WHAT IS A HEALTHY MICROBIOME?

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Abstract

The development of new technologies has resulted in an explosion of studies of the gut microbiome. These studies have revealed a highly complex microbial community, forming an intricate ecosystem with the host and affecting many aspects of host health. In particular dysbiosis, an imbalance within the microbiome, is associated with a wide variety of diseases, and with ageing. Studies in laboratory animals show these links are not just associative, and that the microbiome can directly cause health and disease states in the host. These findings beg the question of what "healthy" microbiomes look like, and how we can use the microbiome to promote human health. Efforts to understand healthy microbiomes have revealed that microbiome composition varies widely between healthy individuals, and that there is no such thing as a single healthy microbiome. Current research shows that qualities of the microbiome ecosystem, such as diversity, robustness, resilience and ability to resist perturbations, are important for host health. Identification of the molecular basis of these qualities, as well as the genetic and biochemical functions of the microbiome ecosystems, will enable us to understand the core functions that define healthy microbiomes.

Keywords

Microbiome, microbiota, microbe, holobiont, health, ageing, disease, metagenomics, immunity

The holobiont – an ecosystem consisting of the human body and its microbes

The *microbiota* is the community of bacteria, fungi and viruses inhabiting different niches of the body, such as the skin, mouth, vagina and gut. The gut microbiota is by far the largest and most dense community of microbes that colonises our bodies, consisting of hundreds of microbial species which until relatively recently were largely unstudied and uncharacterised. The development of new technologies has resulted in an explosion of studies of the gut microbiome. These advances have revealed a highly complex microbial community. It has been estimated that the microbial cells that colonise the human body are at least as abundant as our own somatic cells, and that our bodies host between 500-1000 bacterial species (Sender, Fuchs and Milo, 2016). Each of these species has a genome containing thousands of genes, meaning that in addition to our biology being affected by our own genes, it is affected by millions/billions of microbial genes (Gilbert *et al.*, 2018). Thus, our biology is not only governed by our genes and our environment, but in fact by our genes, our environment *and* the genes and functions of our microorganisms. The combined genome of our microorganisms is referred to as the *microbiome*, and this is the term we will mostly be using in this text.

Gut microorganisms perform a diverse set of functions important for the host, such as extracting energy from a wide array of host-indigestible carbohydrates, producing vitamins, promoting immunity and preventing colonisation of the gut by pathogens. The presence of intricate interactions between a host and its microbes has led to the concept of the "*holobiont*", meaning the biological entity of a host and its associated microorganisms. Accordingly, the human body is an ecosystem highly affected by dynamic host-microbiome interactions and is a 'superorganism' rather than an individual (Simon *et al.*, 2019). The implication is that many aspects of our physiology and health are dependent on interactions within this ecosystem. Indeed, many studies have associated dysbiosis, that is disturbances of the composition of the microbiome, with a wide range of diseases, including intestinal disorders, metabolic syndrome, mood disorders and neurodegenerative diseases, suggesting that disturbances in the microbiome could be directly contributing to ill-health.

So, what do "healthy" microbiomes looks like, and how we can promote healthy microbiomes? These are important and unanswered questions, and there are huge efforts within the field to understand the relationship between the microbiome and health, and to move towards a better understanding of healthy microbiomes. One of the striking aspects of the microbiome is that there is huge variation in microbial composition between healthy individuals, even between genetically identical twins with similar lifestyles (Turnbaugh *et al.*, 2007). This suggests that healthy microbiomes come in many different shapes and forms, and

that what determines if a microbiome is healthy is far more complicated than simply its microbial composition.

The Renaissance of Microbiome Research

Research on the microbiome is booming – the number of studies mentioning "microbiome" or "microbiota" in their title or abstract was 11 in 1980, and has grown to over 13,000 in 2018 ('Hype or hope?', 2019). The reason for this explosion in microbiome studies is the development of affordable whole genome sequencing techniques enabling researchers to profile microbiomes and determine the identity of thousands of species. This is in stark contrast to early studies, in which microbes colonising the human gut were identified by the cultivation and characterisation of their physiological properties. The cultivation-based approaches favoured microbes that grow well in laboratory environments and resulted in a skewed view of the gut microbiome. In the late 1800s and early 1900s the perception was that all healthy adults share a core microbiota, consisting mainly of *Escherichia coli*, which can be isolated from most people and is easily cultivated in aerobic conditions (Rajilić-Stojanović and de Vos, 2014).

