PERSPECTIVE

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A microbial perspective of human developmental biology

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When most people think of human development, they tend to consider only human cells and organs. Yet there is another facet that involves human–associated microbial communities. A microbial perspective of human development provides opportunities to refine our definitions of healthy prenatal and postnatal growth and to develop innovative strategies for disease prevention and treatment. Given the dramatic changes in lifestyles and disease patterns that are occurring with globalization, we issue a call for the establishment of 'human microbial observatories' designed to examine microbial community development in birth cohorts representing populations with diverse anthropological characteristics, including those undergoing rapid change.

A survey of the biological landscape that encompasses human development should consider all facets of what it means to be 'human'. There are at least as many microbial cells as there are human cells in our bodies, and the vast majority of unique genes are microbial[1–3]. As such, we can view ourselves as holobionts[4]. The dynamic microbe–microbe and microbe–host interactions that allow our microbial communities to assemble and endure are as yet largely uncharacterized. Our relationships with microbes begin before birth; they represent potentially modifiable features of postnatal development, and probably contribute to intra- and interpersonal variations in many aspects of normal physiology, metabolism, immunity and neurology, as well as to predisposition to diseases.

The past decade has produced a magnificent and still rapidly evolving toolbox of experimental and computational techniques for culture-independent identification of the microorganisms that comprise our body habitat-associated microbial communities (microbiota), as well as their genes (microbiome) and gene products. These tools allow a number of hypotheses about microbial contributions to human development to be tested. One hypothesis is that maternal microbial ecology affects pregnancy, fetal development and the future health of offspring. If true, the hypothesis suggests the possibility of prenatal prognostic and diagnostic measurements and therapeutic interventions that target the maternal microbiota to guide healthy fetal development and avoid premature birth and other negative outcomes. Another hypothesis is that after birth, there are microbial taxa whose changing patterns of representation can be used to define 'normal' programmes of development of the microbial communities that occupy a given body habitat in biologically unrelated individuals with healthy growth phenotypes (as defined by anthropometric indices). A corollary to this hypothesis is that deviations from these normal programmes of community assembly represent a way to characterize abnormal development, including states of immaturity or precocious maturation. Establishing a causal relationship between the state of microbial community development and healthy growth would allow deviations from normal microbiota development to be used as a parameter for risk assessment or classification of a number of diseases that may manifest themselves early or later in life, yield insights about disease pathogenesis, and provide a starting point for developing microbiota-directed therapeutic interventions or new approaches for disease prevention.

In this Perspective, we discuss evolving concepts about the relationship between maternal microbial ecology (before, during and after pregnancy) and pregnancy outcomes as well as the relationship between human breast milk oligosaccharides, the establishment and expressed functions of the gut microbiota and healthy postnatal growth. We also address the need for long-term birth cohort studies to identify both shared and distinctive features of microbial community development, within and across populations, and delineate how normal execution (and perturbations) of this facet of human developmental biology is related to health status.

Maternal microbial ecology

The structure and function of maternal microbial communities, and the impact of these communities on maternal and infant health outcomes has been considered in several body habitats, including the vagina, the distal gut and the mouth.

Vaginal microbiota

For decades, culture-based studies have suggested that lactobacilli are the most prevalent constituents of the vaginal microbiota in non-pregnant and pregnant women[5]. More recently, culture-independent studies have demonstrated that most vaginal communities are dominated numerically by a single *Lactobacillus* species. This finding has prompted some investigators to assign vaginal communities to a relatively limited number of discrete 'community state types' (CSTs). These CSTs are classified either by which *Lactobacillus* species is dominant (CST I, II, III and V) or by the presence of a relatively diverse, *Lactobacillus*-poor community (CST IV)[6]. The resolution and veracity of the vaginal CST model remains unsettled: some investigators have proposed other stable or transitional states beyond the five described initially[7]. Others have highlighted potential pitfalls, including the extent to which the detection of state types is dependent on the analytical workflow[8]. Irrespective of the ultimate usefulness of the CST model, the limited diversity

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of abundant taxa in vaginal communities suggests that a deterministic process of community assembly, such as habitat filtering, governs the overall structure of the adult vaginal microbiota.

CST IV is similar to the microbiota structure encountered in bacterial vaginosis, a dysbiosis that is associated with adverse health outcomes, including preterm birth[9,10]. In non-pregnant North American women, the prevalence of CSTs varies with self-reported race and ethnicity. CST IV is observed in about 40% of African American and Hispanic women, but only about 20% of Asian American women and about 10% of Caucasian women[6]. This skewed distribution suggests that a diverse, non-*Lactobacillus*-dominated community (CST IV) might represent a normal variant in a subset of women and argues for an expanded assessment of what comprises a healthy vaginal microbiota.

