymph nodes (MLNs), thinner lamina propria, fewer plasma cells, smaller numbers of cytotoxic T cells ($CD8^+$) with reduced cytotoxicity, and reduced production of mucosal immunoglobulin A (IgA) and antimicrobial peptides (Round and Mazmanian, 2009; Chung and Kasper, 2010). The numbers of T-helper cells ($CD4^+$) in the lamina propria and regulatory T cells ($CD4^+CD25^+foxp3^+$) in MLNs are also reduced (Round and Mazmanian, 2009). Lastly, intestinal epithelial cells of GF animals also have reduced mRNA expression of the major histocompatibility complex (MHC) Class II, TLR9, and interleukin (IL)-25 genes (Round and Mazmanian, 2009). All of these abnormalities are corrected within weeks after colonizing GF mice with a commensal microbiota (Macpherson and Harris, 2004).

The gut microbiota influence the development and function of several cell populations of the adaptive immune system, including IgA-secreting plasma cells, Th17 T cells, and regulatory T cells (Treg). IgA is the predominant antibody produced by plasma cells in the mucosa tissue (Pabst *et al.*, 2008). At the intestinal mucosal surfaces, IgA is mainly secreted as a dimer across the epithelial cell layer and serves as the first line of defense against microorganisms through a mechanism known as immune exclusion (Corthësy, 2009). GF mice have reduced numbers of IgA-secreting plasma cells and lower levels of secretory IgA in the intestine. Colonization of GF mice with mouse microbiota or with specific bacterial species increased IgA-secreting cells in the intestinal mucosa and stimulated IgA production (Moreau *et al.*, 1978; Klaasen *et al.*, 1993).

The intestinal microbiota also regulate Th17 and Treg differentiation and Th17/Treg balance. Th17 cells are a subset of helper T cells (Bettelli *et al.*, 2007). Th17 differentiation in mice is initiated by transforming growth factor beta 1 (TGF-β1), IL-6, and IL-21, which activate Stat3 and induce the expression of the transcription factor retinoic acid-related orphan receptor (RORγt) (Chen and O'Shea, 2008). Th17 secretes proinflammatory cytokines, including IL-17, IL-17F, IL-21, and IL-22 (Ivanov *et al.*, 2008; Eisenstein and Williams, 2009). Th17 plays an essential role in the host defense against extracellular pathogens and in the pathogenesis of autoimmune diseases (Chen and O'Shea, 2008).

In CV mice, Th17 cells are abundant in lamina propria in the small intestine of CV mice, whereas the proportions of Th17 cells are significantly reduced in GF or antibiotic-treated mice (Ivanov *et al.*, 2008). Mono-associating GF mice with a segmented filamentous

bacterium, such as *Candidatus arthromitus*, for 10 days induced a robust accumulation of Th17 cells (Ivanov *et al.*, 2009). Interestingly, induction of Th17 cell numbers in the lamina propria of GF mice appears to be bacterial species specific, since colonization with *Bacteroides* species or a mixture of *Clostridium* did not induce Th17 cells in GF mice (Ivanov *et al.*, 2009).

Treg cells are a subpopulation of T cells that are thought to prevent autoimmune diseases, suppress allergy, asthma, and pathogen-induced immunopathology and feto-maternal tolerance, and induce oral tolerance (Corthay, 2009). The numbers of colonic Treg cells and the level of IL-10 expression by Treg cells are reduced in GF mice (Atarashi *et al.*, 2011). Mono-associating GF mice with *Bacteroides fragilis* doubled the percentage of IL-10-producing Treg cells in the colon (Round and Mazmanian, 2010). Recently, Atarashi *et al.* (2013) reported that colonization of GF mice with a mixture of 17 clostridia strains isolated from healthy human feces increased the number of Treg cells and IL-10 production in the lamina propria of the colon. Taken together, these results indicate that microbiota profoundly influence the induction of Treg cells.

As noted above, Th17 and Treg are two subsets of T cells with opposite actions. Th17 cells contribute to host defense against infections and pathogenesis of autoimmune diseases, whereas Treg cells play a vital role in suppression of immunopathology and prevention of autoimmunity diseases. In addition, TGF-β is essential for both Th17 and inducible Treg differentiation (Eisenstein and Williams, 2009). Under normal conditions, Th17 and Treg cells coexist in a well-regulated balance (Ivanov *et al.*, 2008), and alteration of the balance between Th17 and Treg cells may lead to tissue inflammation and autoimmune disease (Eisenstein and Williams, 2009).

Our understanding of the importance of the gut microbiota in the balance of Th17 and Treg cells came initially from the study of Ivanov and colleagues, who examined these cell populations in mice from different sources (Ivanov *et al.*, 2008). C57BL/6 mice obtained from one commercial supplier had 10–15% small intestinal lamina propria Th17 cells, whereas C57BL/6 mice obtained from another commercial supplier had only 1–2% lamina propria Th17 cells. The animals with decreased Th17 cells had an increased proportion of $Fox3^+$ Treg cells and fewer IgA-producing cells. In addition, when mice from the two suppliers were housed together for 2 weeks (allowing for horizontal transfer of intestinal bacteria between the two mouse populations), an increase in small intestinal lamina propria Th17 cell numbers was observed in mice from the second supplier. Taken together, these findings suggest that the composition of gut microbiota, not potential genetic differences between mice from the two suppliers, regulates the Th17/Treg balance in the lamina propria.

11.5.1.4 *Gut–brain axis*

The bidirectional communication system between the central nervous system (CNS) and the enteric nervous system (ENS) is referred to as the gut–brain axis (GBA). It integrates emotional and cognitive centers of the brain with GIT functions and plays an important role in the transfer of information from the CNS to the ENS, and vice versa (Carabotti *et al.*, 2015). For example, stress modulates GIT functions such as motility, secretion, blood flow, and mucosal immune function (Taché and Perdue, 2004; de Jonge, 2013; Browning and Travagli, 2014). Conversely, alteration of GIT function can be perceived as nausea, anorexia, and pain (Drossman, 1998). Disruption of the GBA has been implicated in the pathogenesis of

functional GIT disorders, including irritable bowel syndrome (IBS) and IBD, and also anxiety and depression (Taché and Bernstein, 2009; Foster and McVey Neufeld, 2013).

The importance of the gut microbiota in modulating these bidirectional interactions between the gut and brain (e.g., microbiota–gut–brain axis) and ensuing effects on brain function and behavior have recently come to the forefront. Studies in GF animals have shown that absence of gut microbiota during early life detrimentally impacts the development of the hypothalamic–pituitary–adrenal (HPA) axis (Sudo *et al.*, 2004). For example, GF mice had an exaggerated HPA stress response with elevated plasma levels of adrenocorticotropic hormone and corticosterone compared with CV mice and the enhanced HPA response of GF mice was partially reversed by reconstitution with microbiota from CV animals at an early developmental stage. In addition, GF mice had lower levels of brain-derived neurotropic factor (BDNF), a key neurotrophin involved in neuronal growth and survival, in the cortex and hippocampus (Sudo *et al.*, 2004). Furthermore, GF animals displayed increased motor activity and reduced anxiety-like behavior (Diaz Heijtz *et al.*, 2011). Moreover, microbiota can influence CNS serotonergic neurotransmission. For example, male GF animals have significantly higher concentrations of serotonin (5-hydroxytryptamine), a neurotransmitter, and its main metabolite in the hippocampus than CV animals (Clarke *et al.*, 2013).

The impact of microbiota in the regulation of brain function and behavior has been further supported by studies in animals exposed to antibiotics or probiotics. Male CV mice exposed to a mixture of neomycin and bacitracin (5 mg/ml each) together with an antifungal agent, pimaricin, for 7 days altered the composition of the gut microbiota and increased exploratory behaviors and expression of BDNF in the hippocampus and amygdala (Bercik *et al.*, 2011). However, when the same antimicrobial treatment was administered orally to GF animals, the behavior changes were not observed, suggesting that they were mediated by the alteration of gut microbiota (Bercik *et al.*, 2011). Several studies have demonstrated the impact of probiotic administration on behavior. For example, administration *L. rhamnosus* JB-1 to mice for 28 days altered region-dependent γ-aminobutyric acid (GABA) expression in the brain and reduced stress-induced corticosterone and anxiety- and depression-related behavior (Bravo *et al.*, 2011). In another study, rats treated with *Bifidobacterium infantis* 35624 reversed the impact of maternal separation on behavioral despair in the forced swim test (Desbonnet *et al.*, 2010).

Possible mechanisms for communication between gut microbes and the brain have been reviewed previously (Collins *et al.*, 2012; Cryan and Dinan, 2012; Forsythe and Kunze 2013; Carabotti *et al.*, 2015). The first is via immune activation. As described previously, microbiota can affect host immune responses. Previous studies have also shown that the host immune system has a bidirectional interaction with the CNS, thereby making it a primary target for transducing bacterial effects to the brain (Dantzer *et al.*, 2008). The second is directly through the vagal nerve. The vagal nerve connects the network of nerves and other organs (such as heart, liver, pancreas, stomach, and intestine) to the brain. It delivers information from the GIT to the brain (Forsythe *et al.*, 2014). Several probiotic strains have been shown to exert their behavioral effects via the vagal nerve (Bercik *et al.*, 2011; Bravo *et al*., 2011). For example, mice treated with *L. rhamnosus* JB-1 induced region-dependent alterations in GABA receptor expression and reduced anxiety- and depression-related behavior. Moreover, these neurochemical and behavioral effects were dependent on vagal integrity (Bravo *et al.*, 2011). The third mechanism likely involves microbial metabolites, such as SCFAs, including acetate, propionate, and butyrate. Propionate has been shown to influence

behavior in animals (MacFabe *et al.*, 2011). In addition, bacteria have the capacity to produce neurotransmitters and neuromodulators. For example, species of *Lactobacillus* and *Bifidobacterium* produce GABA and *Escherichia*, *Streptococcus*, and *Enterococcus* spp. produce serotonin (Lyte, 2011; Barrett *et al.*, 2012). GABA is the main CNS inhibitory neurotransmitter and GABAergic neurotransmission in the hippocampus has been related to the modulation of behavior and memory processes (Crestani *et al.*, 1999). Serotonin modulates developmental processes such as neurogenesis, neuronal maturation, innervations, synaptogenesis, and signaling in the CNS, leading to behavioral manifestations (Jaiswal *et al.*, 2015).

11.5.2 Proposed molecular and metabolomic mechanisms

As reviewed above, gut microbiota exert widespread effects on host phenotype and physiology. These actions may be mediated through direct interactions between microbes and host receptors or through the actions of metabolites secreted by the microbes (El Aidy and Kleerebezem, 2013; Marcobal *et al.*, 2013). For example, the host expresses specific pattern recognition receptors (PRRs) that recognize conserved regions on bacteria, known as pathogen-associated molecular patterns (PAMPs) to signal host responses. Examples of the best characterized families of PRRs are the Toll-like receptors (TLRs), which recognize a wide variety of PAMPs from bacteria, fungi, parasites, and viruses, C-type lectin receptors (CLRs), which bind to carbohydrates in a calcium-dependent manner, RIG-I-like receptors (RLRs), which are intracellular receptors involved in the recognition of viruses, and the NOD-like receptors (NLRs), which are intracellular receptors that recognize cytoplasmic PAMPs. TLR4-mediated signaling is key to innate immune responses (Ahmed *et al.*, 2015). The binding of LPS to TLR4 initiates a signaling complex by the recruitment of MyD88 and TIR domain-containing adaptor protein inducing IFN-β (TRIF), to the receptor. The MyD88-dependent signaling pathway induces transcription of proinflammatory cytokines via NF-κB and mitogen-activated protein kinases (MAPKs). The MyD88-independent pathway is mediated by TRIF and culminates in the production of type-1 interferons. In conjunction with the activation of NF-κB, the stimulation of TLRs also activates major MAPK subfamilies: the extracellular signal-regulated kinase (ERK), p38, and Jun N-terminal kinase (JNK). Following an inflammatory stimulation, MAPK activation induces the expression of multiple genes that together regulate inflammatory responses and other transcriptional pathways (Ahmed *et al.*, 2015).

