# The Early Settlers: Intestinal Microbiology in Early Life

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## Abstract

The human intestinal microbiota forms an integral part of normal human physiology, and disturbances of the normal gut microbiology have been implicated in many health and disease issues. Because newborns are essentially sterile, their microbiota must establish and develop from the very first days of life. The first colonizers play an important role in the development of the ecosystem and may impact the long-term composition and activity of the microbiota. These first settlers obviously develop and proliferate dependent on host characteristics and diet, but other factors can also significantly contribute to this vital biological process. Considering the importance of the microbiota for the human immune, metabolic, and neurological systems, it is important to understand the dynamics and driving determinants of this development. This review gives a global overview of our current understanding of the different factors impacting the intestinal microbiology in early life.

## HUMAN INTESTINAL MICROBIOTA: A SYMBIOSIS SHAPED BY EVOLUTION

**Microbiota:** a collection of a variety of microorganisms, commonly referred to according to the habitat that it occupies (e.g., the gut microbiota or vaginal microbiota)

**Xenobiotics:** chemical compounds that are foreign to a living organism and that can be toxic, even at low concentrations

The human intestinal tract harbors a complex microbial ecosystem consisting of an amazing 100 trillion bacteria. This microbiota represents 10 times more bacterial cells than the number of cells in the human body (Savage 1977) and can make up as much as 50% of fecal matter. The importance of microbiota for human health has been known since the late nineteenth century when Metchnikoff developed the theory that senility is due to poisoning of the body by the products of certain gut bacteria and that rebalancing the microbiota with lactic acid bacteria would be beneficial in this respect (Metchnikoff 1907). Knowledge of the composition of the intestinal microbiota has taken huge steps since the application of molecular techniques that complement traditional culturing methods, which are slow and biased. These new methods have revealed that the intestinal microbiota consists of many more types of bacteria than originally thought (more than 1,000 species) (Zoetendal et al. 2008). The most recent development is the use of high-throughput sequencing technologies to determine genome characteristics of the types of microorganisms present and their potential functional capacities (Eckburg et al. 2005, Kurokawa et al. 2007, Qin et al. 2010, Turnbaugh et al. 2007). In healthy individuals, the normal dominant microbiota, or commensal microbiota, is relatively stable and engage in a symbiotic association with the host, meaning that the host is able to determine the composition and development of the intestinal microbiota and that the microbiota can impact certain developments or functions of the host.

The commensal microbiota is able to use components from exogenous origin (e.g., from the diet) or endogenous origin (e.g., intestinal mucus) to sustain growth in this competitive environment. The metabolism and fermentation of these luminal components result in the formation of a variety of metabolites, including, for example, short chain fatty acids (SCFAs), vitamins, and several gasses (Cummings & Macfarlane 1991). SCFAs can be absorbed in the colon, resulting in the salvage of energy that would otherwise be lost and excreted in feces. It is estimated that 2 kcal of energy is retrieved per gram of fiber, making the microbiota an important metabolic organ (Livesey 1990). The types of metabolites that are formed are dependent on the types of bacteria and substrates that are present, with some bacteria thriving very well on a specific substrate and others failing to use that specific substrate (Cummings & Macfarlane 1991). This selective usage enables modulation of the composition of the intestinal microbiota in response to dietary habits during infancy. A number of potentially beneficial bacteria in the gut, including bifidobacteria and lactobacilli, have been linked to health benefits, such as the development and function of the immune system (Duarte et al. 2004, Souza et al. 2004), the resistance to infections by preventing colonization by pathogens or excessive development of pathogens (van der Waaij et al. 1971, Wells et al. 1988), detoxification of xenobiotic compounds, and (bio)activation of beneficial constituents such as polyphenols (Miene et al. 2011, Fekadu et al. 1994).

The human host-microbe symbiosis is initiated in early life, and its establishment is an intriguing biological process. It appears that mammals have coevolved with their intimate friends, and it is likely that the inoculation and colonization leading to the microbiota that we find in adults are not solely a process of chance. At the higher taxonomic level, the microbiota are very simple, containing two dominant phyla, the Firmicutes and Bacteroidetes. Some species seem to find their natural niche only in the gastrointestinal (GI) tract of mammals (e.g., *Bifidobacterium* and *Akkermansia* spp.) (van Passel et al. 2011, Ventura et al. 2007). It makes sense that in evolution, mechanisms have evolved that direct the development of the gut microbiota in a very specific way, with an important role of the parental microbiota in transferring to the offspring.

Infants are born with an essentially sterile gut, and colonization starts immediately during and after delivery (**Figure 1**). The initial contacts with the extrauterine environment, when the infant