Little is known about the development of the vaginal microbiota before and after puberty, or how different vaginal community 'fates' (structural and functional states) in adulthood are determined. One area that should be investigated is the relationship between the glycan content of the vaginal mucosa and the community state, including the biogeographical features of each state. In addition, much remains to be learned about the effects of bacterial and eukaryotic taxa (and the viruses they host) on vaginal epithelial-cell differentiation, vaginal mucosal metabolism and the activities of components of the innate and adaptive arms of the immune system that are represented in this habitat. The development of microarrays composed of purified microbial glycans[11] provides one way to characterize immunological responses to the bacterial antigens represented in the vaginal microbiota and thus creates another approach to classify community states. Representative preclinical models are needed for testing whether causal relationships exist between these and other environmental factors and community states. They will also help to characterize the mechanisms that shape community assembly, that determine community responses to various perturbations, that underlie community resiliency and that mediate the effects of community states on host biology.

A compelling question is whether there is a discernable programme of change in the properties of the vaginal microbiota before, during and after pregnancy and, if there is, the extent to which such change recapitulates features of the original developmental biology of the community. A related question is whether and how functional alterations in vaginal microbial community states and in the microbiota at other body sites during pregnancy affect intrauterine growth of the fetus (Box 1). Work has focused on bacterial taxonomic composition of these communities rather than on the functional features they express. Studies currently suggest that the bacterial composition of the microbiota is more stable during pregnancy than at other times during adulthood[12–15]. The diverse CST IV seems to be the least stable community state during pregnancy: it exhibits a substantially higher rate of transition to alternative CSTs on a week-to-week timescale than do the four *Lactobacillus*-dominated CSTs[14]. A note of caution is that vaginal microbial community composition has not been defined in time-series studies in which samples are taken from the same women before conception, during and after pregnancy, and during subsequent pregnancies. In addition, little is known about the non-bacterial membership of the pregnancy-associated microbiota.

Some factors are thought to promote vaginal microbiota structural stability during pregnancy, such as a lack of menses. However, many factors remain unknown, as is the degree to which structural stability is accompanied by functional stability and how this relates to the transfer of taxa from mothers to infants during the immediate postpartum period. From an anthropological perspective, it is interesting to note that prescribed diets during pregnancy are an important part of the cultural traditions of some populations[16]. It is unclear how these treatments affect vaginal (and gut) microbial community structure, function and stability. The answers to these questions could yield fresh approaches for deliberate manipulation of the vaginal microbiota.

In contrast to the structural stability seen during pregnancy, studies of women in the United States, Europe, Africa and Asia have shown that, after delivery, the vaginal microbiota commonly undergoes an abrupt and striking alteration in its taxonomic composition[14,17–19]. This alteration is characterized by a significant increase in within-community (or α) diversity and is driven by a decrease in the abundance of *Lactobacillus* species and a commensurate increase in a wide range of anaerobic species. Although many features of altered postpartum microbial communities remain to be elucidated (such as the time it takes to return to the 'baseline' state), it seems that they can persist for at least 1 year in many women[14]. A short interval (less than 12 months) between pregnancies is associated with an increased risk of preterm birth; whether a persisting altered postpartum vaginal community contributes to this risk warrants further study.

BOX 1

The evolving maternal–fetal microbial landscape

Much remains to be learned about the assembly, host interactions and transmission of maternal and early childhood microbial communities. Hypotheses about the evolving maternal–fetal microbial landscape deserve further attention and testing.

- Some activities of the maternal microbiota might have a beneficial impact on fetal nutrition and development.
- Altered compositions and expressed activities of the maternal microbiota might contribute to gestational outcomes, including adverse events such as premature labour and birth.
- Microbes that are transferred to offspring before or during delivery might reflect environmental exposures of the mother during pregnancy (for example, diet).
- Persisting disturbances in the vaginal microbiota after giving birth might pose a risk for preterm delivery in subsequent pregnancies.
- Variations in the transfer of microbes from mothers to infants might affect early postnatal development of the child's microbiota, immune system and metabolic processes.

Gut microbiota

Much more information is needed about whether the structural and functional properties of the gut microbiota of women change as a function of pregnancy. If changes do occur, whether and how they relate to maternal and fetal health, as well as the subsequent health of infants and children, should be investigated. The relationship between maternal nutritional status at the time of conception and the health of the newborn is well established[20]. A study of pregnant Finnish women reported a significant increase in faecal energy content, as determined by bomb calorimetry, between the first and third trimesters despite stable diets and energy intake[21]. This change in energy content correlated with shifts in taxonomic composition[21]. However, studies of women residing in the United States[14] and in Tanzania[17], which were conducted at higher temporal resolution, found that the women's faecal microbiota manifested compositional stability throughout pregnancy (as measured by trends of α diversity, week-to-week variation in bacterial composition within subjects and β diversity across gestational time). The reasons for these divergent findings are unclear. The maternal microbiota and diet also have the potential to influence both fetal and maternal epigenomes, although a discussion of this topic is beyond the scope of this Perspective.