Recent advances in spectroscopy and mass spectrometry have enabled metabolomic and metaproteomic analyses to be used to assess the influence of the gut microbiota on human health at a whole-systems level (Aw and Fukuda, 2013; Lichtman *et al.*, 2013). Integrating microbial metagenomic and metabolomic technologies has furthered our understanding of how the gut microbiota contributes to host overall health status and the critically important role of diet (Dutton and Turnbaugh, 2012; El Aidy and Kleerebezem, 2013; Ursell *et al.*, 2014; Shoaie *et al.*, 2015). Changes in diet have been shown to affect rapidly the composition of the gut microbiota (David *et al.*, 2014) and microbiota–diet interactions impact host physiology through the generation of a number of bioactive metabolites, including choline, bile acids, phenols, amines, aromatic metabolites, and SCFAs, which have been implicated in maintaining host health and in the etiology of various pathological states (Bäckhed, 2012; Marcobal *et al.*, 2013; Shoaie *et al.*, 2015).

11.5.2.1 *Effect of the microbiota on intestinal gene expression in GF and GN mice*

GF and GN mice have been instrumental in furthering our understanding of host responses to the resident microbiota (El Aidy *et al.*, 2013a,b). The beauty of this approach is the ability to assess spatial, temporal (El Aidy *et al.*, 2012; Marcobal *et al.*, 2015), and site-specific (Larsson *et al.*, 2012; El Aidy *et al.*, 2013b; Sommer *et al.*, 2015) responses to specific microbes (Zocco *et al.*, 2007; Shima *et al.*, 2008; Lichtman *et al.*, 2013) or complex murine (El Aidy *et al.*, 2013a,b; Lichtman *et al.*, 2013; Marcobal *et al.*, 2013) or human microbiomes (Lichtman *et al.*, 2013; Marcobal *et al.*, 2013; Ridaura *et al.*, 2013). Although most of the cited studies were conducted in murine models, GF and GN piglets have also been used to test the effects of the microbiota on intestinal gene expression (Chowdhury *et al.*, 2007). The ability to measure metabolites in stool, urine, or blood further allows translational studies from animal models to humans (El Aidy and Kleerebezem, 2013).

Summarized in Table 11.3 are key observations on intestinal gene expression or function in which GF animals were colonized with either defined mono-cultures or microbiota from mice or humans. The dependence of normal immune development and function on microbial colonization (Chung *et al.*, 2012) and a robust immune response to colonization are well documented in GF and GN mice (El Aidy *et al.*, 2012). For example, approximately 50% of the genes differentially expressed in the intestine of GN mice in response to colonizing gut microbiota regulate T-cell development (Gaboriau-Routhiau *et al.*, 2009). El Aidy *et al.* (2012) documented the temporal response to colonization in GN mice. GF mice were administered a fecal inoculum from CV mice on day 14 of age and intestinal samples were taken on days 1, 2, 4, 8, 16, and 30 post-conventionalization. They observed both region-specific (jejunum, ileum, and colon) transient and permanent changes in intestinal morphology (increased crypt depth), cell proliferation, and mucin expression within 4 days of conventionalization and onwards. These gut maturational changes were accompanied by an upregulation of antimicrobial defense proteins, including lysozyme P, regenerating islet-derived protein (*Reg*) *IIIβ* and -γ, resistin-like beta (*Retnlβ*), and phospholipase A_2, and also cell surface receptors, including the LPS receptor CD14, MyD88, and TLRs, within 4 days post-conventionalization (El Aidy *et al.*, 2012). The timing of the development of the innate and adaptive arms of the immune system was also investigated. From day 4 post-conventionalization onwards, a core set of major gene categories that were strongly induced were detected, including nodes belonging to bacterial recognition (CD14), proinflammatory cytokines, chemokines, and MHC Class I. Genes for MHC Class II molecules, T-cell differentiation, maturation and cell surface markers (CD3, CD4, and CD8), and B-cell differentiation were upregulated by day 8 in the colon and day 16 in the small intestine. The authors concluded that "… homeostasis had been reached in the colon within 8 to 16 days, whereas establishment of homeostasis in the small intestine required 16 to 30 days of conventionalization, roughly double the amount of time" (El Aidy *et al.*, 2012).

Larsson *et al.* (2012) investigated whether microbially induced host gene expression responses required the adaptor molecule, MyD88, which is involved in Toll-like receptor signaling. They compared transcriptional profiles of duodenum, jejunum, ileum, and colon of GF and CV wild-type (WT) and *Myd88*$^{-/-}$ mice. Gut microbiota modulated the expression of 2000 to >5000 genes in the three regions of the small intestine and ~2000 genes in the colon. Of the 500 most significant microbiota-related genes, the majority were associated with adaptive immunity. These changes were attributed to lymphocyte migration to the

Table 11.3 Host gene or activities regulated by transfaunation of defined microbial species or complex microbiota.

Species	Microbiota or bacteria introduced	Activity or gene expression affected by microbiota	Ref.
2-week-old C57Bl/6J, male mice; GF vs. GN	Feces of CV mice	• Samples were taken on days 1, 2, 4, 8, 16, and 30 post-conventionalization • Region-specific (jejunum, ileum, and colon) transient and permanent changes in intestinal morphology (increased crypt depth) and cell proliferation and mucin expression at day 4 • Upregulation of antimicrobial defense proteins and cell surface receptors for bacterial recognition at day 4 Upregulation of adaptive immunity by day 8 in the colon and day 16 in the small intestine	El Aidy *et al.* (2012)
12-week-old wild-type and *Myd88*$^{-/-}$ C57Bl/6J mice; GF vs. CV	N/A, studied CV mice	• Gene expression 4 (duodenum, jejunum, ileum, and colon) was compared • Hierarchical clustering revealed clusters representing the four regions and further subclustering based on GF vs. CV and genotype (wild-type or *Myd88*$^{-/-}$) • Genes were upregulated in the small intestine (2844–5653 genes) and the colon (2124 genes) of CV vs. GF mice. • Myd88 had small effects on mediating microbially-induced gene expression changes • Microbial regulation of REG3β and REG3γ was Myd88 dependent in the colon. but not the ileum • Microbial diversity was lower in SFB abundance, greater in *Myd88*$^{-/-}$ CV than WT CV mice. • *Myd88*$^{-/-}$ CV mice had increased susceptibility to norovirus infection than WT CV mice. • Genes for lipid and fatty acid metabolism and nutrient absorption were upregulated in CV vs. GF mice, but mitochondrial and peroxisome genes were downregulated in CV vs. GF mice	Larsson *et al.* (2012)
12-week-old C57Bl/6J, female mice; GF vs. CV or GN	Feces of CV mice	• 10% of the host's transcriptome was microbially-regulated • Most genes were associated with immunity, cell proliferation, and metabolism • The impact of colonization on host gene expression was site specific • In general, the gut microbiota induced a more rapid response in the colon than in the ileum	Sommer *et al.* (2015)

Adult, male mice[a]; GF vs. GN	*Bacteroides thetaiotaomicron*	• Samples collected 10 days after inoculation. *B. thetaiotaomicron* upregulated expression of genes involved in intestinal maturation, nutrient absorption, fatty acid transport and metabolism, mucosal barrier fortification, xenobiotic metabolism, angiogenesis, and gut–brain axis	Hooper *et al.* (2001)
Mice[a]; GF vs. GN	1. Feces of CV mice 2. Monocolonized with *E. coli*, *Proteus* sp., *L. acidophilus*, *L. fermentum*, *L. murini*, *Streptococcus faecalis*, *Bacteroides* sp., or *Bifidobacterium* sp.	• FUT2 gene: induction of α(1–2) asialo GM1 fucosyltransferase (FT) activity in small intestine of GN mice that were colonized with feces from CV mice, but not in monocolonized GN mice • Expansion of $CD8^+$ T cells and MCH Class II expression in GN mice with CV microbiota.	Umesaki (2014)
Balb/c mice[a]; GF vs. GN	1. Feces of CV mice 2. *Lactobacillus casei* strain Shirota 3. *Bifidobacterium breve* strain Yakult 4. SFB	• Bacteria- and region-specific effects on gene expression • *L. casei* Shirota affected gene expression to a greater extent in the ileum and to a lesser extent in the colon than *B. breve* Yakult • SFB increased gene expression in both regions more than either probiotic • In the ileum, expression of cryptdins and intestinal fatty acid-binding protein genes was greater in *L. casei* Shirota mice than *B. breve* Yakult mice • In the colon, downregulation of inflammatory response, transport, metabolism and defense/immunity genes and upregulation of guanine nucleotide-binding protein greater in *B. breve* Yakult than *L. casei* Shirota mice	Shima *et al.* (2008)
Piglet, domestic cross-bred; GF vs. GN	Feces from CV pig	• Genes involved in transcription, cell proliferation and differentiation, nutrient transport and metabolism, xenobiotic metabolism, and immune responsiveness were upregulated in GN piglets bearing a microbiota from CV piglets vs. GF piglets	Chowdhury *et al.* (2007)

Abbreviations: CV, conventionally raised; GF, germ free; GN, gnotobiotic; SFB, segmented filamentous bacteria; WT, wild type.
[a] Additional information on the strain, sex, or age of animal was not provided.

mucosa and differentiation in response to the gut microbiota. Unexpectedly, few microbiota-regulated genes required functional MyD88 signaling. However, MyD88 was essential for microbiota-induced colonic expression of the antimicrobial genes Reg3b and Reg3g. Additionally, MyD88 deficiency reduced bacterial diversity and increased the proportion of segmented filamentous bacteria (SFB) in the small intestine. In addition, CV MyD88-deficient mice had norovirus infection and increased expression of antiviral genes in the colon (Larsson *et al.*, 2012). Additionally, genes related to lipid and fatty acid metabolism, and also genes involved in nutrient absorption and metabolism, were regulated in response to microbiota throughout the gut. Interestingly, genes related to cholesterol biosynthesis responded differentially to the microbiota depending on genotype (e.g., WT or *Myd88*$^{-/-}$). Furthermore, genes associated with energy-yielding compartments, mitochondrion and peroxisome, were among those downregulated in the duodenum of CV compared with GF mice (Larsson *et al.*, 2012), potentially providing insight into how the microbiota regulates host energy utilization.

Sommer *et al.* (2015) also described region-specific effects of the gut microbiome on the intestinal transcriptome in GF and CV mice at a single time point and temporal changes in GN mice colonized with CV feces. They applied laser capture microdissection (LCM) to collect cells from the ileal colonic tips and crypt fractions of GF and CV mice. When comparing GF with CV mice, they found that the latter had slightly more Paneth and goblet cells, but the differences were minor. Turning to transcriptional differences, they found that 10% of the host transcriptome was microbially regulated and that epithelial cells were the primary responders to microbial signals. However, microbiota-induced responses were site specific. Consistent with the observations of El Aidy *et al.* (2012) and Larsson *et al.* (2012), half of all the gene ontology (GO) terms associated with differentially regulated genes were related to immune functions (adaptive immunity, innate immunity, virus response, and immune regulation). Immune genes were predominantly increased in the tip fractions of both the ileum and colon, with differences in antigen presentation and interferon-γ responses being enriched in both ileum and colon. In contrast, processes related to T-cell regulation were restricted to the ileum tip whereas virus response functions were mainly enriched in the colon tip (Sommer *et al.*, 2015). Other GO categories that were differentially expressed were proliferation, apoptosis, protein biosynthesis, metabolism, angiogenesis, and others. Next, the time course of colonization on gene expression was assessed by creating GN mice with CV feces and taking samples daily for 1 week. On average, the colonic epithelium responded faster than the ileal epithelium, which is consistent with previous findings (El Aidy *et al.*, 2012). Interestingly, many of the transcriptional regulators that were enriched among the microbially regulated genes involved changes in the methylation and acetylation status of histones, including the histone deacetylase (HDAC) target H3k9ac. The authors speculated that many of the microbially responsive genes might be regulated epigenetically, potentially via SCFA-repressed HDACs or a folate-induced increase in DNA methylation (Sommer *et al.*, 2015).