We now know that the vast majority of microorganisms in the human gastrointestinal tract are strict anaerobes, and that early cultivation studies only provided a partial view of the microbiome as it did not enable cultivation of anaerobes. In the 1970s, strictly anaerobic techniques were developed, allowing the recovery of more than 300 bacterial species from the gut. Studies during this period identified major gastrointestinal bacterial groups, including *Bacteroides, Clostridium, Eubacterium, Veillonella,, Bifidobacterium, Fusobacterium, Lactobacillus* and anaerobic *coccus* (Rajilić-Stojanović and de Vos, 2014; Lloyd-Price, Abu-Ali and Huttenhower, 2016). More recently, culture-independent techniques based on DNA sequencing provided molecular tools to identify microbial taxonomy. Early sequencing studies in the late 1990s showed a large diversity in the microbiome composition in healthy people. These early studies also found that the majority of the microbial DNA sequences did not match any documented species at the time, revealing not only unexpected diversity but also a large number of previously unstudied bacterial species, sparking a revived scientific interest in the microbiome (Rajilić-Stojanović and de Vos, 2014).

The microbiome research field evolved to another phase through the development of high-throughput sequencing techniques, including next-generation sequencing of entire genomic material of the microbiome. Next-generation sequencing showed that the human gut carries over 3 million microbial genes, mainly bacterial, of which the majority had not been previously characterised. The evolution of microbiome research has resulted in a view of the microbiota

composition which is quite different from the view that existed prior to the molecular revolution. There are now huge amounts of studies demonstrating associations between the composition of our microbiome and diseases, and large amounts of information regarding the complexity of the microbiome and variation between individuals. As these advances implicate that the microbiome plays an important role in host health, they have resulted in a huge interest in the microbiome both by the scientific community, the media and the general public. The current explosion in microbiome research has also resulted in scepticism and raised questions regarding the state of the field and whether microbiome research is overhyped. Is the role of the microbiome as extensive as associative studies suggest, or are the current expectations overoptimistic? Only by dissecting the genes and biochemical functions of our microbiomes and pinning down the molecular mechanisms underlying the interactions between our bodies and our microbiomes will we be able to move from association to causation and to establish a real understanding of healthy microbiomes (Hanage, 2014).

From cradle to grave – Changes in the microbiome throughout life

The microbiome changes throughout our life course, playing an important role in human development but also for health outcomes later in life. In early life, the microbiome plays a central role in development of the immune system, and also in the nervous system and bone tissue (Sommer and Bäckhed, 2013). In late life, immune dysfunction and dysbiosis contribute to inflammation and disease development.

Microbial diversity increases in early life

Humans babies are first colonised by their mothers' microbiomes and this transfer occurs mainly through birth, during which they are exposed to the vaginal microbiome of the mother, and through breast feeding, exposing them to microorganisms in the breast milk. Babies born by C-section have microbiomes that differ from those with vaginal delivery and resemble skin microbiomes. Feeding babies with formula is also likely to give rise to microbiomes that differ from microbiomes from breast milk (Kundu *et al.*, 2017). What the implications of different delivery and feeding methods for new-borns are on microbiome composition and overall health in later life are important and largely unanswered questions.

As infants start feeding on solid foods and rely less on breast milk, they are exposed to more microbes and the relatively simple microbiome acquired from the mother matures into a more complex microbiome (Figure 1). During the first three years of life the microbiome undergoes a dramatic shift, from domination by *Bifidobacteria*, bacteria suited to process carbohydrates

from milk, to a wider variety of species. During childhood and puberty the microbiome continues to develop and increase in complexity, until adulthood, when it reaches relative stability (Kundu *et al.*, 2017). However, the human microbiome is highly dynamic and the composition can change in response to diet and other factors within hours and days (Gilbert *et al.*, 2018), adding to the complexity of studying microbiome composition.