Oral microbiota

Mothers harbour complex microbial communities in their mouths. The composition[22] and transcriptional activities[23] of these communities are altered in the setting of periodontitis, a condition also associated with

intrauterine growth restriction, preterm birth and low birthweight[24]. A study of the oral microbiota of women living in the United States and Africa indicated that the taxonomic composition remains stable during pregnancy[14,17]. However, pre-conception data from the same women were unavailable for comparison. Microbial taxa have been detected in amniotic fluid[25–28] and in the placenta[29] that probably originate from the mouth, particularly in women who are unhealthy or had adverse outcomes such as preterm labour with intact fetal (chorioamniotic) membranes or premature rupture of these membranes. Disentangling adverse effects on pregnancy that originate from the oral microbiota is challenging, especially if disease results from a perturbation in relatively minor constituents of the community[30].

Development of the oral microbiota has not been comprehensively defined through time-series studies of healthy infants and children. For example, the effects of maternal prenatal history, gestational age, route of delivery and milk-feeding history remain to be characterized. One study attempted to define 'normal' development by following 50 children from the ages of 4 years to 6 years[31]. A strong effect of chronological age was observed on the taxonomic composition of the oral microbiota. This effect was more pronounced for bacterial communities in supragingival plaque than in saliva, which suggests body-habitat-specific differences in community assembly programmes. Deviations from early, normal community compositions were predictive of subsequent development of dental caries[31].

Preterm delivery and fetal exposure to microbes

The extent to which the fetal environment is sterile has been pondered since the birth of the field of microbiology[32]. Early studies suggested that the amniotic cavity was universally sterile before labour[33], although subsequent, indirect evidence has challenged that assumption[34]. Culture-based and later, polymerase-chain-reaction-based studies indicated that microbial invasion of the amniotic cavity occurs more frequently and involves a greater diversity of microbes than was originally thought[25,26]. Endometrial sampling of the intrauterine cavity in non-pregnant women has yielded widely varying rates (0–89%) of microbial recovery across culture-based studies[35]. Molecular-based studies suggest that most uteruses harbour microbes, with *Lactobacillus*, *Prevotella* and *Bacteroides* among the genera that are most commonly encountered[35,36]. However, data obtained during pregnancy are lacking.

At the time of delivery, the basal plate of the placenta contains intracellular bacteria in about a quarter of women, but in about half of those who deliver spontaneously before 28 weeks of pregnancy[37]. One study has shown that the placenta harbours a complex set of microbial DNA sequences[38]. But unlike more densely colonized body sites such as the gut and mouth, placental samples are overwhelmingly negative in culture-based assays[39]. DNA-based assessments of potential microbes in the placenta, and other low microbial biomass sites, are particularly prone to confounding findings from 'background' DNA[40,41] and should be interpreted with caution in the absence of appropriate controls. The degree to which the fetal–placental environment has evolved to serve as a venue for programmed engagement of diverse microbes, as opposed to being a site that simply tolerates stochastic low-level microbial exposures, remains unclear and merits further study.

A report published this year suggests that in women who experienced spontaneous preterm birth, those with histological evidence of severe chorioamnionitis have fewer species of bacteria on the fetal side of the placental membrane than do those who do not have severe chorioamnionitis[42]. This difference might be driven by a high abundance of a limited number of clonal pathogens (as is typical of many clinical infections) in women with severe chorioamnionitis. Further studies with appropriate negative controls are needed to corroborate these findings and to resolve unanswered questions such as the body site of origin of the detected microbes, as well as the direction and timing of their translocation across adjacent tissues[43].

Microbes have been detected in first-pass meconium samples from approximately two-thirds of healthy, vaginally delivered, breastfed full-term babies, but at very low levels[44]. Detection is more common in meconium from neonates who are born before 33 weeks of gestation, and there is considerable taxonomic overlap with the microbes found in the amniotic fluid[25,45]. Molecular evidence for microbial invasion of the amniotic cavity has provided associations of space, time and 'dose' that support a causal relationship with preterm birth[25]. Microbial taxa that are associated with preterm birth most frequently originate from the mother and exploit one of three natural routes for invading the amniotic cavity[46]: ascension from the vagina and cervix; transfer through the fallopian tubes; or translocation from more distant sites of colonization in the body, presumably through the bloodstream[27]. The majority of invading microbes seem to come from the vagina[25,28,46], although other body habitats, most notably the mouth[27,47] and gut[26], may have a role in some cases. Taxa associated with CST IV communities, such as *Ureaplasma* and *Prevotella* species, are among the more common invaders. By contrast, *Lactobacillus* species are rarely encountered in amniotic fluid, even after membrane rupture[26]. This suggests that features of specific microbial taxa, or groups of taxa that occur together in CST IV communities, underpin factors that promote invasion of the amniotic cavity, such as virulence genes and divergent host immune responses[48]. Whether particular vaginal CSTs or the presence and abundance of individual taxa are associated with preterm birth is an unresolved question of great interest. Studies have produced conflicting results[12,14]. If vaginal CST IV communities are indeed associated with preterm birth in some women, this would be broadly consistent with epidemiological evidence that links bacterial vaginosis, which shares taxonomic similarity with CST IV communities, to an increased risk of preterm birth[9].