Several groups have investigated how monoassociation with commensal or probiotic bacteria modifies intestinal gene expression relative to GF or CV states. In the late 1990s, the Gordon laboratory conducted a series of experiments to uncover how *B. thetaiotaomicron*, a prominent component of the normal mouse and human intestinal microbiota, influences intestinal gene expression and function (Hooper *et al.*, 2001). Ten days after colonization, samples of ileal and colon tips and crypts were collected by LCM and gene microarrays were conducted. A total of 118 probe sets were differentially regulated in GN versus GF mice,

of which 81% (95 probe sets) were upregulated. *B. thetaiotaomicron* upregulated expression of genes involved in nutrient absorption (Na^+/glucose cotransporter, SGLT-1) and fatty acid transport and metabolism [pancreatic lipase-related protein-2, colipase, L-FABP (liver-type fatty acid-binding protein), and apolipoprotein A-IV]. Genes related to mucosal barrier fortification were also induced, particularly small proline-rich protein-2 (sprr2a), which was the most upregulated gene (280-fold) in response to *B. thetaiotaomicron* colonization. Several genes involved in intestinal maturation were induced following colonization, including adenosine deaminase and ornithine decarboxylase. Expressions of genes involved in xenobiotic metabolism were also differentially regulated, suggesting that colonization affected the host's capacity to metabolize and excrete xenobiotics. Angiogenin-3 expression was increased ~60-fold specifically in the ileal crypt of GN versus GF mice, supporting the increased angiogenesis observed in CV versus GF animals. Lastly, a 2–5-fold increase in mRNAs encoding several genes that could be implicated in the microbiome–gut–brain axis (Cryan and Dinan, 2012; Forsythe and Kunze 2013), including L-glutamate transporter, L-glutamate decarboxylase (converts glutamate to γ-aminobutyric acid), vesicle-associated protein-33 (a synaptobrevin- binding protein involved in neurotransmitter release), and enteric γ-actin and cysteine-rich protein-2, was observed (Hooper *et al.*, 2001).

Umesaki (2014) generated GN mice by transfaunating GF mice with feces from a CV mouse or the following isolated strains: *Escherichia coli*, *Proteus* sp., *Lactobacillus acidophilus*, *Lactobacillus fermentum*, *Lactobacillus murini*, *Streptococcus faecalis*, *Bacteroides* sp., and *Bifidobacterium* sp. Induction of α(1–2) asialo GM1 fucosyltransferase (FT) activity, a product of the *FUT2* gene, in the small intestine of GN mice colonized with feces from CV mice, but not in monocolonized GN mice, was observed. Monoassociation of GF mice with *B. thetaiotaomicron*, a non-spore-forming microbe of human origin, in GF mice was also reported to induce FT activity (Bry *et al.*, 1996). FT activity was correlated with the differentiation of epithelial cells. Interestingly, intestinal bacteria that induce FUT2 expression have the potential to stimulate colonization by beneficial *Bifidobacterium* (Wacklin *et al.*, 2011). An expansion of T cells, particularly $CD8^+$ populations, was also observed after introduction of the CV microbiota (Umesaki, 2014). Lastly, the impact of monoassociation with two different probiotic bacteria, SFB or a complex microbiota, were compared (Shima *et al.*, 2008). GF mice were inoculated orally with 10^9 CFU of *Lactobacillus casei* strain Shirota, *Bifidobacterium breve* strain Yakult, SFB, or a fecal suspension from CV mice. After 3 days, intestinal epithelial cells were dissociated from ileal and distal colon samples and gene expression was assessed. They observed bacteria- and region-specific effects on gene expression. Although all bacteria colonized the colon to a greater extent than the ileum, *L. casei* Shirota affected gene expression to a greater extent in the ileum and to a lesser extent in the colon than *B. breve* Yakult. SFB increased gene expression in both regions more than either probiotic. Specific effects of probiotics on gene expression were also observed. For example, expression of cryptdins and intestinal fatty acid-binding protein genes in the ileum was more enhanced in the *L. casei* Shirota mice than in the *B. breve* Yakult mice. In the colon, downregulation of genes involved in inflammatory response, transport, metabolism, and defense/immunity and upregulation of guanine nucleotide-binding protein mRNA was more evident in the mice monoassociated with *B. breve* Yakult than *L. casei* Shirota. Taken together, these findings support previous observations of region- and microbe-specific actions on gut gene expression, while demonstrating that probiotic strains can have very specific actions on epithelial gene expression relative to each other, SFB, and a complex microbiota (Shima *et al.*, 2008).

11.5.2.2 *Effect of the microbiota on intestinal gene expression in GF and GN piglets*

Mice are the most commonly used GF and GN model. Although they have a number of advantages as model systems, there are concerns regarding the appropriateness of mouse models in terms of their native microbiome (Nguyen *et al.*, 2015), immune system (Mestas and Hughes, 2004; Shay *et al.*, 2013), and response to inflammatory diseases (Seok *et al.*, 2013), which can differ markedly from those of humans. For example, while the gut microbiota of both mice and humans are dominated by two main phyla, Bacteroidetes and Firmicutes, 85% of genera and microbial species found in mice are not found in humans (Ley *et al.*, 2005). For those reasons, the use of GF and GN swine for microbiome and infection studies has been gaining traction (Wang and Donovan, 2015). The domestic pig (*Sus scrofa*) is closely related to humans in terms of anatomy, physiology, and genetics, and is considered the preferred non-primate model for humans (Guilloteau *et al.*, 2010; Dawson 2011; Meurens *et al.*, 2012; Odle *et al.*, 2014). In addition, the piglet is an excellent model for infectious diseases (Meurens *et al.*, 2012). From a nutritional perspective, pigs and humans are omnivorous, whereas rodents are granivorous. In terms of GIT anatomy and physiology, pigs are also more similar to humans than are rodents (Guilloteau *et al.*, 2010; Odle *et al.*, 2014). Also, both pigs and humans are colon fermenters whereas fermentation takes place in the cecum of rodents (Heinritz *et al.*, 2013). Pigs are also immunologically similar to humans. For example, porcine immune responses more closely resemble human responses than mouse responses, with >80% of parameters studied, whereas the immune response in mice was similar to that in humans in <10% of comparisons (Dawson 2011).

Consistent with observations in GF mice (Grover and Kashyap, 2014), GF pigs have abnormal intestinal proliferation, structure, and function and immune development compared with CV piglets (Shirkey *et al.*, 2006; Willing and Van Kessel, 2007, 2009). Several studies have investigated the role of bacterial colonization on the host immune development. Haverson *et al.* (2007) compared the immunological structure of the lamina propria in the jejunum of GF piglets with piglets associated with two strains of commensal *E. coli* between 1 and 4 days of age. Monoassociation with *E. coli* increased the numbers of dendritic and T cells in diffuse lymphoid tissue of the jejunum within 2 days compared with GF. Additionally, small intestine (SI) expression of proinflammatory cytokines, interleukin-1β (IL-1β) and IL-6, were higher in GF than CV piglets at postnatal day 13 (Shirkey *et al.*, 2006). Gene microarray profiling of the SI epithelium in GF and CV piglets confirmed the essential role of a commensal microbiota for normal development of the host intestinal transcriptome (Chowdhury *et al.*, 2007) (Table 11.3). Genes involved in transcription, cell proliferation and differentiation, nutrient transport and metabolism, xenobiotic metabolism, and immune responsiveness were upregulated in GN piglets bearing a microbiota from CV piglets versus GF piglets (Chowdhury *et al.*, 2007). These pathways are consistent with what has been reported in GF mice following conventionalization, confirming the utility of the piglet model.

11.5.2.3 *Effect of the microbiota on metabolites in GF and GN mice*

In addition to differences in tissue gene expression profiles, microbial colonization modifies the content and composition of metabolites in the feces, urine, and blood of the host (Griffin *et al.*, 2015; Marcobal *et al.*, 2015). These metabolites originate from the interface of

microbial and host metabolism and have been useful in linking microbial community structures with diet (Shoaie *et al.*, 2015) and human health outcomes (Goodacre, 2007; Griffin *et al.*, 2015). Wikoff *et al.* (2009) compared the plasma metabolome of GF and CV mice and observed that 10% of metabolites differed by more than 50% between the two groups and some metabolites were only detected in CV mice. Amino acid metabolites were particularly affected. For example, the bacterial-mediated production of bioactive indole-containing metabolites derived from tryptophan, such as indoxyl sulfate and the antioxidant indole-3-propionic acid (IPA), and multiple organic acids containing phenyl groups were also greatly increased in the presence of gut microbes. This study was the first to report a drug-like phase II metabolic response of the host to metabolites generated by the microbiome, concluding that the gut microflora has a direct impact on the drug metabolism capacity of the host (Wikoff *et al.*, 2009).

Marcobal *et al.* (2013) compared the fecal and urinary metabolome of GF mice with those transfaunated with human (HM) or murine (CV) fecal microbiota 8 weeks after colonization. Using principal component analysis, the fecal and urinary metabolome of GF, HM, and CV mice discriminated into three distinct clusters. In addition, changes in the relative abundance of fecal metabolites were observed, with tryptamine and indoxyl glucuronide being elevated in HM versus GF mice and creatine and creatinine being lower in HM than GF mice. This study also demonstrated that fecal metabolite profiles in HM mice were changed when mice were switched from a standard diet to one devoid of fermentable polysaccharides. The gut microbiota composition was also altered by the diet, indicating that nutrition represents a potent and promising approach for altering gut microbiota functionality, which in turn can be monitored via metabolomics (Marcobal *et al.*, 2013). As described in the next section, the future of microbiome research will be to integrate these data with host transcriptome and phenotype in order to elucidate bidirectional host–microbe interactions.

11.6 Systems biology approaches to diet-dependent host–microbe interaction

The concept that the host and its microbiome coexist as a "superorganism" is now well accepted (Wu *et al.*, 2015). Both dietary substrates and microbial metabolites that arise from the gut are transported throughout the body and conjointly, the direct chemical interactions between gut microbiota and the host, and the immune-mediated signaling mechanisms, influence various organs such as the gut, the skeletal muscle, the liver, and the brain (Aw and Fukuda, 2015). Attempts to integrate these data sets ideally lend themselves to a systems biology approach, which is based on the understanding that the "whole is greater than the sum of the parts." This holistic approach to deciphering the complexity of biological systems is inherently collaborative and requires transdisciplinary integration of biology, microbiology, statistics, applied mathematics, computer science, and bioinformatics. The ultimate goal of applying systems biology is to develop computational models that can be used to predict how the complex host–microbe ecosystem changes over time, which will allow the microbiome field to move beyond association to mechanisms of interaction and will inform approaches to therapeutically manipulate microbiome composition for human health (Ursell *et al.*, 2014; Aw and Fukuda, 2015).

11.6.1 Integration of multiple omic's data sets

Advances in sequencing and metabolite profiling have permitted investigations into multiple levels of DNA sequencing in the host and microbiota (metagenomics), mRNA expression in the host (transcriptome) or bacteria (metatranscriptome), and metabolites in the feces, blood, and urine of the host, which are catalogued, quantified (metabolomics), and studied in response to stresses such as disease, toxic exposure, or dietary change (metabonomics) (Lindon *et al.*, 2004; Nicholson and Lindon, 2008).

It would be ideal to obtain metabolomic and metagenomic data for every sample for which 16S rRNA data are available; however, these techniques are currently too cost prohibitive and time intensive for most large-scale studies. To overcome this limitation, various software packages have been developed that exploit the strong association between bacterial phylogeny and function. One program, Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt), uses an extended ancestral-state reconstruction algorithm to predict which gene families are present and subsequently combines gene families to estimate the composite metagenome (Langille *et al.*, 2013). Using 16S rRNA information, PICRUSt accurately predicts (average Spearman $r=0.82$) inferred metagenomes from 16S marker genes compared with fully sequenced metagenomes obtained from the Human Microbiome Project.