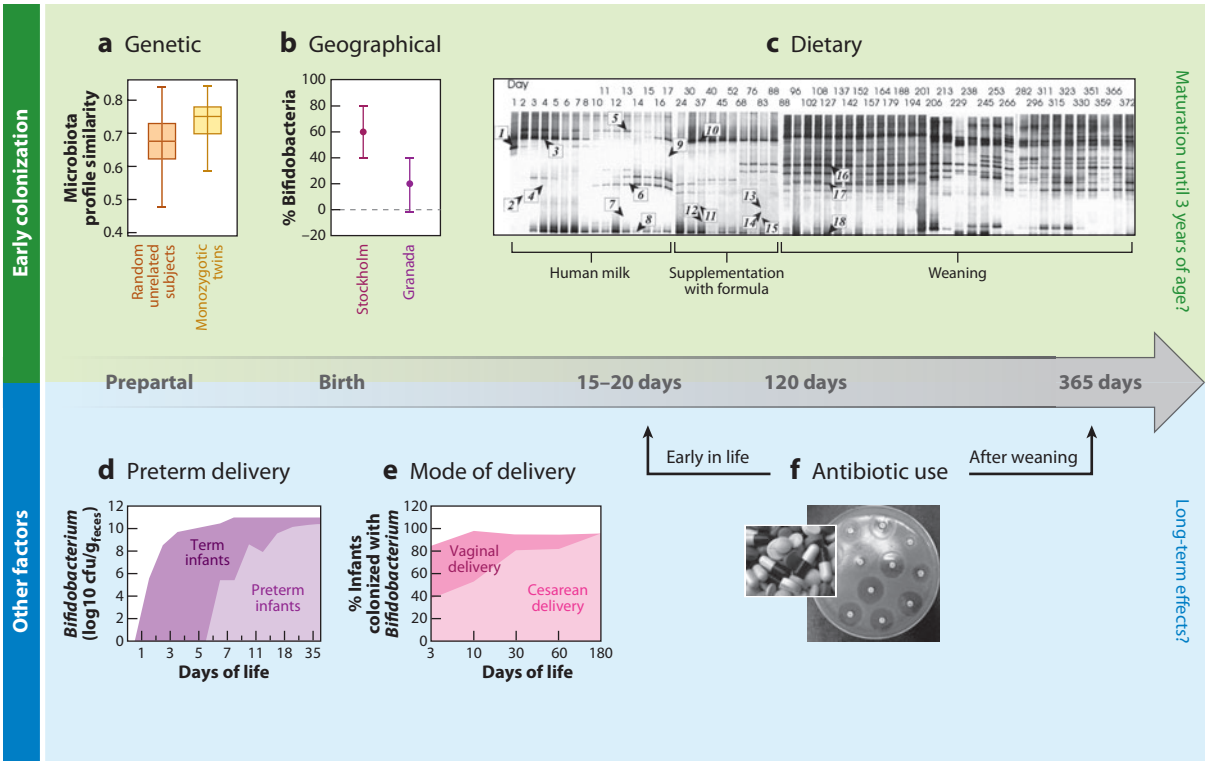


**Figure 1**

Factors affecting the development of the intestinal microbiota in early life. (*a*) The genetic background determines the composition of the microbiota (Zoetendal et al. 2001). (*b*) Bifidobacteria are more dominant in northern countries (Fallani et al. 2010). (*c*) Dietary changes affect diversity in the gut microbiota of a newborn during the first year of life. Reproduced with permission from the American Society for Microbiology (Favier et al. 2002). (*d*) Preterm versus term delivery can lead to lower diversity and delayed colonization, especially by bifidobacteria, and often colonization by pathogens (Butel et al. 2007). (*e*) Cesarean versus vaginal delivery can lead to lower diversity and delayed colonization, especially by bifidobacteria (Huurre et al. 2008). (*f*) Use of antibiotics is linked to disrupted colonization and pathogen overgrowth (Sullivan et al. 2001).

is for the first time exposed to high levels of live microorganisms, facilitate the inoculation of infants, including that of the GI tract. During the first years of life, their intestinal microbiota is still relatively dynamic. Its development depends mainly on the diet, which can change quite significantly during this time period from human milk to a complex adult diet. The initial inoculation and subsequent development of the intestinal microbiota in early life is clearly a key step because these initial processes will most probably impact the composition of the human microbiota throughout life. Knowing that the microbiota can significantly interfere with the human metabolic, cognitive, and immune systems, the initiation of the symbiosis seems a crucial step for preparing optimal health later in life. Consequently, understanding the early interaction between the intestinal microbiota and the human body opens new avenues for important nutritional innovations, particularly for infants and young children. In contrast to our genetic heritage, our intestinal microbiota may be readily modified in the first two years of life and possibly throughout pregnancy via the maternal diet. Thus, by shaping the intestinal microbiota during this so-called permissive period, one can expect to interfere with the immune maturation as well as with the metabolic programming of individuals. It has been postulated that our genetic heritage influences

**Gut/immune maturation:** certain aspects of the adaptive and innate immune system that are not fully functional at birth but develop thereafter

our susceptibility to obesity at a level of 30%, and environmental factors, such as dietary intake in early life, may have a more predominant effect (Lanigan et al. 2010). This review provides an overview of the development of the intestinal microbiota in infants, its importance for infant health, and its possible role in impacting health later in life.

## DEVELOPMENT OF THE INTESTINAL MICROBIOTA FROM BIRTH

### The Prenatal Period

It has been shown that the maternal microbiota can directly affect the immune (Donnet-Hughes et al. 2010) and/or metabolic system of neonates (Collado et al. 2010, Luoto et al. 2010). Therefore, there is a major interest in characterizing the composition of the microbiota during pregnancy and the contribution of maternal microbiota to the development of the newborn's microbiota. The GI tract of the newborn infant is thought to be sterile, although a study performed by DiGiulio demonstrated the presence of bacteria in amniotic fluid of preterm infants, suggesting that the newborn gut may not be sterile at birth (DiGiulio et al. 2008). During and shortly after birth, infants are exposed mainly to microbes that originate from the mother; however, the role of the vaginal microbes in the infant gut colonization is debatable. It has been shown that there is a close relationship between the vaginal microbiota of the mother and that of the external ear canal of the newborn (Fanaro et al. 2003, Mandar & Mikelsaar 1996), but it seems that vaginal microbes from the mother usually do not settle in the intestinal tract of the infant. A molecular epidemiological study on the transmission of vaginal *Lactobacillus* species from mother to the newborn infant showed that only less than one fourth of the infants acquired maternal vaginal lactobacilli at birth, and that one month later, these had been replaced by lactobacilli associated with human milk (Matsumiya et al. 2002). However, the intestinal microbiota of the mother is an important microbial source for the newborn (Fanaro et al. 2003). Studies focusing on probiotic supplementation in pregnant women suggest a strong link between maternal microbiota and infant colonization (Schultz et al. 2004) (Gueimonde et al. 2006), whereas there is only one known study that focused on the effect of prebiotic supplementation on initial colonization of the newborn infant. In this study, a significant increase in the numbers of bifidobacteria was shown in the mother, but no direct effect was observed on the child's intestinal microbiota (Shadid et al. 2007).

### Pre-Weaning Period: 0–4 Months

The sequential process of colonization has been reviewed before and indicates that in general the first settlers that initially colonize infants are facultative anaerobic bacteria (for example *E. coli* and *Streptococci* sp.) often reaching $10^8$–$10^{10}$ cfu $g^{-1}$ feces from 24 to 48 hours after birth (Fanaro et al. 2003, Mackie et al. 1999). Thereafter, colonization by *Staphylococcus*-, *Enterococcus*-, and *Lactobacillus*-like species takes place, and these contribute to generating an anaerobic environment by consuming the oxygen and thus providing a favorable condition for more anaerobic bacteria (Orrhage & Nord 1999). After one week of life, *Bifidobacterium*, *Bacteroides*, and *Clostridium* are detected in the feces and at this time in human milk–fed infants, *Bifidobacterium* species become dominant, usually concomitantly with a decrease in *Enterobacteriaceae* (Yoshioka et al. 1983). The major problem faced by gut microbiologists is the inevitable bias introduced by culture-based enumeration, in particular for anaerobic bacteria because of their vulnerability to oxygen but also considering the fact that many microbes in the gut appear unculturable for the time being. The limitations of different methods, including newly developed molecular tools, must be taken into consideration as controversial results have been reported (Fanaro et al. 2003). For instance, Palmer et al. (2007) failed to demonstrate a predominance in bifidobacteria early in life using

culture independent techniques. The authors, however, indicated that inadequate cell lysis of gram-positive bacteria may have prevented efficient extraction of DNA, causing disturbed results (Salonen et al. 2010). It is now generally accepted that bifidobacteria are usually highly abundant in human milk–fed infants and that studies that deviate from this suffer from methodological or sampling errors (Sela 2011). Culture-independent techniques have also given many new insights into the early colonization dynamics. For example, Favier et al. (2002) described the presence of other bacterial species, such as clostridia and *Ruminococcus*, as important in the early colonization process. More recently, the presence of ruminococci in the feces of human milk–fed infants was confirmed by a study by Coppa et al. (2011) in which these species were observed in 25 of 39 studied infants. Ruminococci have been linked to the production of ruminococcin A, which can inhibit the growth of *Clostridium* species, and therefore it has been hypothesized that the presence of ruminococci in human milk–fed infants inhibits the colonization of certain species of clostridia (Morelli 2008).