The effect of preterm delivery on the development of microbial communities in premature babies has been examined mainly from the perspective of the infant. Comparing the development of microbial communities in premature and full-term infants could lead to amended or new definitions of biological immaturity. Such definitions are confounded, however, by the frequent pre-emptive administration of antibiotics to babies who are born prematurely. Maternal microbial communities may also exert a significant influence. An elegant study demonstrated that transient microbial colonization of pregnant, germ-free mice was sufficient to modulate the function of innate immune cells in the small intestines of their germ-free offspring[49]. Microbial products were detected in both the dam's milk and placenta, which suggests that 'indirect' exposure to microbes through the mother is sufficient to shape neonatal development. Such findings suggest that systematic characterization of multiple body-habitat-associated microbial communities in mothers who have preterm versus full-term pregnancies creates opportunities to examine whether there are identifiable programmes of change in maternal microbial ecology during pregnancy and whether disruption of these programmes affects initial transfer of microbes to their offspring (and the subsequent development of the children's microbiota). This knowledge could change clinical practice so that more attention is placed on careful stewardship of microbial resources in women who have a high risk of preterm delivery[50]. Deliberate efforts could be made to transfer these microbes to their offspring, with the potential for supplementation with important taxa that are missing.

Breast milk and infant gut microbiota

Researchers are beginning to uncover how breast milk composition changes over time after parturition and how it shapes the structural and functional maturation of infant-associated microbial communities.

Microbes associated with breast milk

Studies of milk-associated microbiota reveal highly individualized assemblages[51]. These groups of microbes are routinely dominated by skin-associated bacteria, such as staphylococci and streptococci, which generally do not persist in the infant gut in significant numbers for more than a few weeks[52]. Some anaerobic species, such as *Bifidobacterium*, have been isolated from breast milk, which suggests a route for transit of specific strains that eventually colonize the infant colon. The factors

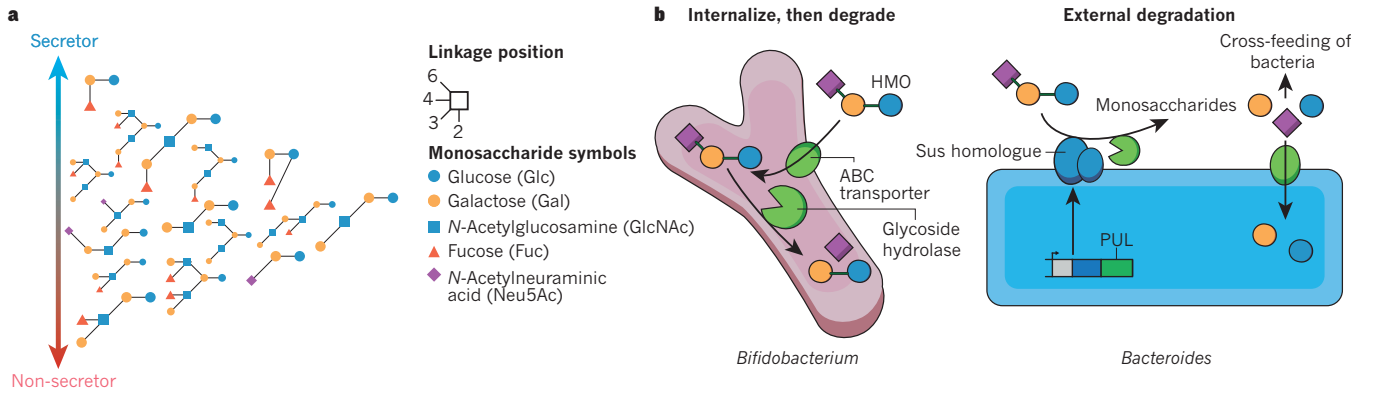

Figure 1 | Oligosaccharides in human breast milk and strategies for their degradation by the infant microbiota. a, HMOs that are most abundant in the breast milk of mothers who are secretors are indicated by the blue arrow; those that are most abundant in the breast milk of non-secretors are indicated by the red arrow. Structures at the intersection of the arrows are found in both secretor and non-secretor mothers in similar abundances. Monosaccharides in HMOs, as well as their glycosidic linkages, are described by the inset key. **b,** Most strains of *Bifidobacterium* (left) use an 'internalize, then degrade' strategy in which HMO structures are first imported using ABC transporters and then degraded by intracellular glycoside hydrolases. Strains of *Bacteroides* (right) typically employ an 'external degradation' strategy for HMO structures, which involves cell-surface-associated carbohydrate-binding proteins and secreted glycoside hydrolases that are encoded by polysaccharide utilization loci (PULs). These PULs have features similar the prototypic starch utilization system (Sus) of *Bacteroides thetaiotaomicron*. This external degradation can result in 'cross-feeding' of secondary consumers, including potentially pathogenic bacteria, in the infant gut microbiota.

that contribute to the strain-specific composition in breast milk are unclear and are subject to debate.