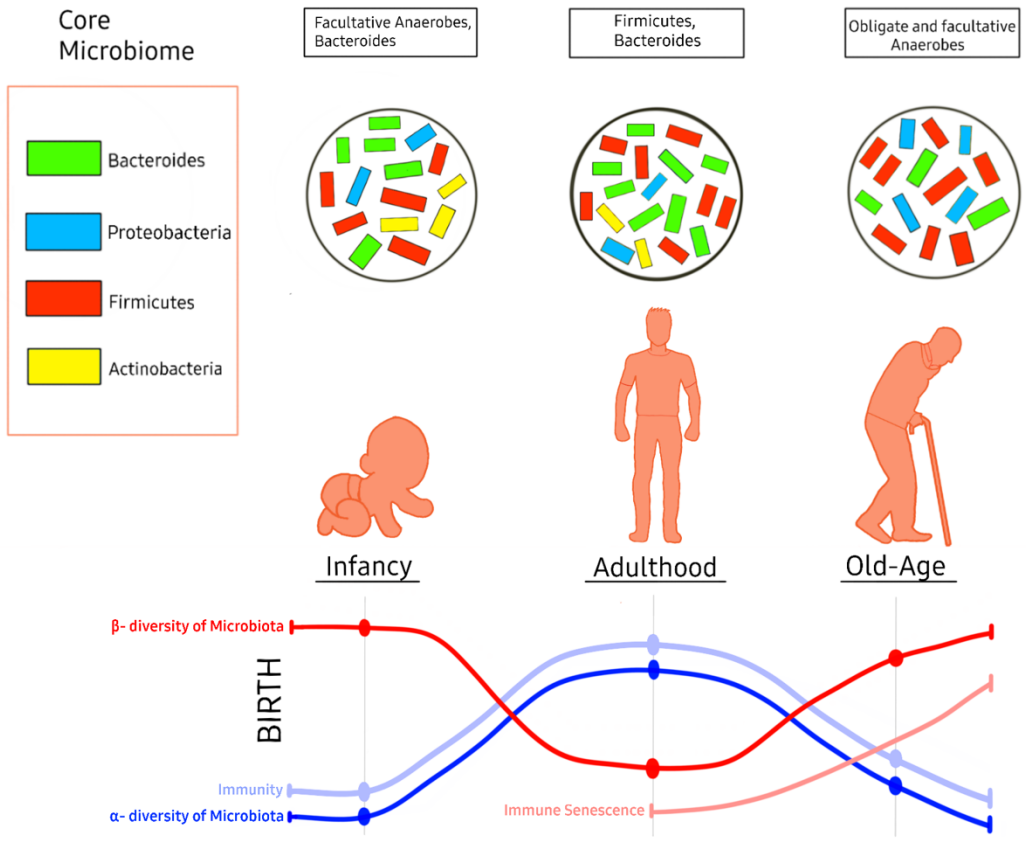

Figure 1: The microbiome throughout the life course. Human foetuses are largely sterile, and babies are first colonised by their mothers' microbiomes during birth and through breast feeding. During early life, the microbiome undergoes a dramatic shift, increasing in diversity and including a wider variety of species (α-diversity). During childhood and puberty, the microbiome continues to develop and increase in complexity until adulthood, when it reaches relative stability. As we get old, the composition of the microbiota changes again, resulting in decreased diversity, expansion of pathogenic bacterial species and alterations in microbial functionality, while variation between individuals (ß-diversity) and immune senescence increase.

Microbial diversity decreases in late life

After relative stability during adulthood, the microbiome changes again as we get old. Microbiotas in healthy individuals are characterised by high bacterial taxonomic diversity, but during ageing the composition of the microbiota changes, resulting in decreased diversity, expansion of pathogenic bacterial species, and alterations in microbial functionality. Also, variation between individuals increases, meaning that age-related changes are different in different individuals and might be linked to individual ageing trajectories (Vaiserman, Koliada and Marotta, 2017; Nagpal *et al.*, 2018) (Figure 1 and Figure 2). Studies in which the microbiomes of centenarians, that is people who live to a 100 years of age or more, have shown that centenarians have microbiomes more similar to those of young adults than other elderly people, and other studies indicate that age-related frailty is associated with particular bacterial species/genera (Biagi *et al.*, 2010; Nagpal *et al.*, 2018). These studies link microbiome composition to ageing, and raise the question whether changes in the microbiome directly contribute to the ageing process or are merely a result of it. In addition to changing with age, microbiome composition is strongly affected by lifestyle, and by diet in particular. The implication is that we could use diet to acquire a healthy microbiome, but also that some of the detrimental effects that diet have on health might be acting through the microbiome.