**Human milk as a driver for early gut colonization.** Human milk is normally the first dietary exposure in infancy, and it is considered the best nutrition for growth and healthy development of the newborn. Human milk contains a wide range of health-promoting constituents, including but not limited to carbohydrates, nondigestible oligosaccharides, nucleotides, fatty acids, immunoglobulins, cytokines, immune cells, lysozyme, lactoferrin, and other immune-modulatory factors (Boehm & Moro 2008, Goldman & Smith 1973, Walker 2010). Human milk oligosaccharides (HMOs) are nondigestible carbohydrates that are fermented in the colon, stimulating the growth of specific fecal bacteria (including bifidobacteria) in infants receiving human milk (Thurl et al. 2011). This prebiotic effect can be considered as a major health benefit because it shapes the microbiota that is believed to stimulate the (developing) immune and metabolic system. The fermentation of HMOs does not only lead to the presence and absence of specific microorganisms but also to specific metabolic profiles, such as the use of HMOs by bifidobacteria and *Bacteroides* (Sela & Mills 2010, Marcobal et al. 2011). In general, the metabolic profile of infants receiving human milk is characterized by relatively higher proportions of acetate and lower proportions of propionate and almost complete absence of butyrate, compared with adults (Knol et al. 2005b). Butyrate is considered a beneficial metabolite in adult gastrointestinal health, and increased butyrate levels have been linked to several health benefits. However, low levels of butyrate in infant feces fit with the microbial ecosystem established (dominance of bifidobacteria and lactic acid bacteria that do not produce butyrate) and are consistently found in human milk–fed infants. Also, the conversion of acetate and lactate into butyrate (Morrison et al. 2006) seems not yet developed in young infants. It can be suggested that butyric acid is not yet that important in infants and that infant enterocytes use an alternative substrate (Parrett & Edwards 1997). Interestingly, new techniques, such as mixed-species genomic microarray analysis of fecal samples, have revealed that the genome of bifidobacteria includes multiple types of genes, such as genes coding for carbohydrate metabolism, but also genes coding for other activities, such as genes involved in folate biosynthesis (Klaassens et al. 2009). In addition to being a source of prebiotic oligosaccharides, human milk has also been shown to be a continuous source of live bacteria. This includes staphylococci, streptococci, bifidobacteria, and lactic acid bacteria (Collado et al. 2009, Martin et al. 2009, Martin et al. 2003, Perez et al. 2007). It has been estimated that an infant consuming approximately 800 ml of human milk per day will ingest approximately $1 \times 10^5$–$1 \times 10^7$ bacteria (Heikkila & Saris 2003, Martin et al. 2003). However, the origin of the bacteria present in human milk remains controversial. It seems more generally accepted that the infant acquires the mother's fecal microbiota during delivery, transfers these bacteria to the breast skin and the nipple and from there to the milk ducts while breastfeeding. Although skin contamination

**Probiotics:** live microorganisms that, when consumed in adequate amounts, confer a health benefit on the host

is almost unavoidable, several studies have shown that the bacteria present in the breast skin differ from that of human milk (Martin et al. 2009, Martin et al. 2003). It has been suggested that at least some of the bacteria present in the maternal gut can reach the mammary gland through an endogenous route, the so-called enteromammary pathway (Martin et al. 2004). This hypothesis has been substantiated by Perez and colleagues (Perez et al. 2007). These authors showed that fresh human milk contains a number ($<3$ log cfu $ml^{-1}$) of viable bacteria and a wide range of free bacterial DNA signatures, including bifidobacterial DNA, which may program the neonatal immune system to accept or reject colonizing microbes after birth. The translocation of bacteria from the gut to the mammary gland via mesenteric lymph nodes has been confirmed in animal models (Fernandez et al. 2004, Perez et al. 2007).

**Influence of pre- and probiotics in early colonization.** As described above, the composition of the intestinal microbiota can be modulated as a result of dietary exposure as well as by intentional diet interventions with specific components. The main strategies to modulate the gut microbiota of infants have been the administration of specific growth substrates or live bacteria directly (prebiotics or probiotics if health benefits have been demonstrated). Combinations of these two concepts (synbiotics) have been used as well as interventions based on products made by or involving bacteria but only containing metabolites or nonviable microorganisms (sometimes referred to as postbiotics). Prebiotic oligosaccharides are defined as nondigestible carbohydrates that reach the colon intact and are known for their ability to selectively stimulate the growth and/or activity of bacteria that impact health positively (Gibson & Roberfroid 1995). Nondigestible carbohydrates or HMOs, representing 15% to 20% of the total amount of carbohydrates in human milk, are thought to exert this function in human milk. Bovine milk, the most common base for infant formulas, contains insignificant levels of these complex carbohydrates, and this partly explains the differences observed between human milk–fed babies and formula-fed babies. Consequently, a significant number of studies have been performed with different types of prebiotic oligosaccharides, usually obtained from plant extracts or lactose, and supplemented in infant formulas that are based on bovine milk. The use of prebiotics in infant formulas has been recently reviewed (Rao et al. 2009). One specific mixture of short chain galacto-oligosaccharides (scGOS) and long chain fructo-oligosaccharides (lcFOS) in a 9 to 1 ratio is specifically relevant in this context given that it has been extensively studied. These specific prebiotics have been shown to selectively stimulate the growth of bifidobacteria and lactobacilli as well as generating metabolic profiles (specifically SCFA and pH profiles) closer to profiles observed in human milk–fed infants (Haarman & Knol 2005, 2006; Knol et al. 2005b; Scholtens et al. 2008). Several studies have demonstrated beneficial effects on the composition of the intestinal microbiota, including lower levels of potentially pathogenic bacteria (Knol et al. 2005a). Interestingly, infant milk formulas with scGOS/lcFOS reduced the risk of atopic dermatitis in infants with a familiar history of atopy (Arslanoglu et al. 2008) and reduced the number of infectious episodes in healthy term infants (Bruzzese et al. 2009) or infants with a high risk of developing allergy (Arslanoglu et al. 2008), also underlining the importance of the gut microbiota in infant health.