Human milk oligosaccharides

From a molecular perspective, breast milk is the best-characterized food that humans consume. The most abundant component in dried samples of breast milk is lactose, which provides nutrition for the infant, although many bacterial taxa can also digest this disaccharide. Lactose is made available specifically to bacterial colonizers of the infant gut by extending it by 3–20 monosaccharide units to yield structures that are known collectively as human milk oligosaccharides (HMOs)[53,54]. All HMOs contain this lactose core together with various combinations of glucose, galactose, *N*-acetyl galactosamine, fucose and sialic acid (*N*-acetylneuraminic acid or Neu5Ac)[55]. HMOs are often terminated by fucose or sialic acid (Fig. 1a). Approximately 60% of HMO structures are fucosylated, and 5–20% are sialylated[56,57].

The role of HMOs has become more apparent through the application of nanoflow liquid chromatography mass spectrometry. This method has detected more than 300 HMO structures in breast milk samples pooled from several mothers, with the concentrations of these structures spanning four orders of magnitude. The number of HMO structures found in the milk of a particular mother is often more than 100, although the profile of the structures varies between mothers[53,54]. HMOs contain varying amounts of Lewis-blood-group antigens Le^a, Le^b, Le^x and Le^y (ref. 58). Individuals who produce Le^b epitopes (α(1,2)-fucosylated structures) in their secretions, due to the presence of an active fucosyltransferase 2 (*FUT2*) gene, are known as secretors[59]. Secretors tend to have higher amounts of HMOs than do non-secretors (as much as 20% more). They also produce higher levels of fucosylated structures (nearly twofold more). However, non-secretors often have higher levels of sialylated structures[57] (Fig. 1a). The percentage of non-secretors varies geographically: they comprise about 20% of the population in Europe and up to 40% in West Africa[57].

Whether the HMO profiles of breast milk change as a function of time after delivery, and how differences in HMO composition relate to the development of the gut microbiota and healthy growth of the infant are important unanswered questions. Nanoflow liquid chromatography mass spectrometry has only been applied to HMO profiling during the past several years and the assay imposes constraints on throughput[60]. Limited information is therefore available about how specific HMOs change with time in healthy mothers, and whether consistent differences exist in the HMO profiles across groups of women representing different ages, parities, geographic locations, nutritional states, culinary traditions and socio-economic statuses. The general trend during lactation is a decrease in levels of total HMOs as the mother progresses from the production of colostrum to mature milk, with the largest drop occurring in the first month postpartum[61]. However, the total amount of milk that is delivered as colostrum is quite small compared with the volume of mature milk (matching the size of the infant's stomach and intestine). Therefore, throughout lactation, the amount of each class of HMO — and even each specific HMO structure — provided to the infant remains relatively constant[61–63].

Giving birth prematurely can significantly affect the profile of a mother's HMO structures[64]. HMO profiles cannot yet be predicted in these mothers. Many mothers who deliver preterm have fucosylated HMOs that are as low as 20–40% of total HMOs, but some have levels of greater than 60%. This discrepancy is not corrected over time.

A study published this year demonstrated that the HMO content of Malawian mothers' milk correlates with infant growth outcomes[65]. Breast milk samples collected at 6 months postpartum were divided into two groups: those from mothers whose infants exhibited healthy growth at the time of collection (as defined by anthropometry), and those from mothers whose offspring exhibited severe stunting. Liquid chromatography–time-of-flight mass spectrometry revealed that mothers of infants with stunted growth had significantly lower concentrations of total, sialylated and fucosylated HMOs, with the most growth-discriminatory sialylated HMO being sialyllacto-*N*-tetraose b, and the most discriminatory fucosylated HMOs being 2′-fucosyllactose and lacto-*N*-fucopentaose I.

Sialic acids constitute a group of nine-carbon monosaccharides that are derived from neuraminic acid, and include Neu5Ac. UDP-*N*-acetylglucosamine-2-epimerase, which is the rate-limiting enzyme in the biosynthesis of sialic acid, is produced at low levels in the livers of infants[66]. Breast milk is therefore an important source of these sugars. The availability of sialic acids affects many organs, including the brain, where Neu5Ac is a component of gangliosides and is covalently linked to neural cell-adhesion molecules (NCAMs) that mediate cell–cell interactions involved in synaptogenesis and memory[67,68]. Supplementation of the diet with sialylated glycoproteins and sialyllactose increases the polysialylation of NCAM and sialyated gangliosides, with some reports showing improved memory in animal models[69]. A preclinical model has demonstrated that 6′-sialyllactose also increases muscle mass and contractility[70].

Several HMO structures have been produced chemically and

enzymatically[71]. However, producing the wide array of structures encountered in human milk is not yet commercially feasible. There is an approximately 25% overlap between bovine milk oligosaccharide and HMO structures. Sialylated oligosaccharides are present in mature human milk at concentrations that are up to 20-fold greater than in mature bovine milk[72,73]. Therefore, bovine-milk-derived infant formulas, as well as complementary or therapeutic foods that are used to treat children with undernutrition, are deficient in these compounds. However, bovine milk oligosaccharides (BMOs) that have structural similarity to HMOs are present in the by-products of dairy processing, providing an opportunity to purify them at a scale sufficient for preclinical and clinical studies, and potentially for wider distribution should such studies demonstrate sufficient safety and efficacy, and yield an understanding of their mechanism of action.