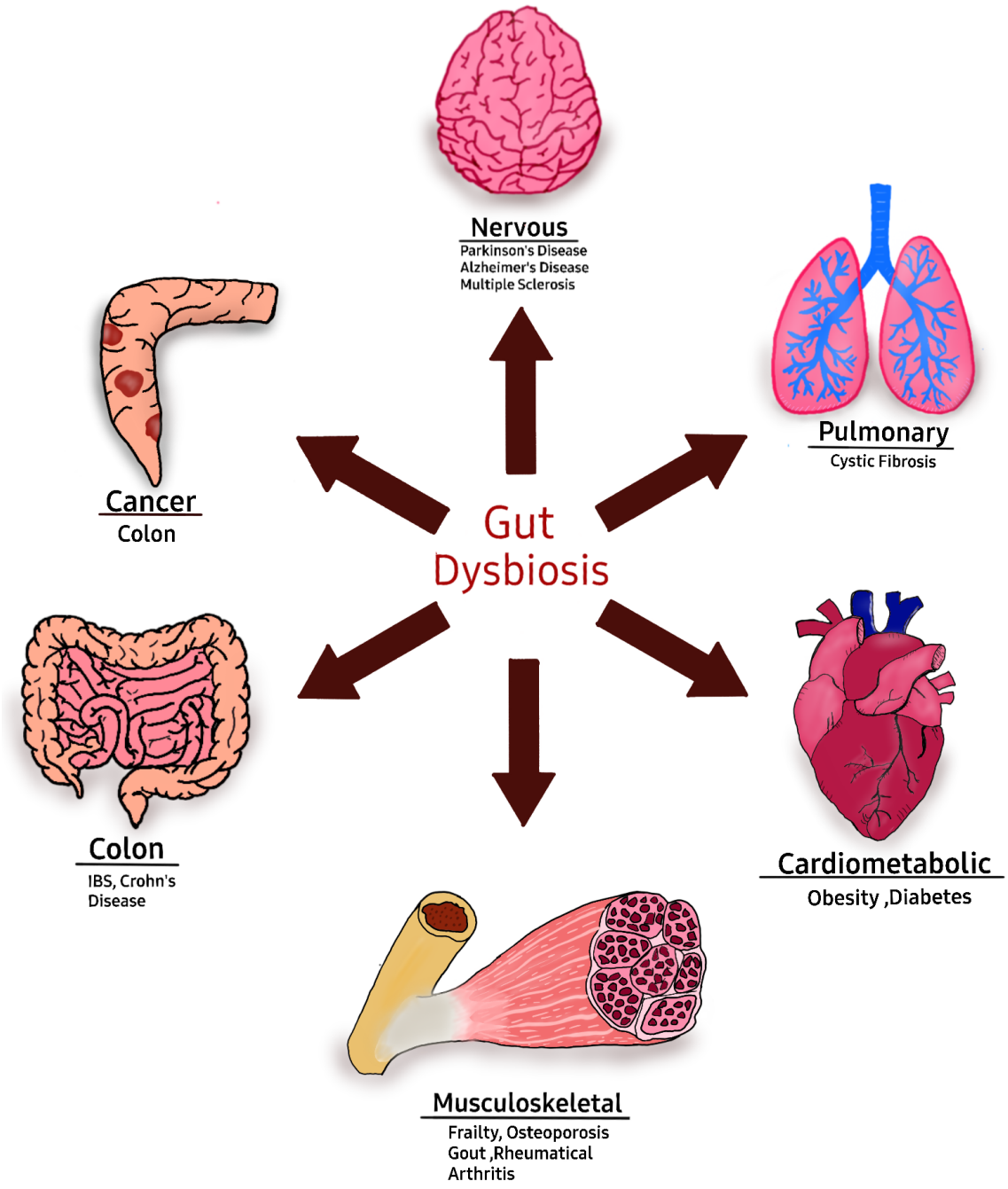

Figure 2: Dysbiosis contributes to ageing and disease. The symbiotic relationship between the microbiome and the host is central to host health. Disruption of this relationship contributes to dysbiosis and imbalanced immune responses, resulting in autoimmune and inflammatory diseases. During ageing, a negative cycle of immune senescence and dysbiosis result in chronic inflammation, fuelling age-related diseases.