An alternative approach that has received considerable attention is the concept of oral administration of viable bacteria (when a health effect is demonstrated, it is referred to as probiotics) (Parracho et al. 2007). Recently, the committee on nutrition of the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition has reviewed the published evidence related to safety and health effects of the administration of formulas supplemented with probiotics. In the systematic review, 20 randomized controlled trials carried out in healthy infants were included, and another 13 trials were excluded (Braegger et al. 2011). Many studies have been carried out using infant formulas or follow-on formulas supplemented with probiotics in the past years. The

available studies varied in methodological quality, the specific probiotics studied, the durations of the interventions, and the doses used. The most commonly studied probiotic was *Bifidobacterium animalis* ssp. *lactis* CNCMI-3446 (previously known as *Bifidobacterium lactis* Bb12), administered either alone or in combination with *Streptococcus thermophilus* or with *S. thermophilus* and *Lactobacillus helveticus* (13 trials). Other probiotics used were *Lactobacillus acidophilus johnsonii La1* (2 trials), *Bifidobacterium longum BL999* (also known as BB536) in combination with *Lactobacillus rhamnosus LPR* (1 trial), *L. rhamnosus GG* (LGG) alone or in combination with *B. longum BL999* (2 trials), *Lactobacillus reuteri ATCC 55730* (2 trials), and *Lactobacillus salivarius CECT5713* (1 trial). The committee concluded that, for healthy infants, the available scientific data suggest that the administration of currently evaluated probiotic-supplemented formula to healthy infants does not raise safety concerns with regard to growth and adverse effects.

The administration of a few probiotics (single or in combination) supplemented to infant or follow-on formulas and given beyond early infancy may be associated with some clinical benefits, such as a reduction in the risk of nonspecific GI infections, a reduced risk of antibiotic use, and a lower frequency of colic and/or irritability (Braegger et al. 2011). The effects of live bacteria appear to be very much strain dependent and condition specific and therefore need further exploration. Lately, combinations of prebiotics and probiotics (synbiotics) or (fermentation) products from microorganisms (postbiotics) are also gaining further interest as ways to beneficially modify the composition and activity of the gut microbiota in infants.

## Weaning

After four to six months of only receiving human milk or infant milk formulas, many infants in industrialized countries are gradually introduced to solid weaning foods. This starts with foods such as cereals, fruits, and vegetables and introduces new types of nondigestible carbohydrates (NDCs) that are specific to these types of foods (Edwards & Parrett 2002). Although human milk and infant milk formulas mainly consist of soluble NDCs, solid weaning foods also contain insoluble NDCs (Edwards & Parrett 2002). Colonic fermentation has been shown to be dependent on both the presence of specific bacteria and the delivery of potential substrates for fermentation (Macfarlane & Macfarlane 1997). It has been suggested that infants partly develop the ability to ferment NDCs other than prebiotic oligosaccharides during the weaning period (Midtvedt & Midtvedt 1992, Parrett et al. 1997). To be able to do this, different bacteria work closely together to get the highest yield from the substrates provided; for example, primary degraders are able to break down plant cell walls to reveal other nondigestible fractions for secondary degraders (Flint et al. 2008). The introduction of solid weaning foods with new NDCs that have not been part of the diet before is a dietary factor that can induce a major change in the composition of the intestinal microbiota. One of the first studies that aimed to observe the changes in the intestinal microbiota during weaning was performed by Stark & Lee (1982). In this study, 14 infants were followed during the first year of life, and solid weaning foods were introduced between the 10th and the 28th week of life (Stark & Lee 1982). It was shown that counts of *Bacteroides*, *Clostridium*, and anaerobic streptococci increased after the introduction of solid weaning foods in human milk–fed infants, whereas facultative anaerobes, such as bifidobacteria, remained high, and colonization with anaerobes other than bifidobacteria continued (Stark & Lee 1982). Similar results were observed in other observational studies, such as in a study by Amarri et al. (2006) in which the effects of introduction of solid weaning foods to four-month-old human milk–fed infants until the age of nine months were evaluated (Amarri et al. 2006). In their study, stable counts of bifidobacteria and lactobacilli, and increases in enterobacteria and enterococci throughout the first five months of complementary feeding were observed. In a study performed by Roger et al. (2010) it was shown

that human milk–fed infants harbored a more diverse bifidobacteria population when compared with formula-fed infants. In addition, they showed that the diversity of *Bifidobacterium* species increased with the start of weaning, particularly in the human milk–fed group.

The introduction of solid foods also correlates with enrichment in functional genes characteristic of the adult gut microbiome, including enzymes involved in the degradation of xenobiotic compounds and in vitamin biosynthesis. By the age of one year, the infants conserved a unique microbiota in terms of composition but had converged toward a profile characteristic of the adult GI tract regarding functionalities (Koenig et al. 2010). However, some studies claim that stabilization occurs later during the second year of life, based on measurements of functional traits related to bacterial metabolism. Such is true for SCFAs, butyrate taking 12 months to reach adult levels, mucin degradation taking 12 months, and bilirubin-to-urobilinogen conversion taking 24 months. Some bacterial activities may take longer to develop or stabilize, probably also depending on the further diversification of the diet. The conversion of cholesterol to coprostanol appears around six months of age and is still below half of adult specific activity at 15 months (Norin et al. 1985). Conversely, it has been shown that dietary intervention can modulate the composition of the intestinal microbiota during weaning. In a study by Scholtens et al. (2006), the effect of solid weaning foods with added scGOS/lcFOS on the composition of the intestinal microbiota in formula-fed infants during weaning was studied. It was shown that the percentage of fecal bifidobacteria significantly increased during the intervention period and that the percentage of bifidobacteria was significantly higher in the group of infants that received solid weaning foods with scGOS/lcFOS when compared with infants that received control weaning food without scGOS/lcFOS (Scholtens et al. 2006).

## OTHER FACTORS AFFECTING EARLY COLONIZATION

### Effect of Delivery Mode on Colonization

In addition to other factors that shape the early microbial colonization, the mode of delivery plays a key role in the establishment of the gut microbiota. It is well known that the maternal vaginal and perineal microbiota are the main contributors to the colonization of vaginally delivered neonates, whereas the skin and nosocomial environment play a significant role in Caesarian section (CS)-born infants. A recent study comparing vaginally and CS-born babies showed that vaginally delivered infants acquired bacterial communities resembling their own mother's vaginal microbiota, dominated by *Lactobacillus* spp., *Prevotella* spp., and *Sneathia* spp., whereas infants delivered by CS had bacterial communities that were more similar to that of the mother's skin and were dominated by *Staphylococcus* spp., *Corynebacterium* spp., and *Propionibacterium* spp. (Dominguez-Bello et al. 2010). Other authors have shown that infants delivered by CS are colonized later and less frequently by bifidobacteria (Adlerberth et al. 2007, Biasucci et al. 2008, Gronlund et al. 1999, Huurre et al. 2008, Penders et al. 2006). The delay in the bifidobacterial colonization has been shown to sustain until one month of age, whereas in vaginally delivered infants it occurs at 10 days (Gronlund et al. 1999). Chen and coworkers (2007) suggest that the differences could be due not only to the CS itself, but also to the prophylactic use of antibiotics, highly recommended during a Caesarian delivery.