A study of gnotobiotic animals has provided direct evidence that sialylated milk oligosaccharides are causally related to growth[65]. Young germ-free mice and newborn germ-free piglets were colonized with members of the gut microbiota of a Malawian infant who exhibited stunted growth. Recipient animals were fed a diet representative of foods consumed after weaning by Malawians, with or without supplementation with sialylated BMOs that had been purified from a whey waste stream generated during the manufacture of cheese. The study revealed that sialylated BMOs promote lean body-mass gain, improve metabolic flexibility[74] and affect bone growth. These effects were not ascribable to differences in food consumption. They were also microbiota-dependent: they were not observed in germ-free animals. Moreover, growth promotion was not observed when the animals were provided an isocaloric Malawian diet supplemented with a mixture of fructo-oligosaccharides, a component of some infant formulas.

The milk-oriented microbiota

The initial microbiota of nursing infants is an assemblage of microbes derived from mother's faecal, vaginal and skin microbiota[52]. Within weeks, promicrobial and antimicrobial agents in breast milk help to guide development of a milk-oriented microbiota. A common enrichment involves members of the Actinobacteria, mainly *Bifidobacterium* species, that frequently dominate the gut microbiota of breastfed infants, in some cases representing 70–90% of the faecal community[75]. Intriguingly, this enrichment is less pronounced in infants from more industrialized countries[75–78]. Bifidobacterial enrichment is linked to maternal genotype; the breast milk of secretors seems to enrich bifidobacteria more rapidly[76].

Several beneficial functions have been attributed to a milk-oriented microbiota that is dominated by bifidobacteria. For example, lactate and acetate, the primary end products of bifidobacterial fermentation, are important sources of energy for colonocytes. They also lower intestinal pH and contribute to gut barrier function[79]. Robust colonization by a single bifidobacterial subspecies, *Bifidobacterium longum* subsp. *infantis*, correlates with improved vaccine responses during the first year of life[77]. Intestinal bifidobacteria also produce essential nutrients, including folate and riboflavin[80].

Two dominant species of *Bifidobacterium*, *B. longum* and *B. breve*, routinely colonize breastfed infants throughout the world, although other species, including *B. bifidum*, *B. catenulatum* and *B. pseudocatenulatum* are also commonly observed. In general, bifidobacteria are prolific consumers of HMOs; they possess an array of glycoside hydrolases (notably fucosidases and sialidases[81]) that catalyze the cleavage of key glycosidic linkages, permitting metabolism of some or all of the sugar monomers that are embedded in HMOs. The mechanisms for HMO consumption by these organisms follow two different strategies[82]. *B. longum* subsp. *infantis* and, to a lesser extent, *B. longum* subsp. *longum*, *B. breve* and *B. pseudocatenulatum*, transport HMOs directly into the cell through ATP-binding cassette (ABC) transporters and cleave these oligosaccharides with intracellular glycoside hydrolases (Fig. 1b, left)[83]. By contrast, *B. bifidum* deploys glycoside hydrolases to the cell wall for extracellular cleavage of HMOs before importing selected products of degradation. Similarly, *Bacteroides* species, another important set of HMO consumers (and frequent members of milk-oriented microbiota), also deploy external glycoside hydrolases to degrade these structures before they are internalized (Fig. 1b, right)[83].

The 'internalize, then degrade' approach for HMO consumption adopted by the majority of infant-borne bifidobacteria can be viewed as an ingenious strategy for protecting the neonate. These bacteria prevent growth of competitor strains by simple sequestration of available sugar substrates in the colon, a concept consistent with the inverse correlation observed between faecal HMO concentrations and the level of bifidobacterial colonization[76,84]. An important consideration is whether there are deleterious consequences of harbouring a milk-oriented microbiota that is dominated by bacteria that degrade HMO externally. An antibiotic-treated mouse model has been used to show that mucins, large glycoproteins that contain structures similar to those of HMOs, can be externally degraded by *Bacteroides* spp. to release fucose and sialic acid monomers that cross-feed various pathogenic bacteria[85]. External degradation of HMOs could lead to growth of pathogens or pathobionts in the low-diversity neonatal gut microbiota.Three recent studies point to this potential risk. In gnotobiotic mice that were colonized with the microbiota from a Malawian infant with stunted growth, external degradation of sialylated BMOs by *Bacteroides fragilis* released the constituent monosaccharides, including sialic acid, that cross-fed *Escherichia coli* populations[65]. Others have observed *Bacteroides* cross-feeding Enterobacteriaceae in mice that are fed sialyllactose (an oligosaccharide common to mammalian milks) and in nursing piglets[86,87]. Enterobacteriaceae are considered by some researchers to be a harbinger of dysbiosis[88].

These findings suggest that the potential for bacterial cross-feeding on HMOs may be a risk factor for neonates. They also illustrate the extreme caution that should be afforded when composing diets for neonates that harbour low-diversity gut microbiota during early stages of community development. In cases in which a single oligosaccharide prebiotic is being considered, such as fucosyllactose or sialyllactose in infant formula, it would help to know the composition of the infant milk-oriented microbiota to avoid potential cross-feeding of enteropathogens. Alternatively, this problem might be alleviated by the use of synbiotics (a combination of pre- and probiotics) in which the probiotic component is known to readily consume the oligosaccharides provided or derived monomers.