Several recent studies have investigated correlations between microbial diversity and host transcriptomic and metabolomics responses when all data sets are available. Using GF and GN mice, El Aidy *et al.* (2013a) employed combined transcriptome, metabonome, and microbial profiling tools to analyze the dynamic responses of the colonic mucosa to colonization by normal mouse microbiota (CV) at different time points over 16 days. The observed dynamic changes in the microbial composition from early colonizers (days 1 and 2) belonging predominantly to the phyla Bacteroidetes, Firmicutes (notably the class Bacilli), Proteobacteria, and Actinobacteria to later colonizers (days 4, 8, and 16), where specific subgroups of the Firmicutes, particularly the members of *Clostridium* clusters IV and XIVa, increased in abundance. The shift in the composition of the microbiota was reflected by differences in local and systemic metabolite profiles and host transcriptomics. For example, at 1, 2 and 4 days post-conventionalization, succinate and lactate were in high abundance in the colon, but were absent at days 8 and 16, which was consistent with an increased abundance of butyrate-producing bacteria (butyryl-CoA-transferase positive) on days 8 and 16. Correlations of host transcriptomes, metabolite patterns, and microbiota composition were investigated using the O2-PLS method (Trygg and Wold, 2003) and revealed associations between Bacilli and Proteobacteria and differential expression of host genes involved in energy and anabolic metabolism (El Aidy *et al.*, 2013a). Additionally, differential host colonic gene expression correlated with scyllo- and myo-inositol, glutamine, glycine, and alanine levels in colonic tissues during conventionalization. One of the strengths of this study was the combined time-resolved analyses of multiple data sets that expanded the understanding of host–microbe molecular interactions during the microbial establishment (El Aidy *et al.*, 2013a).

Going a step further, mathematical modeling of the human gut microbiome at a genome scale has been shown to be a useful tool to untangle microbe–microbe, diet–microbe, and microbe–host interactions. Recently, Shoaie *et al.* (2015) described the use of the CASINO (Community And Systems-level Interactive Optimization) toolbox, a new computational platform that comprises an optimization algorithm integrated with diet analysis to predict the

phenotypes and related dietary intake within the human gut microbiota.The toolbox was tested by simulating and testing the performance of single bacteria or five bacterial species that are representative of the predominant phyla in the human GIT *in vitro* before applying it to an existing data set from a diet-intervention study of 45 obese and overweight individuals with either low (LGC) or high (HCG) microbial gene count (Cotillard *et al.*, 2013). In these *in vitro* studies, the model simulations matched fecal metabolomics data, but more importantly, it correctly correlated with changes in serum levels of 10 amino acids and one SCFA (acetate), suggesting that the five species captured some of the major metabolic functions of the human gut microbiome (Shoaie *et al.*, 2015). Focusing on metabolic interactions between diet, gut microbiota, and host metabolism, the predictive power of the CASINO toolbox for fecal and plasma metabolites profiles was observed. For example, obese individuals with an LGC microbiome had a more impaired metabolic phenotype and elevated levels of plasma amino acids were associated with insulin resistance compared with those with an HGC gut microbiome, suggesting either production of these amino acids by bacteria in the LGC individuals or utilization of these amino acids by bacteria in the HCG subjects. Furthermore, the HGC microbiome exhibited a higher capacity to produce SCFAs (Cotillard *et al.*, 2013). Using CASINO, the relative contribution of each bacterial species to the production of specific metabolites was calculated. Using the LGG microbiome and poorer phenotype, the investigators were also able to model the simulated consumption of eight essential amino acids by the LGG versus HGG gut microbiome and subsequently calculate the required dietary changes to achieve an essential amino acids intake required to improve metabolic control. This recommendation involved reducing the intake of breads, cereals, and rice and increasing the intake of protein-rich meat and dairy (Shoaie *et al.*, 2015). Although this approach needs to be validated through a specifically designed nutrition intervention study, information generated from CASINO shows the potential of using modeling to aid in the design of nutritional, prebiotic, or probiotic interventions to fortify the microbiota and improve the gut microbiome metabolic profile (Shoaie *et al.*, 2015).

11.6.2 Non-invasive molecular fingerprinting of host–microbiome interactions in human infants

The early postnatal period is a critical window for colonization of the GIT, intestinal development, and immune education (Donovan, 2006; Donnet-Hughes *et al.*, 2010). These processes are mediated by early nutrition and dysbioses can have long-term health consequences (Li *et al.*, 2014b). Mechanistic examination of host–microbe relationships in healthy infants has been hindered by ethical constraints surrounding tissue biopsies, hence a statistically rigorous analytical framework to examine simultaneously both host and microbial responses to dietary/environmental factors using exfoliated intestinal epithelial cells was developed (Donovan *et al.*, 2014). The initial goal was to investigate genome-wide markers by interrogating the intestinal transcriptome of human infants. Each day, up to one-third of normal adult epithelial cells (~10 billion cells) are shed. The Chapkin laboratory developed a non-invasive method using host exfoliated cell mRNA directly isolated from feces, which contains sloughed SI and colon cells (Davidson *et al.*, 1995). This highly sensitive technique is capable of isolating and quantifying specific mRNAs under various intestinal conditions and has been tested in adult humans (Davidson *et al.*, 1995; Zhao *et al.*, 2009). It was hypothesized that this approach could also be applied to infants and would be sensitive to dietary

intake (Chapkin *et al.*, 2010). Stool was collected from the diapers of 3-month-old breast- and formula-fed infants and host mRNA isolated and analyzed by gene microarray. A total of 1214 genes were differentially expressed between the two groups of infants. Analysis of gene networks identified differential expression of genes related to signal transduction, cytoskeletal remodeling, cell adhesion, and immune response (Chapkin *et al.*, 2010). The glucocorticoid receptor, Z01 (a critical tight junction protein) and genes encoding proteins involved in cell–cell interactions, including integrins, cadherins, and syntaxin, and proteins involved in cell proliferation and apoptosis were differentially expressed (Chapkin *et al.*, 2010). More recently, this approach was applied to stool samples obtained from extremely preterm infants (gestational age 24–30 weeks) and it was demonstrated that RNA sequencing (versus gene microarray) can also be used to query host gene expression (Knight *et al.*, 2014). For comparison, host mRNA from term infants (Chapkin *et al.*, 2010) was also sequenced to study developmental differences in gene expression. On average, 5500 genes were detected in both term and preterm samples; however, the transcriptional landscape was dramatically altered in the preterm versus term infant intestine. For example, the intestinal cells of preterm infants overexpressed IL-1β and IL-33 and genes related to macrophage activation, but had lower expression of genes promoting T-cell development than term infants (Knight *et al.*, 2014).

Next, the multivariate relationship between the microbiome metagenomic functional profile and the host transcriptome in infants was examined (Schwartz *et al.*, 2012). The bacterial 16S rRNA and DNA were sequenced in the same stool samples from the term infants (Chapkin *et al.*, 2010). Microbiota functional characteristics were mapped to functional SEED categories. Interestingly, the "virulence" SEED category differed significantly between the bacterial metagenome of breastfed and formula-fed infants. To examine the intrinsic relationship between host and microbiome, host transcriptome and metagenomic data were combined and integrated using the multivariate technique of canonical correlation analysis (CCA) (Schwartz *et al.*, 2012). A robust multivariate structure relating microbiota virulence genes and host immunity and defense genes was observed. Seven of the top 11 immunity and defense host genes that were related to the microbiota were downregulated in breastfed versus formula-fed infants, including ALOX5, a lipoxygenase involved in arachidonic acid and leukotriene synthesis, the cytokine IL1α, and binding proteins for natural killer cells, T cells, and LPS. In these comparisons, the overall effect of breastfeeding was to reduce inflammatory genes in the gut, potentially promoting tolerance to luminal microbes (Schwartz *et al.*, 2012).

Taken together, these studies validate a novel molecular methodology that utilizes stool samples containing intact sloughed epithelial cells to quantify non-invasively intestinal gene expression profiles that can be applied from infancy (Chapkin *et al.*, 2010) through adulthood (Davidson *et al.*, 1995; Zhao *et al.*, 2009). This approach allows repeated assessment of the same individual over time to assess temporal changes in gene expression and can be combined with the bacterial metagenome to investigate host–microbe cross-talk (Schwartz *et al.*, 2012). By examining the multivariate structure underlying the bacterial metagenome or metatranscriptome and gut exfoliated cell transcriptome, this approach leverages richer and fuller information content compared with analyses focusing on single data sets. Moreover, the approach is responsive to dietary intake (Chapkin *et al.*, 2010; Cho *et al.*, 2011; Donovan *et al.*, 2014), stage of development (Knight *et al.*, 2014), and disease state (Davidson *et al.*, 1995; Zhao *et al.*, 2009) and can be effectively used as part of an integrative systems biology approach to identify host- and microbe-specific biomarkers that are sensitive to modifications to changes in the dynamics of the ecosystem. Recently, integration of

the host and microbial transcriptomes has also been reported in epithelial cells and microbes isolated from the lungs of asthmatic and non-asthmatic children (Castro-Nalla *et al.*, 2015; Pérez-Losada *et al.*, 2015).

11.7 Summary and conclusions

Mammals have never existed in the absence of microbes and as such it is expected that our species and our resident microbiota have co-evolved a mutualistic existence in which we are dependent upon each other for optimal function. Over the past two decades, our understanding of the diversity of microbes that inhabit unique niches in the human habitus has rapidly expanded through sequencing of bacterial 16S rRNA sequences in complex communities. Additional advances in microbial DNA (metagenomics) and mRNA (metatranscriptomics) sequencing has provided insight into the genetic potential and functions of the microbiome (Dave *et al.*, 2012; El Aidy and Kleerebezem, 2013). Up to 10% of the metabolites in the serum of mammals are substantially contributed to by the microbiota (Wikoff *et al.*, 2009), hence integrating metagenomic and metabonomic data using a systems biology approach is needed to permit a fuller understanding of the interplay between gut microbiome and host metabolism (Ursell *et al.*, 2014; Aw and Fukuda, 2015; Wu *et al.*, 2015). In the future, integration of information on the host genome/transcriptome, microbiome metagenome/metatranscriptome, and metabolome will inform a more complete understanding of the complex mammalian superorganism (Shoaie *et al.*, 2015). Meaningful interpretation of the gut microbiome requires that host genetic and environmental influences be controlled or accounted for, hence model animal systems will continue to be important for discovery (Wu *et al.*, 2015). However, in order to identify microbial taxa and gene functions that are consistently associated with human diseases, standardization of sample collection and the analytical procedures is required. Improved catalogs of cultured human gut microbes are essential to allow their functional characterization (Wu *et al.*, 2015) and more complete metabolomics and proteomic databases to annotate human samples. Ultimately, well-designed studies in humans that take into account the multiplicity of genetic, geographical, and dietary factors that shape the human gut microbiota are essential to define causal relationships between the gut microbiota and various diseases and to identify new therapeutic targets that could be captured and utilized for improved human health and well-being (Nagpal *et al.*, 2014).

References

AAP (2012) American Academy of Pediatrics, *Section on Breastfeeding. Breastfeeding and the use of human milk. Pediatrics*, **129**, e827–e841.

Abrams, G.D., Bauer, H., and Sprinz, H. (1963) Influence of the normal flora on mucosal morphology and cellular renewal in the ileum. A comparison of germ-free and conventional mice. *Laboratory Investigation*, **12**, 355–364.

Abrams, G.D. and Bishop, J.E. (1967) Effect of the normal microbial flora on gastrointestinal motility. *Proceedings of the Society for Experimental Biology and Medicine*, **126**, 301–314.

Ahmed, A.U., Williams, B.R., and Hannigan, G.E. (2015) Transcriptional activation of inflammatory genes: mechanistic insight into selectivity and diversity. *Biomolecules*, **5**, 3087–3111.

Aomatsu, T., Imaeda, H., Fujimoto, T., *et al.* (2012) Terminal restriction fragment length polymorphism analysis of the gut microbiota profiles of pediatric patients with inflammatory bowel disease. *Digestion*, **86**, 129–135.

Aron-Wisnewsky, J., and Clément, K. (2016) The gut microbiome, diet, and links to cardiometabolic and chronic disorders. *Nature Reviews Nephrology*, **12**, 169–181. DOI: 10.1038/nrneph.2015.191.