The results obtained regarding other bacterial groups and the type of delivery are more controversial. It has been shown that infants born by CS are more frequently colonized by *Clostridium* species and less by *Bacteroides*, respectively (Adlerberth et al. 2007, Penders et al. 2006). However, Huurre et al. (2008) did not find differences regarding clostridia or *Bacteroides*. *Enterobacteriaceae* were found more often in infants born by CS by Adlerberth and coworkers (2007); however, they

also showed that *E. coli* was lower in those infants. This last result is in contrast with the results obtained by Penders and coworkers (Penders et al. 2006). The differences observed between vaginally delivered and CS-born infants can still be observed months after birth (Gronlund et al. 1999, Penders et al. 2006) and perhaps even longer. Salminen et al. showed that at seven years of age, the main divergence found in the intestinal microbiota of infants born by CS was in the lower numbers of clostridia (Salminen et al. 2004).

Moreover, it has been shown that the intestinal microbiota of infants born by CS is less diverse and contains lower bacterial numbers than the microbiota of those infants born vaginally (Biasucci et al. 2008, Gronlund et al. 1999, Huurre et al. 2008). In addition, Biasucci et al. reported greater intergroup and intragroup profile variations, particularly in infants born by vaginal delivery, whereas those born by CS displayed more constant profiles (Biasucci et al. 2008).



## Preterm Infants

One additional factor that can affect the infant gut colonization is the gestational age, although relatively little is known about the colonization process in preterm infants (Mshvildadze et al. 2010, Westerbeek et al. 2006). A major consequence of premature birth appears to be a delayed colonization with a limited number of species (de la Cochetiere et al. 2004) (Jacquot et al. 2011, Rougé et al. 2010, Schwiertz et al. 2003). It has been suggested that this difference in timing and diversity is mainly due to the aseptic neonatal intensive care environment and the extensive use of antibiotics shortly after birth. It should be noted that a high proportion of preterm infants receive antibiotics in the first days of life (Jacquot et al. 2011).

Other than the differences mentioned above, the colonization dynamics in preterm infants are different from that of term infants. The first colonizers seem to be predominantly coliforms, enterococci, and *Bacteroides*. It has been shown that preterm infants are often colonized by potentially pathogenic species, including *Klebsiella*, *Enterobacter*, and *Clostridium* species, with a reduced degree of colonization by normal commensal microbiota, such as *Bifidobacterium* and *Lactobacillus* (Blakey et al. 1982, Butel et al. 2007, Gewolb et al. 1999, Magne et al. 2006, Sakata et al. 1985). As mentioned above, the mode of delivery has a significant impact on the establishment of the gut microbiota in term infants. As a high number of preterm infants (50%–70%) are born by CS, differences in initial colonization may partly be explained by the mode of delivery (Bin-Nun et al. 2005, Underwood et al. 2009). It seems that the delayed colonization and lower diversity in preterm infants are even more pronounced in Caesarian infants versus vaginally delivered preterm infants (Gewolb et al. 1999, Hallstrom et al. 2004, Magne et al. 2006). However, recent studies could not confirm the effect of the mode of delivery on gut colonization of preterm infants (Jacquot et al. 2011, Mshvildadze et al. 2010). This discrepancy is probably due to the small sample size and the added factors that might play a role in the colonization of preterm infants, such as length of hospital stay, intubation, and type and amount of enteral feeding (Siggers et al. 2011).

Neonatal sepsis is a large problem in preterm infants with a high mortality and morbidity. The main reasons for the increased risk of infections might be the immature immune system and the abnormal gut microbiota. As mentioned before, an impaired intestinal colonization might play an important role in overall health. The delayed colonization and reduced diversity described in preterm infants leave those infants more susceptible to bacterial disturbances and therefore at higher risk of GI disorders and diseases, such as necrotizing enterocolitis (Claud & Walker 2001, de la Cochetiere et al. 2004). Moreover, Jacquot and colleagues showed a positive relationship between diversity of intestinal microbiota and digestive tolerance and weight gain (Jacquot et al. 2011). This may suggest that supporting the development of the microbiota could be a means for improving catch-up growth in preterm infants.

## Influence of Antibiotics on the Colonization Pattern

The increased use of antibiotics during the past decades has been positively correlated with increased inflammatory diseases (Greer & O'Keefe 2011). At the same time, the prevalence of microorganisms that are intimately involved in human physiology is changing through medical practices and lifestyle changes that may not be beneficial for human health. The early exposure to antibiotics has significant immediate effects and probably also sustainable effects on the gut microbiota composition. Several studies have investigated the impact of antibiotics on the gut microbiota equilibrium, often leading to the proliferation of (opportunistic) pathogenic strains (Bartosch et al. 2004, Nord et al. 2006, Sullivan et al. 2001). Parameters associated with antibiotics use, such as the specificity, dose, length of treatment, and route of administration (Sullivan et al. 2001), are often variable, which makes it difficult to draw strong conclusions on the exact impact on the microbiota. It seems however that antibiotic treatment causes disturbances in the expected patterns of early colonization by *Bifidobacterium* species, whereas an overgrowth of *Enterococcus* and *Enterobacteriaceae* species can occur (Bennet et al. 2002, Fallani et al. 2010, Hussey et al. 2011, Mangin et al. 2010, Penders et al. 2006, Tanaka et al. 2009). Recently, it has been shown that antibiotic treatment, administered either orally or parenterally, does not only alter the total counts of *Bifidobacterium* but can also have an impact at the species level (i.e., relative reduction of *Bifidobacterium adolescentis* and *Bifidobacterium bifidum*) (Hussey et al. 2011, Mangin et al. 2010). A recent study has shown that those infants whose mothers received antibiotics perinatally and/or during breastfeeding presented lower proportions of *Bacteroides* and *Atopobium*. Interestingly, this effect disappeared when weaning started, suggesting that the effect of early antibiotic administration can also fade upon weaning rather than persist when treatment has stopped (Mackie et al. 1999).

Although early antibiotic treatment appears to have no major long-term impact (dysbiosis) on the fecal intestinal microbiota, it has been indicated that exposure to antibiotics early in life may trigger the subsequent development of certain diseases, especially immune disorders, e.g., asthma, wheezing, and other allergic diseases (Droste et al. 2000, Kozyrskyj et al. 2007, Kummeling et al. 2007, Marra et al. 2009). Perhaps the microbiota in specific niches in the gut (e.g., small intestine) can be changed, although this aberration is not detectable in the feces, or the short term change of the microbiota has long-term consequences on the immune system and its development.