Are we losing our healthy microbiome? The disappearing microbiota hypothesis

A striking example of how the microbiome is connected to lifestyle is the difference in microbiome composition in different geographical populations. People living in Western industrialised countries have reduced gut microbiome diversity compared with native populations living traditional lifestyles, such as hunter-gatherers. The Hadza in Tanzania are one of the last remaining hunter-gatherers in Africa, hunting animals and foraging berries, roots and plants, and living on a diet consisting of around 600 plant and animal species (Spector and Leach, 2017). Compared to European urban dwellers they have distinct microbiomes with higher levels of microbiome diversity. The Hadza share microbiome features, such as bacterial families, with other traditional societies across the globe, meaning that these bacteria are not a feature of the local environment, but of adaptations to the host. Uncontacted Amerindians have substantially higher bacterial diversity than urban US populations, but also higher than semi-transculturated Amerindians (Yatsunenko *et al.*, 2012; Dominguez-Bello *et al.*, 2016). These microbiomes that are preserved across traditional populations are lost in industrialised nations, suggesting that a modern lifestyle has resulted in some bacteria going extinct in the human gut, an idea called the "disappearing microbiota hypothesis" (Schnorr *et al.*, 2014; Smits *et al.*, 2017; Fragiadakis *et al.*, 2019).

What does the loss of our indigenous microbes mean for our health? As the large majority of studies of the human microbiome has been conducted in modern societies, we know very little about the bacteria that have been extinct by our modern lifestyle and we have no understanding of their biological functions. But there are good reasons to think that these microbes are beneficial for us and that their loss might have contributed to illnesses such as asthma, allergies, celiac disease and obesity (Dominguez-Bello *et al.*, 2016). Most of the microbes in our microbiota appear to be host-specific microbes that need their host in order to survive. This means that the microbes benefit from the reproductive success of their host and that natural selection will favour microbes that have beneficial effects on the host, improving the success of both the host and the microbes. Indeed, the existence of indigenous microbiomes living in symbiosis with the host is ancient. Co-evolution of animals and bacteria can be traced back in our evolutionary tree and has existed for more than 800 million years, and there are many examples of how co-evolution provides the hosts with evolutionary advantages. Microbes produce essential vitamins, protect against pathogens and aid digestion. Overall, their beneficial activities affect many metabolic, physiological and immunological functions in our bodies (Blaser, 2006).

The disappearing microbiota hypothesis parallels and competes with the well-known hygiene hypothesis, which suggests that improved hygiene and access to vaccines and

antibiotics in modern society reduces exposure to parasites and pathogens early in life. This lack of pathogen exposure affects the development of the immune system, skewing towards autoimmune responses and resulting in an increase of allergic and autoimmune disorders in later years (Blaser, 2006). The degree to which bacteria extinct in Western societies play a role in our health remains unknown, but that gut microbes play an important role in host health is becoming increasingly clear.

Emerging links between the microbiome and host health

The recent development of sequencing techniques has enabled profiling of microbiomes in humans and resulted in an explosion of studies demonstrating associations between the composition of our microbiome and many different diseases. Some of these links are not surprising (intestinal infections and inflammatory bowel diseases), whereas others are less expected (obesity, cardiovascular disease) or completely unexpected (major depression, neurodegenerative diseases and autism spectrum disorder) (Gilbert *et al.*, 2016, 2018; Sharon *et al.*, 2016).

Protection against intestinal infections

For some diseases the links are well-established and direct, and microbiome-based approaches are already being used as treatments. The most striking example is intestinal infections with the bacteria *Clostridium difficile*. *C. difficile* infections are characterised by severe diarrhoea and can be deadly – an estimated 15,000 deaths a year in the United States alone are directly attributable to *C. difficile* infection. The first-line treatment for *C. difficile* infections is antibiotics, which wipes out *C. difficile* but also a large part of the healthy microbiome. Antibiotics leave the patients susceptible to new infections as *C. difficile* can easily establish itself in absence of a healthy microbiome, and about 20% of patients have reoccurring *C. difficile* infections. Faecal microbiota transplants are now becoming accepted as an effective treatment. Controlled trials of faecal transplants, in which stool from a healthy donor is transplanted to help re-establish a healthy microbiota, have reported over 90% efficacy in clearing reoccurring *C. difficile* infections. Due to the complexity and variability in donor stools, combined with a limited understanding of the ecological forces that shape the microbiota, faecal transplants are not free of risk for the receiver. For these reasons, next-generation microbiota-based medicines will likely become the preferred option. As we learn more about how the microbiome protects the host against pathogenic infections, defined

interventions with rationally selected mixtures of microorganisms or their products that can be more reliably managed will be used (Giles, D'Adamo and Forster, 2019; Hui *et al.*, 2019).