Atarashi, K., Tanoue, T., Shima, T., *et al.* (2011) Induction of colonic regulatory T cells by indigenous *Clostridium* species. *Science*, **331**, 337–341.

Atarashi, K., Tanoue, T., Oshima, K., *et al.* (2013) Treg induction by a rationally selected mixture of *Clostridia* strains from the human microbiota. *Nature*, **500**, 232–236.

Aw, W. and Fukuda, S. (2015) Toward the comprehensive understanding of the gut ecosystem via metabolomics-based integrated omics approach. *Seminars in Immunopathology*, **37**, 5–16.

Azad, M.B., Konya, T., Persaud, R.R., *et al.* (2016) Impact of maternal intrapartum antibiotics, method of birth and breastfeeding on gut microbiota during the first year of life: a prospective cohort study. *BJOG*, **123**, 983–993. DOI: 10.1111/1471-0528.13601.

Bäckhed, F. (2012) Host responses to the human microbiome. *Nutrition Reviews*, **70**(Suppl. 1), S14–S17.

Bäckhed, F., Ding, H., Wang, T., *et al.* (2004) The gut microbiota as an environmental factor that regulates fat storage. *Proceedings of the National Academy of Sciences of the United States of America*, **101**, 15718–15723.

Bäckhed, F., Manchester, J.K., Semenkovich, C.F., and Gordon, J.I. (2007) Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 979–984.

Barrett, E., Ross, R.P., O'Toole, P.W., *et al.* (2012) γ-Aminobutyric acid production by culturable bacteria from the human intestine. *Journal of Applied Microbiology*, **113**, 411–417.

Benson, A.K., Kelly, S.A., Legge, R., *et al.* (2010) Individuality in gut microbiota composition is a complex polygenic trait shaped by multiple environmental and host genetic factors. *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 18933–18938.

Bercik, P., Denou, E., Collins, J., *et al.* (2011) The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. *Gastroenterology*, **141**, 599–609.

Berrington, J.E., Stewart, C.J., Cummings, S.P., and Embleton, N.D. (2014) The neonatal bowel microbiome in health and infection. *Current Opinion in Infectious Diseases*, **27**, 236–243.

Bettelli, E., Korn, T., and Kuchroo, V.K. (2007) Th17: the third member of the effector T cell trilogy. *Current Opinion in Immunology*, **19**, 652–657.

Bindels, L.B., Delzenne, N.M., Cani, P.D., and Walter, J. (2015) Towards a more comprehensive concept for prebiotics. *Nature Reviews Gastroenterology & Hepatology*, **12**, 303–310.

Blekhman, R., Goodrich, J.K., Huang, K., *et al.* (2015) Host genetic variation impacts microbiome composition across human body sites. *Genome Biology*, **16**, 191. DOI: 10.1186/s13059-015-0759-1.

Bode, L. (2006) Recent advances on structure, metabolism, and function of human milk oligosaccharides. *Journal of Nutrition*, **136**, 2127–2130.

Bravo, J.A., Forsythe, P., Chew, M.V., *et al.* (2011) Ingestion of *Lactobacillus* strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 16050–16055.

Browning, K.N. and Travagli, R.A. (2014) Central nervous system control of gastrointestinal motility and secretion and modulation of gastrointestinal functions. *Comprehensive Physiology*, **4**, 1339–1368.

Bry, L., Falk, P.G., Midtvedt, T., and Gordon, J.I. (1996) A model of host-microbial interactions in an open mammalian ecosystem. *Science*, **273**, 1380–1383.

Cani, P.D. and Delzenne, N.M. (2009) Interplay between obesity and associated metabolic disorders: new insights into the gut microbiota. *Current Opinion in Pharmacology*, **9**, 737–743.

Carabotti, M., Scirocco, A., Maselli, M.A., and Severi, C. (2015) The gut–brain axis: interactions between enteric microbiota, central and enteric nervous systems. *Annals of Gastroenterology*, **28**, 203–209.

Carlisle, E.M. and Morowitz, M.J. (2013) The intestinal microbiome and necrotizing enterocolitis. *Current Opinion in Pediatrics*, **25**, 382–387.

Carlstedt-Duke, B., Midtvedt, T., Nord, C.E., and Gustafsson, B.E. (1986) Isolation and characterization of a mucin-degrading strain of *Peptostreptococcus* from rat intestinal tract. *Acta Pathologica, Microbiologica, et Immunologica Scandinavica. Section B, Microbiology*, **94**, 293–300.

Castro-Nallar, E., Shen, Y., Freishtat, R.J., *et al.* (2015) Integrating microbial and host transcriptomics to characterize asthma-associated microbial communities. *BMC Medical Genomics*, **8**, 50. DOI: 10.1186/s12920-015-0121-1.

Chapkin, R.S., Zhao, C., Ivanov, I., *et al.* (2010) Noninvasive stool-based detection of infant gastrointestinal development using gene expression profiles from exfoliated epithelial cells. *American Journal of Physiology. Gastrointestinal and Liver Physiology*, **298**, G582–G589.

CDC (2014) *Breastfeeding Report Card*. Centers for Disease Control and Prevention, http://www.cdc.gov/breastfeeding/data/reportcard.htm (accessed 30 November 2015).

Chakraborti, C.K. (2015) New-found link between microbiota and obesity. *World Journal of Gastrointestinal Pathophysiology*, **6**(4), 110–119. DOI: 10.4291/wjgp.v6.i4.110.

Chen, Z. and O'Shea, J.J. (2008) Th17 cells: a new fate for differentiating helper T cells. *Immunologic Research*, **41**, 87–102.

Chichlowski, M., German, J.B., Lebrilla, C.B., and Mills, D.A. (2011) The influence of milk oligosaccharides on microbiota of infants: opportunities for formulas. *Annual Review of Food Science and Technology*, **2**, 331–351.

Cho, Y., Kim, H., Turner, N.D., *et al.* (2011) A chemoprotective fish oil- and pectin-containing diet temporally alters gene expression profiles in exfoliated rat colonocytes throughout oncogenesis. *Journal of Nutrition*, **141**, 1029–1035.

Chow, J., Lee, S.M., Shen, Y., *et al.* (2010) Host-bacterial symbiosis in health and disease. *Advances in Immunology*, **107**, 243–274.

Chowdhury, S.R., King, D.E., Willing, B.P., *et al.* (2007) Transcriptome profiling of the small intestinal epithelium in germfree versus conventional piglets. *BMC Genomics*, **8**, 215.

Chung, H. and Kasper, D.L. (2010) Microbiota-stimulated immune mechanisms to maintain gut homeostasis. *Current Opinion in Immunology*, **22**, 455–460.

Chung, H., Pamp, S.J., Hill, J.A., *et al.* (2012) Gut immune maturation depends on colonization with a host specific microbiota. *Cell*, **149**, 1578–1593.

Clarke, G., Grenham, S., Scully, P., *et al.* (2013) The microbiome–gut–brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner. *Molecular Psychiatry*, **18**, 666–673.

Collado, M.C., Isolauri, E., Laitinen, K., and Salminen, S. (2010) Effect of mother's weight on infant's microbiota acquisition, composition, and activity during early infancy: a prospective follow-up study initiated in early pregnancy. *American Journal of Clinical Nutrition*, **92**, 1023–1030.

Collins, S.M., Surette, M., and Bercik, P. (2012) The interplay between the intestinal microbiota and the brain. *Nature Reviews Microbiology*, **10**, 735–742.

Corthay, A. (2009) How do regulatory T cells work? *Scandinavian Journal of Immunology*, **70**, 326–336.

Corthësy, B. (2009) Secretory immunoglobulin A: well beyond immune exclusion at mucosal surfaces. *Immunopharmacology and Immunotoxicology*, **31**, 174–179.

Cotillard, A., Kennedy, S.P., Kong, L.C., *et al.* (2013) Dietary intervention impact on gut microbial gene richness. *Nature*, **500**, 585–588.

Cox, L.M., Yamanishi, S., Sohn, J., *et al.* (2014) Altering the intestinal microbiota during a critical developmental window has lasting metabolic consequences. *Cell*, **158**, 705–721.

Crestani, F., Lorez, M., Baer, K., *et al.* (1999) Decreased GABAA-receptor clustering results in enhanced anxiety and a bias for threat cues. *Nature Neuroscience*, **2**, 833–839.

Cryan, J.F. and Dinan, T.G. (2012) Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nature Reviews Neuroscience*, **13**, 701–712.

Dantzer, R., O'Connor, J.C., Freund, G.G., *et al.* (2008) From inflammation to sickness and depression: when the immune system subjugates the brain. *Nature Reviews Neuroscience*, **9**, 46–56.

Dave, M., Higgins, P.D., Middha, S., and Rioux, K.P. (2012) The human gut microbiome: current knowledge, challenges, and future directions. *Translational Research*, **160**, 246–257.

Davenport, E.R., Cusanovich, D.A., Michelini, K., *et al.* (2015) Genome-wide association studies of the human gut microbiota. *PLoS One*, **10**(11), e0140301. DOI: 10.1371/journal.pone.0140301.

David, L.A., Maurice, C.F., Carmody, R.N., *et al.* (2014) Diet rapidly and reproducibly alters the human gut microbiome. *Nature*, **505**(7484), 559–563.

Davidson, L.A., Jiang, Y.H., Lupton, J.R., and Chapkin, R.S. (1995) Noninvasive detection of putative biomarkers for colon cancer using fecal messenger RNA. *Cancer Epidemiology, Biomarkers & Prevention*, **4**(6), 643–647.

Dawson, H. (2011) A comparative assessment of the pig, mouse, and human genomes: structural and functional analysis of genes involved in immunity and inflammation. In The Minipig in Biomedical Research (eds. P.A. McAnulty, A.D. Dayan, N.-C. Ganderup, and K.L. Hastings), CRC Press, Boca Raton, FL, pp. 321–341.

De Filippo, C., Cavalieri, D., Di Paola, M., *et al.* (2010) Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 14691–14696.

de Jonge, W.J. (2013) The gut's little brain in control of intestinal immunity. *ISRN Gastroenterology*, **2013**, 630159. DOI: 10.1155/2013/630159.

De Leoz, M.L., Kalanetra, K.M., Bokulich, N.A., *et al.* (2015) Human milk glycomics and gut microbial genomics in infant feces show a correlation between human milk oligosaccharides and gut microbiota: a proof-of-concept study. *Journal of Proteome Research*, **14**, 491–502.

Desbonnet, L., Garrett, L., Clarke, G., *et al.* (2010) Effects of the probiotic *Bifidobacterium infantis* in the maternal separation model of depression. *Neuroscience*, **170**, 1179–1188.

Deshpande, G., Rao, S., Patole, S., and Bulsara, M. (2010) Updated meta-analysis of probiotics for preventing necrotizing enterocolitis in preterm neonates. *Pediatrics*, **125**, 921–930.

Dethlefsen, L., Huse, S., Sogin, M.L., and Relman, D.A. (2008) The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. *PLoS Biology*, **6**(11), e280. DOI: 10.1371/journal.pbio.0060280.

Diaz Heijtz, R., Wang, S., Anuar, F., *et al.* (2011) Normal gut microbiota modulates brain development and behavior. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 3047–3052.

Dominguez-Bello, M.G., Costello, E.K., Contreras, M., *et al.* (2010) Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 11971–11975.

Donnet-Hughes, A., Perez, P.F., Doré, J., *et al.* (2010) Potential role of the intestinal microbiota of the mother in neonatal immune education. *Proceedings of the Nutrition Society*, **69**, 407–415.

Donovan, S.M. (2006) Role of human milk components in gastrointestinal development: current knowledge and future needs. *Journal of Pediatrics*, **149**(Suppl. 3), 49–61.

Donovan, S.M., Wang, M., Monaco, M.H., *et al.* (2014) Noninvasive molecular fingerprinting of host-microbiome interactions in neonates. *FEBS Letters*, **588**, 4112–4119.

Drossman, D.A. (1998) Presidential address. Gastrointestinal illness and the biopsychosocial model. *Psychosomatic Medicine*, **60**, 258–267.