## Geographical Influence

Knowing that the bacteria colonizing the infant gut during the first days of life originate mainly from the mother and the environment, the hygiene that surrounds birth may be of major importance for the initial colonization dynamics. Infants in industrialized countries are born and grow up with high hygienic standards, whereas infants born and raised in developing countries encounter heavier bacterial exposures, which may have consequences for the gut colonization process. Even though only few direct comparative studies are available in the literature, it is well accepted that colonization by usual commensal species, such as *Escherichia coli*, is often delayed in industrialized countries compared with the past and compared with developing countries (Nowrouzian et al. 2003). Fallani et al. have recently shown in a large scale study that diet and lifestyle differences even between European countries may lead to different patterns of the gut colonization (Fallani et al. 2010). Their observations suggest a possible north-south geographic gradient characterized by higher proportion of bifidobacteria, *Atopobium*, *Clostridium perfringens* + *Clostridium difficile*, and sum of total bacteria in northern European cities (Glasgow, Stockholm) and by higher *Bacteroides*, enterobacteria and lactobacilli in southern European cities (Granada, Reggio Emilia).

Another study has shown differences regarding lactobacilli and eubacteria comparing Swedish to Estonian children (Bjorksten et al. 1999). Strikingly, the well-documented bifidogenic effect associated with human milk was not observed in two recent studies that took place in North America, raising the hypothesis of possible decreasing bifidobacteria in the commensal microbiota in this part of the world (Koenig et al. 2010, Palmer et al. 2007). All together there is clear evidence that, because of different lifestyles and food habits, the geographical context may significantly affect the microbial colonization pattern in early life. However, it appears that the geographical influence may also be driven by the specific human genotypes, as it has been shown that a higher similarity index is found between homozygote twins compared with fraternal twin pairs and unrelated paired controls (Stewart et al. 2005, Zoetendal et al. 2001). Weaning practices also differ greatly among countries and cultures, especially regarding the starting age, duration, and types of foods introduced, which may at least partly explain the geographical differences that have been observed (De Filippo et al. 2010).



## LONG-TERM EFFECTS OF EARLY COLONIZATION

Research on the long-term effects of early childhood nutrition has recently received a lot of attention (McMullen & Mostyn 2009). For example, epidemiological studies have reported an increased risk for asthma, food allergy, atopy, and autoimmune diseases (including type 1 diabetes) in children and young adults delivered by CS (Bager et al. 2008). The exact underlying mechanism linking the mode of delivery and the increased risk of disease later in life is still unknown. Several studies suggest that CS may well cause a genetic and microbiological imprint in the immune cells that could play a role later in life (Neu & Rushing 2011). The altered microbial colonization of the neonatal gut in CS may affect the priming of the neonatal immune system, which may explain the link between the mode of delivery and the increased risk for developing allergic and autoimmune diseases (Ly et al. 2006). The tolerance set up in early life coincides with the gut colonization by microbes (Sudo et al. 1997) and together with the presence of a certain type of mucin structure, environmental microbial inoculums, and mode of feeding, makes a stringent selection of the first microbial colonizers that play a prominent role in shaping the early ecosystem. An altered initial colonization of the gut is likely to induce intestinal microbiota dysbiosis throughout adult life. Unfortunately, no follow-up studies are available to demonstrate whether initial differences in fecal microbiota composition (e.g., induced by delivery mode or mode of feeding) can still be observed in children beyond three years of age. One recent study demonstrated that infants that had received an infant formula with scGOS/lcFOS in the first six months of life still had a significantly higher percentage of fecal bifidobacteria at the age of 12 months (Salvini et al. 2011). Another study has investigated the long-lasting effect of early microbiota in an intrauterine growth restriction (IUGR) animal model. The IUGR not only modifies the colonic microbiota in neonatal rats but also imparts a long-lasting alteration of the microbiota activities in young adult rats (Fanca-Berthon et al. 2010). In addition, a human study has recently demonstrated strong associations between some specific constituent of the microbiota at four days after birth and the concentration of specific microbial groups after 120 days, indicating the possible impact of early colonizers on the later microbiota (Eggesbo et al. 2010). Recently, Arumugam et al. (2011) described the enterotypes as a group of microbial groups that together contribute to a preferred ecosystem or type of gut microbiota. The characterization of the enterotypes has revealed that an adult human being can be divided into three categories according to their microbiota. So far, the origin of the enterotypes has not been characterized, but it could be the consequence of long-lasting effect of early alteration of the gut microbiota.

## EARLY COLONIZATION AND HEALTH

### Interaction with the Host Metabolism

Perinatal life is a critical period during which several environmental factors govern the development of organ structures and physiological systems and thus affect its functionality (McMullen & Mostyn 2009). Several epidemiological studies and animal models have supported the principle of "developmental origins of health and disease," which explains that irreversible changes in tissue structure, gene expression patterns, and physiological function in early life alter the risk of disease later in life (Armitage et al. 2004, Barker 1990). Most scientific evidence is based on the role of the fetal period in programming of later-life health and disease risk. However, recent studies have also shown that early postnatal nutrition has programming effects on adult body composition and metabolic homeostasis (Oosting et al. 2010) and thus the postnatal period can be considered as a critical period of life. It raises the question whether the microbiota, often described as a metabolic organ, may also steer metabolic development in early life, thus inducing sustained changes in adult metabolic homeostasis. The gut microbiota is at the intersection between host genotype and diet modulating host physiology and metabolism. In addition, there is increasing evidence of the role of the gut microbiota in obesity. Indeed, the gut microbiota in obese people has been described to be enriched in genes involved in sensing and degrading dietary polysaccharide, transporters of oligosaccharide, and genes involved in their intracellular metabolism (Ley 2010, Turnbaugh et al. 2008). Accordingly, the obese microbiome is described to be more efficient in energy harvesting, resulting in increased level of SCFAs, which is a source of energy as well as a key signaling molecule. SCFAs are associated with increased lipogenesis in the liver as well as LDL production (Turnbaugh et al. 2006). The gut microbiota have been shown to reduce the expression of fasting-induced adipose factor secreted protein (Backhed et al. 2004), resulting in an increase of the lipoprotein lipase and fat storage in white adipose tissue. An overview on the interplay between the intestinal microbiota and host metabolic health is shown in **Figure 2**. Another emerging hypothesis is the signaling role of bile acids that can regulate several metabolic pathways, with the gut microbiota as an important regulator of bile synthesis and secondary bile acid production directly impacting the metabolic homeostasis (Lefebvre et al. 2009). Finally, microbes interacting with the host immune system may either (*a*) lead to the destruction of B-cells and increased risk of type 1 diabetes or (*b*) the inducement of metabolic inflammation via bacterial lipopolysaccharides (Delzenne & Cani 2011). The routes by which the gut microbiota may interfere with cell proliferation and differentiation in early life are numerous and therefore perturbation in the microbiota in early life may be an important risk factor for disease later in life. Following this hypothesis, a longitudinal prospective study done on 28,354 mother-infant couples has recently revealed that a combination of early exposures potentially associated with microbiota alteration, including delivery mode, maternal prepregnancy BMI, and antibiotics in infancy, influences the risk of obesity in later childhood (Ajslev et al. 2011). Optimizing the early gut colonization process may therefore be an opportunity to prevent metabolic disease later in life.