Several challenging questions need to be addressed. First, we know very little about the functions of various HMO structures or why mammalian evolution has produced such a diverse repertoire. Even more diversity could exist given the number of possible glycosidic linkages, suggesting that observed HMO structures were selected for by evolution. Second, we need to better characterize the interactions and relative effect sizes of the antimicrobial and promicrobial components of breast milk on development of the milk-oriented microbiota. One approach for addressing these questions is to use gnotobiotic animals colonized with milk-oriented microbiota from infants representing different gestational ages, milk-feeding histories and growth phenotypes. Alternatively, gnotobiotic animals could be colonized with defined collections of cultured bacterial strains, recovered from a given donor's microbiota; these clonally arrayed collections can be manipulated so that all members, or subsets of members, are added — with or without pathogens and pathobionts — to recipient animals (Fig. 2). Gnotobiotic recipients colonized with these communities can be fed breast milk or infant formula supplemented with defined milk oligosaccharide structures. (Many of the antimicrobial elements of breast milk, including antibodies, lactoferrin and lysozyme, are absent from such formulas.) These models represent one way for determining the rules that govern early phases of development of the human gut microbiota.

The weaning-oriented microbiota and beyond

Culture-independent studies have characterized a programme of gut microbial community development that is executed during the first

2–3 years of postnatal life, as infants move from a diet dominated by milk through a period of complementary feeding to a fully weaned state. In one study, monthly collection of faecal samples from members of a Bangladeshi birth cohort with healthy growth phenotypes allowed the generation of 16S rRNA-sequence-based data sets that described the bacterial composition of their developing gut communities[78]. This study used Random Forests-based models to identify a group of age-discriminatory bacterial strains, the relative abundances of which defined the state of development ('age') of a child's microbiota. Remarkably, many of these age-discriminatory strains were also present in models of normal microbiota development in healthy Malawian infants and children[89].

Deviations from normal can be expressed in the form of a microbiota-for-age *Z*-score (MAZ). Calculating MAZ scores disclosed that microbiota development was impaired in Malawian and Bangladeshi children who presented with moderate or severe acute malnutrition[78,89]. Their microbial communities appeared 'younger' than would be expected from their chronological age. Moreover, this microbiota immaturity is not durably repaired by treatment with current ready-to-use therapeutic foods[78,89]. Transplanting immature microbiota from Malawian children who are stunted or underweight, or from chronologically age-matched donors who have healthy growth phenotypes, to young germ-free mice fed a diet resembling that consumed by the microbiota donors showed that immature microbiota transmit impaired growth phenotypes[89].

These and other studies provide preclinical proof-of-concept that gut microbiota development is causally related to healthy growth[89,90]. They also provide a microbial measure of normal as well as perturbed postnatal development. An important question is how microbiota development affects development of the immune system. This issue can be addressed in part by defining IgA responses of the gut mucosa to members of the microbiota[91], using faecal samples serially collected from members of birth-cohort studies[92]. This approach represents one way to identify relationships between microbial community development, development of the immune system, breast milk HMO content and host growth phenotypes.

A call for human microbial community observatories

Characterizing normal gut microbiota development and the development of other body-habitat-associated microbial communities in members of birth cohorts provides a framework for exploring the degree to which these processes vary across populations of infants and children with healthy growth phenotypes. Whether —and how — perturbations of these programmes are related to growth faltering and the risk for and development of various diseases can also be investigated. These studies should include an examination of the mother and her microbial communities starting at the time of conception, and of the impact of these communities on fetal development. The results could yield insights about as yet unappreciated microbial contributions to a wide range of disorders that are overtly manifest, or foreshadowed, by changes of microbial community structure and function in infancy or childhood (for example, obesity[93–95], immunological disorders including atopic states[96] and neurodevelopmental disorders[97]).

Given the dramatic, myriad and rapid changes in our lifestyles wrought by globalization, as well as the vast differences in sanitation and hygiene experienced by various populations, we propose that a series of 'human microbial observatories' be established to characterize the evolution of microbial communities in mothers before, during and after pregnancy, to monitor fetal development and to characterize the development of microbial communities in their offspring (and perhaps in the future, in the pregnancies of these children and their offspring). We propose that the populations selected for study should not only illustrate currently distinct lifestyles and geographies, but also contain segments that are likely to undergo lifestyle changes within a generation.