Dutton, R.J. and Turnbaugh, P.J. (2012) Taking a metagenomic view of human nutrition. *Current Opinion in Clinical Nutrition and Metabolic Care*, **15**, 448–454.

Eisenstein, E.M. and Williams, C.B. (2009) The T(reg)/Th17 cell balance: a new paradigm for autoimmunity. *Pediatric Research*, **65**, 26R–31R.

El Aidy, S. and Kleerebezem, M. (2013) Molecular signatures for the dynamic process of establishing intestinal host-microbial homeostasis: potential for disease diagnostics? *Current Opinion in Gastroenterology*, **29**, 621–617.

El Aidy, S., van Baarlen, P., Derrien, M., *et al.* (2012) Temporal and spatial interplay of microbiota and intestinal mucosa drive establishment of immune homeostasis in conventionalized mice. *Mucosal Immunology*, **5**, 567–579.

El Aidy, S., Derrien, M., Merrifield, C.A., *et al.* (2013a) Gut bacteria–host metabolic interplay during conventionalisation of the mouse germfree colon. *ISME Journal*, **7**, 743–755.

El Aidy, S., Merrifield, C.A., Derrien, M., *et al.* (2013b) The gut microbiota elicits a profound metabolic reorientation in the mouse jejunal mucosa during conventionalisation. *Gut*, **62**, 1306–1314.

Fallani, M., Amarri, S., Uusijarvi, A., *et al.* (2011) Determinants of the human infant intestinal microbiota after the introduction of first complementary foods in infant samples from five European centres. *Microbiology*, **157**, 1385–1392.

FAO/WHO (2001) *Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria*. Food and Agriculture Organization of the United Nations and World Health Organization, http://iqb.es/digestivo/pdfs/probioticos.pdf (accessed 10 November 2015).

Forsythe, P. and Kunze, W.A. (2013) Voices from within: gut microbes and the CNS. *Cell and Molecular Life Sciences*, **70**, 55–69.

Forsythe, P., Bienenstock, J., and Kunze, W.A. (2014) Vagal pathways for microbiome–brain–gut axis communication. *Advances in Experimental Medicine and Biology*, **817**, 115–133.

Foster, J.A. and McVey Neufeld, K.A. (2013) Gut–brain axis: how the microbiome influences anxiety and depression. *Trends in Neuroscience*, **36**, 305–312.

Gaboriau-Routhiau, V., Rakotobe, S., Lécuyer, E., *et al.* (2009) The key role of segmented filamentous bacteria in the coordinated maturation of gut helper T cell responses. *Immunity*, **31**, 677–689.

Ghaisas, S., Maher, J., and Kanthasamy, A. (2016) Gut microbiome in health and disease: linking the microbiome–gut–brain axis and environmental factors in the pathogenesis of systemic and neurodegenerative diseases. *Pharmacology & Therapeutics*, **158**, 52–62. DOI: 10.1016/j.pharmthera.(2015)11.012.

Gkolfakis, P., Dimitriadis, G., and Triantafyllou, K. (2015) Gut microbiota and non-alcoholic fatty liver disease. *Hepatobiliary & Pancreatic Diseases International*, **14**, 572–581.

Glaister, J.R. (1973) Factors affecting the lymphoid cells in the small intestinal epithelium of the mouse. *International Archives of Allergy and Applied Immunology*, **45**, 719–730.

Gnoth, M.J., Kunz, C., Kinne-Saffran, E., and Rudloff, S. (2000) Human milk oligosaccharides are minimally digested *in vitro*. *Journal of Nutrition*, **130**, 3014–3020.

Goodacre, R. (2007) Metabolomics of a superorganism. *Journal of Nutrition*, **137**(Suppl. 1), 259S–266S.

Goodrich, J.K., Waters, J.L., Poole, A.C., *et al.* (2014) Human genetics shape the gut microbiome. *Cell*, **159**, 789–799.

Gordon, H.A. and Bruckner-Kardoss, E. (1961) Effect of normal microbial flora on intestinal surface area. *American Journal of Physiology*, **201**, 175–178.

Gore, C., Munro, K., Lay, C., *et al.* (2008) *Bifidobacterium pseudocatenulatum* is associated with atopic eczema: a nested case–control study investigating the fecal microbiota of infants. *Journal of Allergy and Clinical Immunology*, **121**, 135–140.

Greiner, T. and Bäckhed, F. (2011) Effects of the gut microbiota on obesity and glucose homeostasis. *Trends in Endocrinology and Metabolism*, **22**, 117–123.

Grice, E.A. and Segre, J.A. (2012) The human microbiome: our second genome. *Annual Review of Genomics and Human Genetics*, **13**, 151–170.

Griffin, J.L., Wang, Z., and Stanley, E. (2015) Does our gut microbiome predict cardiovascular risk? *Circulation*. *Cardiovascular Genetics*, **8**, 187–191.

Grover, M. and Kashyap, P.C. (2014) Germ-free mice as a model to study effect of gut microbiota on host physiology. *Neurogastroenterology and Motility*, **26**, 745–748.

Grześkowiak, Ł., Grönlund, M.M., Beckmann, C., *et al.* (2012) The impact of perinatal probiotic intervention on gut microbiota: double-blind placebo-controlled trials in Finland and Germany. *Anaerobe*, **18**, 7–13.

Guilloteau, P., Zabielski, R., Hammon, H.M., and Metges, C.C. (2010) Nutritional programming of gastrointestinal tract development. Is the pig a good model for man? *Nutrition Research Reviews*, **23**, 4–22.

Gustafsson, B.E., Midtvedt, T., and Strandberg, K. (1970) Effects of microbial contamination on the cecum enlargement of germfree rats. *Scandinavian Journal of Gastroenterology*, **5**, 309–314.

Haarman, M. and Knol, J. (2005) Quantitative real-time PCR assays to identify and quantify fecal *Bifidobacterium* species in infants receiving a prebiotic infant formula. *Applied Environmental Microbiology*, **71**, 2318–2324.

Harmsen, H.J., Wildeboer-Veloo, A.C., Raangs, G.C., *et al.* (2000) Analysis of intestinal flora development in breast-fed and formula-fed infants by using molecular identification and detection methods. *Journal of Pediatric Gastroenterology and Nutrition*, **30**, 61–67.

Haverson, K., Rehakova, Z., Sinkora, J., *et al.* (2007) Immune development in jejunal mucosa after colonization with selected commensal gut bacteria: a study in germ-free pigs. *Veterinary Immunology and Immunopathology*, **119**, 243–253.

Heikkilä, M.P. and Saris, P.E. (2003) Inhibition of *Staphylococcus aureus* by the commensal bacteria of human milk. *Journal of Applied Microbiology*, **95**, 471–478

Heinritz, S.N., Mosenthin, R., and Weiss, E. (2013) Use of pigs as a potential model for research into dietary modulation of the human gut microbiota. *Nutrition Research Reviews*, **26**, 191–209.

Hooper, L.V. and Macpherson, A.J. (2010) Immune adaptations that maintain homeostasis with the intestinal microbiota. *Nature Reviews Immunology*, **10**, 159–169.

Hooper, L.V., Wong, M.H., Thelin, A., *et al.* (2001) Molecular analysis of commensal host–microbial relationships in the intestine. *Science*, **291**(5505), 881–884.

Hooper, L.V., Stappenbeck, T.S., Hong, C.V., and Gordon, J.I. (2003) Angiogenins: a new class of microbicidal proteins involved in innate immunity. *Nature Immunology*, **4**, 269–273.

Hooper, L.V., Littman, D.R., and Macpherson, A.J. (2012) Interactions between the microbiota and the immune system. *Science*, **336**, 1268–1273.

Høverstad, T. and Midtvedt, T. (1986) Short-chain fatty acids in germfree mice and rats. *Journal of Nutrition*, **116**, 1772–1776.

Hunt, K.M., Foster, J.A., Forney, L.J., *et al.* (2011) Characterization of the diversity and temporal stability of bacterial communities in human milk. *PLoS One*, **6**(6), e21313. DOI: 10.1371/journal.pone.0021313.

Ivanov, I.I., de Llanos Frutos, R., Manel, N., *et al.* (2008) Specific microbiota direct the differentiation of IL-17-producing T-helper cells in the mucosa of the small intestine. *Cell Host & Microbe*, **4**, 337–349.

Ivanov, I.I., Atarashi, K., Manel, N., *et al.* (2009) Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell*, **139**, 485–498.

Jaiswal, P., Mohanakumar, K.P., and Rajamma, U. (2015) Serotonin mediated immunoregulation and neural functions: complicity in the aetiology of autism spectrum disorders. *Neuroscience and Biobehavioral Reviews*, **55**, 413–431.

Jakobsson, H.E., Jernberg, C., Andersson, A.F, *et al.* (2010) Short-term antibiotic treatment has differing long-term impacts on the human throat and gut microbiome. *PLoS One*, **5**(3), e9836. DOI: 10.1371/journal.pone.0009836.

Ji, B. and Nielsen, J. (2015) From next-generation sequencing to systematic modeling of the gut microbiome. *Frontiers in Genetics*, **6**, 219. DOI: 10.3389/fgene.2015.00219.

Jiménez, E., Fernández, L., Marín, M.L., *et al.* (2005) Isolation of commensal bacteria from umbilical cord blood of healthy neonates born by cesarean section. *Current Microbiology*, **51**, 270–274.

Jiménez, E., Marín, M.L., Martín, R., *et al.* (2008) Is meconium from healthy newborns actually sterile? *Research in Microbiology*, **159**, 187–193.

Jost, T., Lacroix, C., Braegger, C., and Chassard, C. (2013) Assessment of bacterial diversity in breast milk using culture-dependent and culture-independent approaches. *British Journal of Nutrition*, **110**, 1253–1262.

Kalliomäki, M, Salminen, S, Arvilommi, H, *et al.* (2001) Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. *Lancet*, **357**, 1076–1079.

Kandori, H., Hirayama, K., Takeda, M., and Doi, K. (1996) Histochemical, lectin-histochemical and morphometrical characteristics of intestinal goblet cells of germfree and conventional mice. *Experimental Animals*, **45**, 155–160.

Kang, D.-W., Park, J.G., Ilhan, Z.E., *et al.* (2013) Reduced incidence of *Prevotella* and other fermenters in intestinal microflora of autistic children. *PLoS One*, **8**, e68322. DOI: 10.1371/journal.pone.0068322

Karlsson, C.L., Onnerfält, J., Xu, J., *et al.* (2012) The microbiota of the gut in preschool children with normal and excessive body weight. *Obesity*, **20**, 2257–2261.

Khachatryan, Z.A., Ktsoyan, Z.A., Manukyan, G.P., *et al.* (2008) Predominant role of host genetics in controlling the composition of gut microbiota. *PLoS One*, **3**(8), e3064. DOI: 10.1371/journal.pone.0003064.

Kitajima, H., Sumida, Y., Tanaka, R., *et al.* (1997) Early administration of *Bifidobacterium breve* to preterm infants: randomised controlled trial. *Archives of Disease in Childhood. Fetal and Neonatal Edition*, **76**, F101–F107.

Klaasen, H.L., Van der Heijden, P.J., Stok, W., *et al.* (1993) Apathogenic, intestinal, segmented, filamentous bacteria stimulate the mucosal immune system of mice. *Infection and Immunity*, **61**, 303–306.

Knight, J.M., Davidson, L.A., Herman, D., *et al.* (2014) Non-invasive analysis of intestinal development in preterm and term infants using RNA-sequencing. *Scientific Reports*, **4**, 5453. DOI: 10.1038/srep05453.

Knights, D., Silverberg, M.S., Weersma, R.K., *et al.* (2014) Complex host genetics influence the microbiome in inflammatory bowel disease. *Genome Medicine*, **6**, 107. DOI: 10.1186/s13073-014-0107-1.

Koenig, J.E., Spor, A., Scalfone, N., *et al.* (2011) Succession of microbial consortia in the developing infant gut microbiome. *Proceedings of the National Academy of Sciences of the United States of America*, **108**(Suppl. 1), 4578–4585.