### Function and Development of the Immune System

The intestinal microbiota plays an important role in the digestion of food. However, secondary to these metabolic effects, the intestinal microbiota also plays an important role in the function and development of the immune system, either because of the presence of specific bacteria or the production of metabolic end products.

**Gut maturation and immune maturation.** Newborns have a competent immune system that is not fully developed at birth but develops rapidly thereafter. Both the innate and adaptive immune

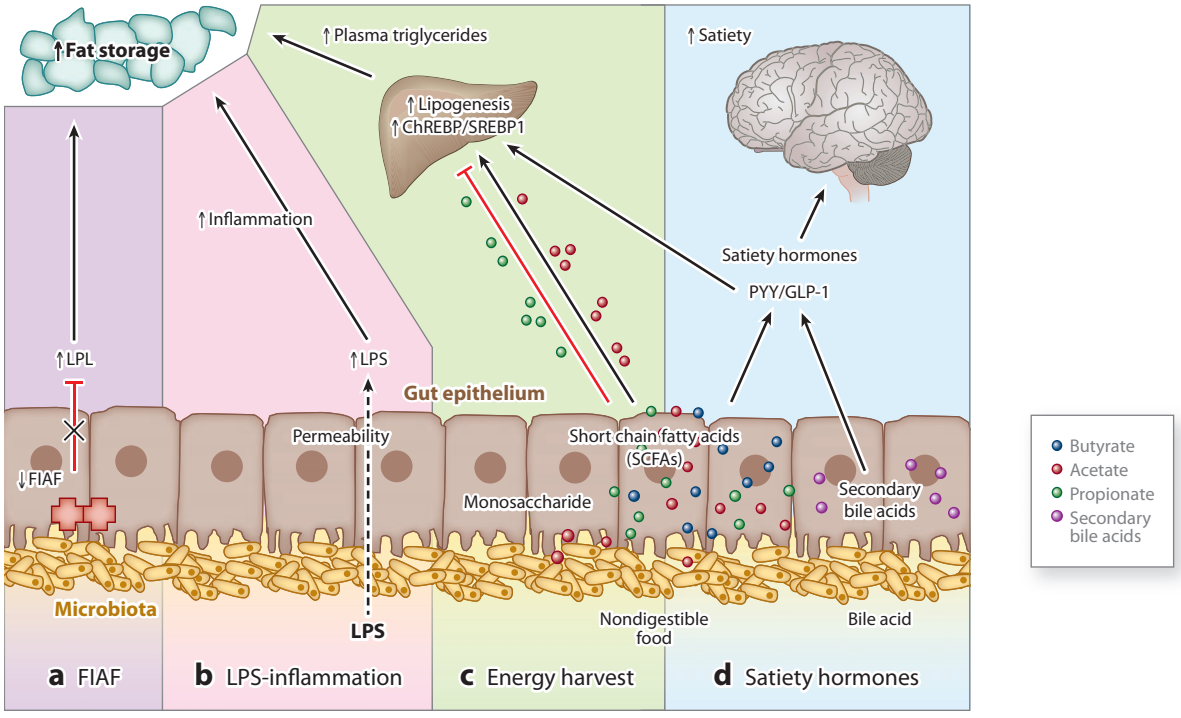


**Figure 2**

Interplay between gut microbiota and host metabolism as recently reviewed (Cani & Delzenne 2009, Greiner & Backhed 2011). (*a*) The gut microbiota suppress enterocyte expression of fasting-induced adipose factor (FIAF), promoting lipoprotein lipase (LPL) mediated triglyceride storage in adipose tissue. (*b*) Bacterial lipopolysaccharide (LPS) has been shown to deregulate the inflammatory tone and trigger body weight gain. (*c*) Short chain fatty acids (SCFAs) produced by gut microbes, a source of energy, are signaling molecules for lipogenesis in the liver. (*d*) Through SCFAs and secondary bile acid, the microbiota may interfere with the production of gut hormones, such as PYY or GLP-1, acting on satiety or liver lipogenesis.

system are not fully functional at birth, which could be explained by the low exposure to antigens up to birth and the need to prevent incompatibility with the mothers's immune system (Martin et al. 2011).

The cells of the immune system in the gut develop in close proximity to large communities of microorganisms in the intestinal lumen. Available evidence has indicated that intestinal bacteria play a crucial role in establishing and regulating the intestinal surface barrier (Hooper et al. 2001, Neish et al. 2000). Intestinal bacteria provide instructive signals for the development of key lymphocyte subsets: They direct class switching in human intestinal B cells (He et al. 2007); govern the development of intestinal Th17 effector T cells (Ivanov et al. 2008); and suppress the production of Treg cells (Hall et al. 2008). Additionally, intestinal bacteria impact the outcome of systemic immune responses by determining the ratio of Th1 and Th2 effector cells (Mazmanian et al. 2005). In the gut, specific homeostatic mechanisms protect resident immune cells against hyperactivation and accompanying inflammation. The important role for microbes in the maturation of the immune system is also being postulated by the so-called hygiene hypothesis (Strachan 1989). Different epidemiological studies support the hygiene hypothesis and have clearly shown that modifications in the pattern of microbial exposure represent a critical factor underlying the rise in the prevalence of atopic disorders. Moreover, it also suggests that this increase in

allergic diseases reflects a decrease in infections during childhood (Singh & Ranjan Das 2010). The composition of the gut microbiota and exposure to foodborne and orofecal pathogens probably have important homeostatic influences, both by enhancing the Secretory IgA (SIgA)-mediated intestinal surface barrier and by promoting oral tolerance through a shift from predominant Th2 cell activity in the newborn period to a more balanced cytokine profile later on (Brandtzaeg 2010).

Given that the gut microbiota is a key source of microbial-driven immune regulation, alterations of the normal bacterial colonization patterns may change the outcome of the immune development and cause predisposition to certain immune-related disorders later in life, such as allergy, obesity, or diabetes. Indeed, differences in gut microbiota composition and activity between healthy and atopic children have been shown in several cross-sectional epidemiologic studies (Bjorksten 2009, Garrett et al. 2010, Round & Mazmanian 2009, Vael & Desager 2009). However, whether the observed changes in the microbiota are the consequences of allergy or perhaps initiate the process leading to allergy remains a key question.