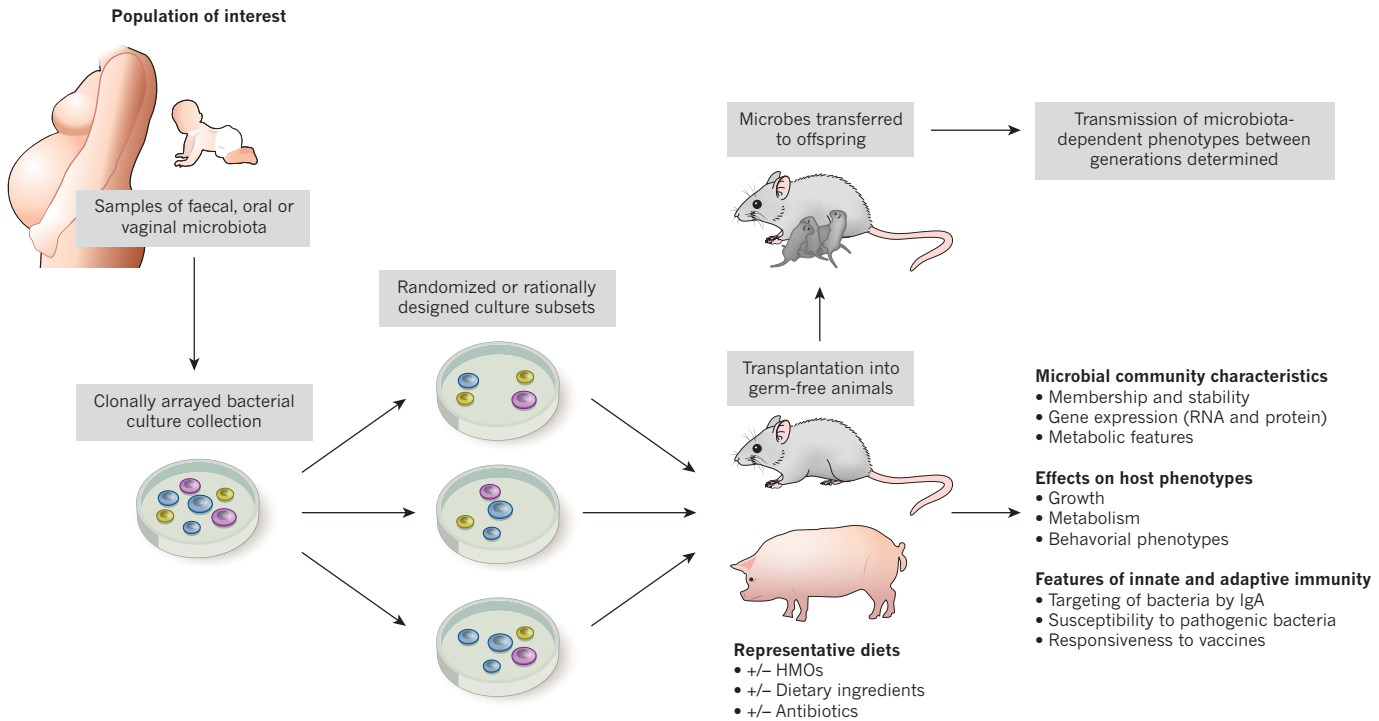

Figure 2 | Discovery pipeline for characterizing the functional properties of developing human microbial communities. Samples of intact, uncultured microbiota are obtained from infants and children with healthy growth phenotypes and normal microbial community development and from those with perturbed community development, or from their mothers. Clonally arrayed collections of cultured organisms are then generated from these microbial communities. The effects of different community configurations on host biology are tested by transplanting these collections, or subsets of the collections, into germ-free animals (mice or other species). Recipient animals are fed diets representative of those consumed by their microbiota donors, or diets designed to test hypotheses about the role of various components, including HMOs, on microbiota-mediated functions. Follow-up studies can be performed by assessing the transmission of microbial communities of interest and associated phenotypes to the offspring of these gnotobiotic animals.

Organizations, both private and public, that are committed to addressing global health challenges have already made investments that have enabled durable, trusting relationships to be established between health-care providers and such populations, as well as the infrastructure required to obtain informed consent and apply validated procedures for collecting and archiving biospecimens and associated metadata. Examples include the Global Enteric Multicenter Study (GEMS)[98]; the Etiology, Risk Factors, and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health (MAL-ED) Study[99]; and various water, sanitation and hygiene (WASH) programmes[100]. These investments should be leveraged for the proposed human microbial observatories, which will require sustained support given the extended period of observation. Effective and innovative strategies for achieving such durable support require expertise from multiple disciplines. In our opinion, the development of these strategies is a compelling challenge whose solutions have broad implications for obtaining answers to this biological question as well as myriad others related to the promotion of human flourishing (eudaimonia) in the broadest sense.

Wise and effective stewardship of human microbial resources is a responsibility that extends across generations and national boundaries. Knowledge of how microbial communities evolve in health and how their development is jeopardized or overtly disrupted provides an opportunity to discover strategies and tools for their timely repair. However, understanding how such repair can be achieved brings great responsibility. The immediate as well as long-term consequences of such interventions applied early in the course of a human life need to be determined. Rigorous tests of safety and efficacy have to be designed and applied in representative animal models when available. Thoughtful consideration must be given to the ethical, regulatory and societal issues and consequences that could arise from early interventions that shape the composition and function of our microbial communities. This is a time for inspiration and awe as we gain insight about how we function as holobionts. It is also a time for mindfulness and sobriety as we consider how to deliberately shape facets of our own developmental biology to improve wellness during our human lifecycle. ■

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