Kozáková, H., Reháková, Z., and Kolínská, J. (2001) *Bifidobacterium bifidum* monoassociation of gnotobiotic mice: effect on enterocyte brush-border enzymes. *Folia Microbiologica*, **46**, 573–576.

Kunz, C., Rudloff, S., Baier, W., *et al.* (2000) Oligosaccharides in human milk: structural, functional, and metabolic aspects. *Annual Review of Nutrition*, **20**, 699–722.

Langille, M.G., Zaneveld, J., Caporaso, J.G., *et al.* (2013) Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nature Biotechnology*, **31**, 814–821.

Larsson, E., Tremaroli, V., Lee, Y.S., *et al.* (2012) Analysis of gut microbial regulation of host gene expression along the length of the gut and regulation of gut microbial ecology through MyD88. *Gut*, **61**, 1124–1131.

Leamy, L.J., Kelly, S.A., Nietfeldt, J., *et al.* (2014) Host genetics and diet, but not immunoglobulin A expression, converge to shape compositional features of the gut microbiome in an advanced intercross population of mice. *Genome Biology*, **15**(12), 552.

Lesher, S., Walburg, H.E., Jr., and Sacher, G.A., Jr. (1964) Generation cycle in the duodenal crypt cells of germ-free and conventional mice. *Nature*, **202**, 884–886.

Ley, R.E., Backhed, F., Turnbaugh, P., *et al.* (2005) Obesity alters gut microbial ecology. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 11070–11075.

Ley, R.E., Turnbaugh, P.J., Klein, S., and Gordon, J.I. (2006) Microbial ecology: human gut microbes associated with obesity. *Nature*, **44**, 1022–1023.

Li, E., Hamm, C.M., Gulati, A.S., *et al.* (2012) Inflammatory bowel diseases phenotype, *C. difficile* and NOD2 genotype are associated with shifts in human ileum associated microbial composition. *PLoS One*, **7**(5), e26284. DOI: 10.1371/journal.pone.0026284.

Li, M., Bauer, L.L., Chen, X., *et al.* (2012) Microbial composition and *in vitro* fermentation patterns of human milk oligosaccharides and prebiotics differ between formula-fed and sow-reared piglets. *Journal of Nutrition*, **142**, 681–689.

Li, M., Monaco, M.H., Wang, M., *et al.* (2014a) Human milk oligosaccharides shorten rotavirus-induced diarrhea and modulate piglet mucosal immunity and colonic microbiota. *ISME Journal*, **8**, 1609–1620.

Li, M., Wang, M., and Donovan, S.M. (2014b) Early development of the gut microbiome and immune-mediated childhood disorders. *Seminars in Reproductive Medicine*, **32**, 74–86.

Lichtman, J.S., Marcobal, A., Sonnenburg, J.L., and Elias, J.E. (2013) Host-centric proteomics of stool: a novel strategy focused on intestinal responses to the gut microbiota. *Molecular & Cellular Proteomics*, **12**, 3310–3318.

Lindon, J.C., Holmes, E., and Nicholson, J.K. (2004) Metabonomics: systems biology in pharmaceutical research and development. *Current Opinion in Molecular Therapeutics*, **6**, 265–272.

Lozupone, C.A., Stombaugh, J.I., Gordon, J.I., *et al.* (2012) Diversity, stability and resilience of the human gut microbiota. *Nature*, **489**, 220–230.

Lyte, M. (2011) Probiotics function mechanistically as delivery vehicles for neuroactive compounds: microbial endocrinology in the design and use of probiotics. *Bioessays*, **33**, 574–581.

MacFabe, D.F., Cain, N.E., Boon, F., *et al.* (2011) Effects of the enteric bacterial metabolic product propionic acid on object-directed behavior, social behavior, cognition, and neuroinflammation in adolescent rats: Relevance to autism spectrum disorder. *Behavioural Brain Research*, **217**, 47–54.

Mack, D.R., Michail, S., Wei, S., *et al.* (1999) Probiotics inhibit enteropathogenic *E. coli* adherence *in vitro* by inducing intestinal mucin gene expression. *American Journal of Physiology*, **276**, G941–G950.

Mackie, R.I., Sghir, A., and Gaskins, H.R. (1999) Developmental microbial ecology of the neonatal gastrointestinal tract. *American Journal of Clinical Nutrition*, **69**, 1035S–1045S.

Macpherson, A.J., and Harris, N.L. (2004) Interactions between commensal intestinal bacteria and the immune system. *Nature Reviews Immunology*, **4**, 478–485.

Mah, K.W., Chin, V.I., Wong, W.S., *et al.* (2007) Effect of a milk formula containing probiotics on the fecal microbiota of Asian infants at risk of atopic diseases. *Pediatric Research*, **62**, 674–679.

Mai, V., Young, C.M., Ukhanova, M., *et al.* (2011) Fecal microbiota in premature infants prior to necrotizing enterocolitis. *PLoS One*, **6**(6), e20647. DOI: 10.1371/journal.pone.0020647.

Makino, H., Kushiro, A., Ishikawa, E., *et al.* (2011) Transmission of intestinal *Bifidobacterium longum* subsp. *longum* strains from mother to infant, determined by multilocus sequencing typing and amplified fragment length polymorphism. *Applied Environmental Microbiology*, **77**, 6788–6793.

Marcobal, A., Barboza, M., Froehlich, J.W., *et al.* (2010) Consumption of human milk oligosaccharides by gut-related microbes. *Journal of Agricultural and Food Chemistry*, **58**, 5334–5340.

Marcobal, A., Kashyap, P.C., Nelson, T.A., *et al.* (2013) A metabolomic view of how the human gut microbiota impacts the host metabolome using humanized and gnotobiotic mice. *ISME Journal*, **7**, 1933–1943.

Marcobal, A., Yusufaly, T., Higginbottom, S., *et al.* (2015) Metabolome progression during early gut microbial colonization of gnotobiotic mice. *Science Reports*, **5**, 11589. DOI: 10.1038/srep11589.

Marques, T.M., Wall, R., Ross, R.P., *et al.* (2010) Programming infant gut microbiota: influence of dietary and environmental factors. *Current Opinion in Biotechnology*, **21**, 149–156.

Martín, R., Langa, S., Reviriego, C., *et al.* (2003) Human milk is a source of lactic acid bacteria for the infant gut. *Journal of Pediatrics*, **143**, 754–758.

Martín, R., Langa, S., Reviriego, C., *et al.* (2004) The commensal microflora of human milk: new perspectives for food bacteriotherapy and probiotics. *Trends in Food Science and Technology*, **15**, 121–127.

Martín, V., Maldonado-Barragán, A., Moles, L., *et al.* (2012) Sharing of bacterial strains between breast milk and infant feces. *Journal of Human Lactation*, **28**, 36–44.

McFarland, L.V. (2014) Deciphering meta-analytic results: a mini-review of probiotics for the prevention of paediatric antibiotic-associated diarrhoea and *Clostridium difficile* infections. *Beneficial Microbes*, **2**, 1–6.

McKnite, A.M., Perez-Munoz, M.E., Lu, L., *et al.* (2012) Murine gut microbiota is defined by host genetics and modulates variation of metabolic traits. *PLoS One*, **7**(6), e39191. DOI: 10.1371/journal.pone.0039191.

Meropol, S.B. and Edwards, A. (2015) Development of the infant intestinal microbiome: a bird's eye view of a complex process. *Birth Defects Research, Part C. Embryo Today: Reviews*, **105**, 228–239. DOI: 10.1002/bdrc.21114. [Epub ahead of print]

Mestas, J. and Hughes, C.C.W. (2004) Of mice and not men: differences between mouse and human immunology. *Journal of Immunology*, **172**, 2731–2738.

Meurens, F., Summerfield, A., Nauwynck, H., *et al.* (2012) The pig: a model for human infectious diseases. *Trends in Microbiology*, **20**, 50–57.

Moreau, M.C., Ducluzeau, R., Guy-Grand, D., and Muller, M.C. (1978) Increase in the population of duodenal immunoglobulin A plasmocytes in axenic mice associated with different living or dead bacterial strains of intestinal origin. *Infection and Immunity*, **21**, 532–529.

Murphy, K., O'Shea, C.A., Ryan, C.A., *et al.* (2015) The gut microbiota composition in dichorionic triplet sets suggests a role for host genetic factors. *PLoS One*, **10**(4), e0122561. DOI: 10.1371/journal.pone.0122561.

Nagpal, R., Yadav, H., and Marotta, F. (2014) Gut microbiota: the next-gen frontier in preventive and therapeutic medicine? *Frontiers in Medicine*, **1**, 15. DOI: 10.3389/fmed.2014.00015.

Nicholson, J.K. and Lindon, J.C. (2008) Systems biology: metabonomics. *Nature*, **455**, 1054–1056.

Nguyen, T.L., Vieira-Silva, S., Liston, A., and Raes, J. (2015) How informative is the mouse for human gut microbiota research? *Disease Models & Mechanisms*, **8**, 1–16. DOI: 10.1242/dmm.017400.

Ninonuevo, M.R., Park, Y., Yin, H., *et al.* (2006) A strategy for annotating the human milk glycome. *Journal of Agricultural and Food Chemistry*, **54**, 7471–7480.

Odle, J., Lin, X., Jacobi, S.K., *et al.* (2014) The suckling piglet as an agrimedical model for the study of pediatric nutrition and metabolism. *Annual Review of Animal Biosciences*, **2**, 419–444.

O'Sullivan, A., Farver, M., and Smilowitz, J.T. (2015) The influence of early infant-feeding practices on the intestinal microbiome and body composition in infants. *Nutrition and Metabolic Insights*, **8**(Suppl. 1), 1–9. DOI: 10.4137/NMI.S29530.

Ou, J., Carbonero, F., Zoetendal, E.G., *et al.* (2013) Diet, microbiota, and microbial metabolites in colon cancer risk in rural Africans and African Americans. *American Journal of Clinical Nutrition*, **98**, 111–120.

Øyri, S.F., Műzes, G., and Sipos, F. (2015) Dysbiotic gut microbiome: a key element of Crohn's disease. *Comparative Immunology, Microbiology and Infectious Diseases*, **43**, 36–49.

Pabst, R., Russell, M.W., and Brandtzaeg, P. (2008) Tissue distribution of lymphocytes and plasma cells and the role of the gut. *Trends in Immunology*, **29**, 206–208.

Penders, J., Vink, C., Driessen, C., *et al.* (2005) Quantification of *Bifidobacterium* spp., *Escherichia coli* and *Clostridium difficile* in faecal samples of breast-fed and formula-fed infants by real-time PCR. *FEMS Microbiology Letters*, **243**, 141–147.

Penders, J., Thijs, C., Vink, C., *et al.* (2006) Factors influencing the composition of the intestinal microbiota in early infancy. *Pediatrics*, **118**, 511–521.

Penders, J., Kummeling, I., and Thijs, C. (2011) Infant antibiotic use and wheeze and asthma risk: a systematic review and meta-analysis. *European Respiratory Journal*, **38**, 295–302.

Pérez-Losada, M., Castro-Nallar, E., Bendall, M.L., *et al.* (2015) Dual transcriptomic profiling of host and microbiota during health and disease in pediatric asthma. *PLoS One*, **10**(6), e0131819.

Peterson, L.W. and Artis, D. (2014) Intestinal epithelial cells: regulators of barrier function and immune homeostasis. *Nature Reviews Immunology*, **14**, 141–153.

Qin, J., Li, R., Raes, J., *et al.*(2010) A human gut microbial gene catalogue established by metagenomic sequencing. *Nature*, **464**, 59–65.

Rautava, S., Luoto, R., Salminen, S., and Isolauri, E. (2012a) Microbial contact during pregnancy, intestinal colonization and human disease. *Nature Reviews Gastroenterology & Hepatology*, **9**, 565–576.

Rautava, S., Collado, M.C., Salminen, S., and Isolauri, E. (2012b) Probiotics modulate host–microbe interaction in the placenta and fetal gut: a randomized, double-blind, placebo-controlled trial. *Neonatology*, **102**, 178–184.

Ridaura, V.K., Faith, J.J., Rey, F.E., *et al.* (2013) Gut microbiota from twins discordant for obesity modulate metabolism in mice. *Science*, **341**(6150), 1241214. DOI: 10.1126/science.1241214.