SIgA is one of the key factors in the development of the immune system. It is part of the mucosal immune system of the gut [gut-associated lymphoid tissue (GALT)]. The mucosal surface is the principal position for microorganisms and antigens to enter the human body, and especially in the GI tract, the exposure to foreign compounds is excessive. Therefore, the GALT is important in the defense against microorganisms and antigens from the environment. The SIgA complexes agglutinate microorganisms and prevent the adherence of pathogenic bacteria and viruses to the mucosal surface (Mazanec et al. 1993). In addition, SIgA can excrete microorganisms or antigens that have crossed the epithelial barrier out of the lamina propria to the lumen (Robinson et al. 2001).

The development of the GALT is impacted by microbial exposure and development of the gut microbiota in early life (Hooper 2004, Hooper & Gordon 2001, Mazmanian et al. 2005, Stappenbeck et al. 2002). In the GI tract, normal commensals should be discriminated from potentially pathogenic bacteria (Brandtzaeg 1996, 1998). Induction of an inflammatory response by the normal gut microbiota should be prevented, but an adequate immune response to pathogens should be maintained. Induction of SIgA expression plays an important role in the establishment of host-microbiota homeostasis and in inducing an immunological tolerance towards the gastrointestinal microbiota (Hapfelmeier et al. 2010).

**Secretory IgA (SIgA):** an immunoglobulin that is secreted by the mucosal immune system into the (gut) lumen

**Gut-associated lymphoid tissue (GALT):** this mucosal immune system is the largest immune component in the body and is of key importance in providing protection from the external environment

**Commensals:** bacteria living in symbiotic relationship with each other, where one benefits without negatively affecting the other

**Colonization resistance:** ecophysiological environment in which it is difficult for entering (pathogenic) bacteria to permanently colonize

**Increased colonization resistance and reduced risk of infections.** Other than the mechanisms described above, the gut microbiota constitutes part of the primary line of defense providing colonization resistance (Laparra & Sanz 2009, Salminen et al. 2005, Stecher & Hardt 2010). The term colonization resistance is commonly used to describe the ability of the colonic microbiota to resist invasion by exogenous microorganisms playing a fundamental role in blocking the colonization of entering pathogens and in preventing the overgrowth of potential pathogens naturally present in very low number in the gut (Endt et al. 2010, Stecher & Hardt 2010). The mechanisms involved in colonization resistance include host factors and bacterial factors, such as competition for adhesion sites and nutrients, together with the production of acidic condition (pH) and metabolites (SCFAs) (Hopkins & Macfarlane 2003, Roy et al. 2006, Vollaard & Clasener 1994). In vitro, studies have shown that the combination of SCFAs with low pH inhibits the growth of pathogenic bacteria (Hopkins & Macfarlane 2003). Therefore, other than the important role that early colonization has in the immune development of the newborn, the bacterial inhabitants of the gut also create an unfriendly environment for potential pathogens, preventing them from growing and colonizing and thus protecting the infant against infections.

## PERSPECTIVES FOR EARLY COLONIZATION

The past ten years have seen a complete reassessment of the phylogenetic make-up of the dominant human intestinal microbiota based on culture-independent molecular approaches. High-resolution and high-throughput tools, such as pyrosequencing-based phylogenetic mapping, are today most appropriate to study the assembly and dynamics of microbiota in early life and to identify common features or conversely specificities in given contexts (see Zoetendal et al. 2008). The information gathered still remains limited to the structure of the ecosystem, answering the question "who is there?", although the study of gene sequences has revealed that at least 80% of all identified species have not been cultivated and that 60% of these identified species have never been described before (Eckburg et al. 2005). In addition to the question "who is there?", current developments using the functional metagenomic, proteomic, and transcriptomic approaches allow us to ask the question "who is doing what?" by registering the complete gene repertoire of the human intestinal microbiota, including gene sequences encoding for specific bacteria, and gene sequences encoding for functional capacities (Kurokawa et al. 2007, Qin et al. 2010). These investigations have already brought extremely insightful information. For example, infection occurrence in the early life is associated with a transient imbalance with an enrichment of the microbiome with genes from virus or fungi (Koenig et al. 2010). Even though there are only a few studies with relatively low numbers of individuals that have investigated the microbiota implementation using functional metagenomics, relevant information has already been described, such as on the capability of the microbiota to metabolize carbohydrate and to produce folate or exopolysaccharide (Koening et al. 2010, Klaassens et al. 2009). In the future, functional metagenomics will give insights into the potential health benefits of the intestinal microbiota in early and later life.

Although mapping the whole gene repertoire of the microbiota is becoming accessible, one may expect that our knowledge regarding the microbial colonization, the link with health and disease, such as obesity, allergy, and autism, and susceptibility to infection will be completely revisited in the coming years with newly generated data. Optimizing the early gut colonization may be crucial for preventing disease later in life, although the window of opportunity or the time-span during which the microbiota can be modulated for host benefit remains to be established.

### SUMMARY POINTS

1. The human intestinal tract harbors a complex microbial ecosystem that develops from birth to the post-weaning period into adulthood.
2. The development of the microbial ecosystem also includes the development of the functional repertoire of the microbiome.
3. Different factors are known to influence the early colonization and development of the intestinal microbiota, including the effects of HMOs, mode and timing of delivery, use of antibiotics, introduction of weaning foods, genetic background, and geographical location.
4. Dietary interventions with prebiotics and/or probiotics can affect the composition of the intestinal microbiota.
5. Early colonization can have potential long-term effects on the adult microbiota and therefore on health.

6. There is an association between the microbial patterns and immune maturation in early life as well as dysbiosis associated with allergic disease.
7. The microbiota affects host metabolism and have a potential role in metabolism programming in infants.

### FUTURE ISSUES

1. The necessary timing for the microbiota to reach a functional profile that is characteristic of the adult GI tract should be assessed.
2. Well-designed clinical studies are needed to describe the whole gene repertoire of the infant microbiome and the correlation of these gene profiles with health parameters.
3. Technologies for mapping of the intestinal microbiota (metaproteomics, metabolomics, and metatranscriptomics) should be further developed and integrated with health data (systems biology).
4. Long-term follow up trials are needed to determine the sustainable impact of early life dysbiosis of the microbiota (e.g., use of antibiotics) on health and microbial prognostic markers associated with immune related or metabolic disease.
5. Current knowledge on the different factors (feeding practices, disease, delivery mode) should be refined to assess how early colonization may predict a certain type of adult microbiota (enterotypes).
6. The effect of different types of food transitions on the metabolic potential of the intestinal microbiota and on the metabolic programming of neonates should be determined.
7. New customized probiotics and/or prebiotics for a rational programming of the infant microbiota should be identified.

## DISCLOSURE STATEMENT

All authors are employees of Danone Research. Danone is a company that produces baby foods.

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