Risnes, K.R., Belanger, K., Murk, W., and Bracken, M.B. (2011) Antibiotic exposure by 6 months and asthma and allergy at 6 years: findings in a cohort of 1401 US children. *American Journal of Epidemiology*, **173**, 310–318.

Roberfroid, M., Gibson, G.R., Hoyles, L, *et al.* (2010) Prebiotic effects: metabolic and health benefits. *British Journal of Nutrition*, **104**(Suppl. 2), S1–S63.

Round, J.L. and Mazmanian, S.K. (2009) The gut microbiota shapes intestinal immune responses during health and disease. *Nature Reviews Immunology*, **9**, 313–323.

Round, J.L. and Mazmanian, S.K. (2010) Inducible Foxp3$^+$ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 12204–12209.

Salvini, F., Riva, E., Salvatici, E., *et al.* (2011) A specific prebiotic mixture added to starting infant formula has long-lasting bifidogenic effects. *Journal of Nutrition*, **141**, 1335–1395.

Samuel, B.S., Shaito, A., Motoike, T., *et al.* (2008) Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, Gpr41. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 16767–16772.

Satokari, R. (2015) Contentious host–microbiota relationship in inflammatory bowel disease – can foes become friends again? *Scandinavian Journal of Gastroenterology*, **50**, 34–42.

Scalabrin, D.M., Mitmesser, S.H., Welling, G.W., *et al.* (2012) New prebiotic blend of polydextrose and galacto-oligosaccharides has a bifidogenic effect in young infants. *Journal of Pediatric Gastroenterology and Nutrition*, **54**, 343–352.

Scheepers, L.E., Penders, J., Mbakwa, C.A., *et al.* (2015) The intestinal microbiota composition and weight development in children: the KOALA Birth Cohort Study. *International Journal of Obesity*, **39**, 16–25.

Schwartz, S., Friedberg, I., Ivanov, I.V., *et al.* (2012) A metagenomic study of diet-dependent interaction between gut microbiota and host in infants reveals differences in immune response. *Genome Biology*, **13**(4), r32. DOI: 10.1186/gb-2012-13-4-r32.

Seok, J., Warren, H.S., Cuenca, A.G., *et al.* (2013) Genomic responses in mouse models poorly mimic human inflammatory diseases. *Proceedings of the National Academy of Sciences of the United States of America*, **110**, 3507–3512.

Shay, T., Jojic, V., Zuk, O., *et al.* (2013) Conservation and divergence in the transcriptional programs of the human and mouse immune systems. *Proceedings of the National Academy of Sciences of the United States of America*, **110**, 2946–2951.

Shima, T., Fukushima, K., Setoyama, H., *et al.* (2008) Differential effects of two probiotic strains with different bacteriological properties on intestinal gene expression, with special reference to indigenous bacteria. *FEMS Immunology and Medical Microbiology*, **52**, 69–77.

Shirkey, T.W., Siggers, R.H., Goldade, B.G., *et al.* (2006) Effects of commensal bacteria on intestinal morphology and expression of proinflammatory cytokines in the gnotobiotic pig. *Experimental Biology and Medicine*, **231**, 1333–1345.

Shoaie, S., Ghaffari, P., Kovatcheva-Datchary, P., *et al.* (2015) Quantifying diet-induced metabolic changes of the human gut microbiome. *Cell Metabolism*, **22**, 320–331.

Smith, K., McCoy, K.D., and Macpherson, A.J. (2007) Use of axenic animals in studying the adaptation of mammals to their commensal intestinal microbiota. *Seminars in Immunology*, **19**, 59–69.

Smith, S.B. and Crouse, J.D. (1984) Relative contributions of acetate, lactate and glucose to lipogenesis in bovine intramuscular and subcutaneous adipose tissue. *Journal of Nutrition*, **114**, 792–800.

Sommer, F., Nookaew, I., Sommer, N., *et al.* (2015) Site-specific programming of the host epithelial transcriptome by the gut microbiota. *Genome Biology*, **16**, 62.

Sudo, N., Chida, Y., Aiba, Y., *et al.* (2004) Postnatal microbial colonization programs the hypothalamic–pituitary–adrenal system for stress response in mice. *Journal of Physiology*, **558**, 263–275.

Szentkuti, L., Riedesel, H., Enss, M.L., *et al.* (1990) Pre-epithelial mucus layer in the colon of conventional and germ-free rats. *Histochemical Journal*, **22**, 491–497.

Szymański, H., Pejcz, J., Jawień, M., *et al.* (2006) Treatment of acute infectious diarrhoea in infants and children with a mixture of three *Lactobacillus rhamnosus* strains – a randomized, double-blind, placebo-controlled trial. *Alimentary Pharmacology & Therapeutics*, **23**, 247–253.

Taché, Y. and Bernstein, C.N. (2009) Evidence for the role of the brain–gut axis in inflammatory bowel disease: depression as cause and effect? *Gastroenterology*, **136**, 2058–2061.

Taché, Y. and Perdue, M.H. (2004) Role of peripheral CRF signalling pathways in stress-related alterations of gut motility and mucosal function. *Neurogastroenterology and Motility*, **16**(Suppl. 1), 137–142.

Takahashi, I. and Kiyono, H. (1999) Gut as the largest immunologic tissue. *Journal of Parenteral and Enteral Nutrition*, **23**(Suppl. 5), S7–S12.

Tanaka, S., Kobayashi, T., Songjinda, P., *et al.* (2009) Influence of antibiotic exposure in the early postnatal period on the development of intestinal microbiota. *FEMS Immunology and Medical Microbiology*, **56**, 80–87.

Thum, C., Cookson, A.L., Otter, D.E., *et al.* (2012) Can nutritional modulation of maternal intestinal microbiota influence the development of the infant gastrointestinal tract? *Journal of Nutrition*, **142**, 1921–1928.

Tong, M., McHardy, I., Ruegger, P., *et al.* (2014) Reprograming of gut microbiome energy metabolism by the FUT2 Crohn's disease risk polymorphism. *ISME Journal*, **8**, 2193–2206.

Trygg, J. and Wold, S. (2003) O2-PLS, a two-block (X–Y) latent variable regression (LVR) method with an integral OSC filter. *Journal of Chemometrics*, **17**, 53–64.

Turnbaugh, P.J., Ley, R.E., Mahowald, M.A., *et al.* (2006) An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature*, **444**, 1027–1031.

Umesaki, Y. (2014) Use of gnotobiotic mice to identify and characterize key microbes responsible for the development of the intestinal immune system. *Proceedings of the Japan Academy. Series B, Physical and Biological Sciences*, **90**, 313–332.

Ursell, L.K., Haiser, H.J., Van Treuren, W., *et al.* (2014) The intestinal metabolome: an intersection between microbiota and host. *Gastroenterology*, **146**, 1470–1476.

Vael, C., Vanheirstraeten, L., Desager, K.N., and Goossens, H. (2011) Denaturing gradient gel electrophoresis of neonatal intestinal microbiota in relation to the development of asthma. *BMC Microbiology*, **11**, 68. DOI: 10.1186/1471-2180-11-68.

Vester Boler, B.M., Rossoni Serao, M.C., Faber, T.A., *et al.* (2013) *In vitro* fermentation characteristics of select nondigestible oligosaccharides by infant fecal inocula. *Journal of Agricultural and Food Chemistry*, **61**, 2109–2119.

Voreades, N., Kozil, A., and Weir, T.L. (2014) Diet and the development of the human intestinal microbiome. *Frontiers in Microbiology*, **5**, 494. DOI: 10.3389/fmicb.2014.00494.

Wacklin, P., Mäkivuokko, H., Alakulppi, N., *et al.* (2011) Secretor genotype (FUT2 gene) is strongly associated with the composition of *Bifidobacteria* in the human intestine. *PLoS One*, **6**(5), e20113. DOI: 10.1371/journal.pone.0020113.

Wang, L., Christophersen, C.T., Sorich, M.J., *et al.* (2013) Increased abundance of *Sutterella* spp. and *Ruminococcus torques* in feces of children with autism spectrum disorder. *Molecular Autism*, **4**(1), 42. DOI: 10.1186/2040-2392-4-42.

Wang, M. and Donovan, S.M. (2015) Human microbiota-associated swine: current progress and future opportunities. *ILAR Journal*, **56**, 63–73.

Wang, M., Karlsson, C., Olsson, C., *et al.* (2008) Reduced diversity in the early fecal microbiota of infants with atopic eczema. *Journal of Allergy and Clinical Immunology*, **121**, 129–134.

Wang, M., Li, M., Wu, S., *et al.* (2015) Fecal microbiota composition of breast-fed infants is correlated with human milk oligosaccharides consumed. *Journal of Pediatric Gastroenterology and Nutrition*, **60**, 825–833.

Wang, M., Monaco, M.H., and Donovan, S.M. (2016) Impact of early gut microbiota on immune and metabolic development and function. *Seminars in Fetal & Neonatal Medicine*, in press.

Wang, Y. and Kasper, L.H. (2014) The role of microbiome in central nervous system disorders. *Brain, Behavior, and Immunity*, **38**, 1–12. DOI: 10.1016/j.bbi.2013.12.015.

White, R.A., Bjørnholt, J.V., Baird, D.D., *et al.* (2013) Novel developmental analyses identify longitudinal patterns of early gut microbiota that affect infant growth. *PLoS Computational Biology*, **9**(5), e1003042. DOI: 10.1371/journal.pcbi.1003042.

Wikoff, W.R., Anfora, A.T., Liu, J., *et al.* (2009) Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 3698–3703.

Willing, B.P. and Van Kessel, A.G. (2007) Enterocyte proliferation and apoptosis in the caudal small intestine is influenced by the composition of colonizing commensal bacteria in the neonatal gnotobiotic pig. *Journal of Animal Science*, **85**, 3256–3266.

Willing, B.P. and Van Kessel, A.G. (2009) Intestinal microbiota differentially affect brush border enzyme activity and gene expression in the neonatal gnotobiotic pig. *Journal of Animal Physiology and Animal Nutrition*, **93**, 586–595.

Wong, J.M., de Souza, R., Kendall, C.W., *et al.* (2006) Colonic health: fermentation and short chain fatty acids. *Journal of Clinical Gastroenterology*, **40**, 235–243.

Wu, G.D., Chen, J., Hoffmann, C., *et al.* (2011) Linking long-term dietary patterns with gut microbial enterotypes. *Science*, **334**, 105–108.

Wu, H., Tremaroli, V., and Bäckhed, F. (2015) Linking microbiota to human diseases: a systems biology perspective. *Trends in Endocrinology and Metabolism*, **26**, 758–770.

Yallapragada, S.G., Nash, C.B., and Robinson, D.T. (2015) Early-life exposure to antibiotics, alterations in the intestinal microbiome, and risk of metabolic disease in children and adults. *Pediatric Annals*, **44**, e265–e269. DOI: 10.3928/00904481-20151112-09.

Yatsunenko, T., Rey, F.E., Manary, M.J., *et al.* (2012) Human gut microbiome viewed across age and geography. *Nature*, **486**, 222–227.

Yurist-Doutsch, S., Arrieta, M.C., Vogt, S.L., and Finlay, B.B. (2014) Gastrointestinal microbiota-mediated control of enteric pathogens. *Annual Review of Genetics*, **48**, 361–382.

Zhao, C., Ivanov, I., Dougherty, E.R., *et al.* (2009) Noninvasive detection of candidate molecular biomarkers in subjects with a history of insulin resistance and colorectal adenomas. *Cancer Prevention Research*, **2**, 590–597.

Zijlmans, M.A., Korpela, K., Riksen-Walraven, J.M., *et al.* (2015) Maternal prenatal stress is associated with the infant intestinal microbiota. *Psychoneuroendocrinology*, **53**, 233–245.

Zocco, M.A., Ainora, M.E., Gasbarrini, G., and Gasbarrini, A. (2007) *Bacteroides thetaiotaomicron* in the gut: molecular aspects of their interaction. *Digestive and Liver Disease*, **39**, 707–712.