Metabolic syndrome

Less expected is that the gut microbiome is associated with metabolic diseases such as obesity, type 2 diabetes and cardiovascular disease. The microbiomes of obese and lean people differ in striking ways; obesity, insulin resistance and fatty liver disease are associated with less microbial diversity and higher levels of particular bacterial groups e.g. *Firmicutes*. Interventions that induce weight loss and improve metabolic functions in both animals and humans result in shifts in microbiota composition, indicating that the microbiome plays a role in metabolic disorder. More direct evidence suggesting that the microbiome directly influences metabolic function in the host includes faecal transfer of microbiotas into recipient human and animal hosts, which in some cases faecal transfer results in recapitulation of the metabolic phenotype of the donors (Everard *et al.*, 2013).

So how does decreased microbial diversity contribute to metabolic disease and weight gain? A dominating current hypothesis is that an abnormal microbiome damages the gut barrier that keeps toxins and pathogens from crossing into the bloodstream. When this occurs, it can set off a cascade of inflammation, contributing to insulin resistance, cardiovascular disease and autoimmune conditions. A diverse microbiome protects and maintains the gut barrier and we are now learning about bacterial species and products that play a role in this protection.

Akkermanisa muciniphia – a bacteria with beneficial effects on host metabolism

One such species is *Akkermanisa muciniphia* (*A. mucinphilia). Akkermansia* is a well-known health-associated genus protecting against inflammation and promoting a healthy metabolic homeostasis. In particular, the species *A. mucinphilia* has been demonstrated to have beneficial effects. Abundance of *A. mucinphila* is decreased in the microbiota of obese animals compared to lean animals and in middle-aged mice. In old animals it has almost completely disappeared (Anhê and Marette, 2017). Oral administration of *A. muciniphila* reverses obesity and related complications in obese mice and strengthens the gut barrier, but surprisingly, pasteurised *A. muciniphila* is even more beneficial. Unlike live *A. muciniphila*, treatment with the pasteurised bacterium has additional beneficial effects, including decreasing adipocyte diameter and a decreased capacity of the host to harvest energy from the diet (Ottman *et al.*, 2016).

Remarkably, *A. muciniphila* has beneficial effects on the host through a single bacterial protein, Amuc_1100, which is highly abundant and located on the pili (a hair-like appendage

found on the surface of many bacteria) on the outer membrane of the cells. Amuc_1100 can be produced and purified in a laboratory, and when administered to animals it reproduces the beneficial effects of pasteurized *A. muciniphila* on obesity and many related parameters such as plasma-triglyceride levels, glucose tolerance and insulin resistance. It also improves the gut barrier function (Plovier *et al.*, 2017). Further testing of Amuc_1100 and other bacterial molecules will reveal if bacterial proteins and compounds can be produced in laboratory settings and used as drugs for their beneficial properties on human health, independently of the gut bacteria that originally produced them. This will enable a more direct and controlled way to use microbiome-based therapies for human health.

Brain and nervous system disorders

For some diseases associated with the microbiome it can be challenging to envision possible causative links at first, such as autism spectrum disorder, major depression and neurodegenerative diseases. However, the gastrointestinal tract and the brain are physically and chemically connected to each other. The vagus nerve directly connects the gut with the brain, providing a means of neuronal transmission between these two organs. In addition, gut microbes produce chemical compounds, including neurotransmitters that directly affect neuronal activity, metabolites with neuroactive properties and other molecules that can be transported through the circulation and reach the brain and other organs (Sherwin, Dinan and Cryan, 2018). Considering that a direct bidirectional crosstalk between the brain and the gut has been demonstrated, with reciprocal influence on each other's physiology and function, it is not surprising that microbiome composition can affect brain health and brain function.

There is evidence, although preliminary and mostly from animal models, for a role for the microbiome in neuropsychiatric conditions, including major depression, autism spectrum disorder, schizophrenia and neurodegenerative diseases such Parkinson's and Alzheimer's diseases (Sharon *et al.*, 2016). Animal models allow controlled experiments such as generating germ-free animals and performing faecal transplants. These kinds of experiments have demonstrated that gut microbes and the molecules they produce can promote or inhibit brain and nervous system disorders. They also show that the crosstalk between the gut microbiome and the brain is a complex network of interactions that we are only starting to understand.

The immune system – the mediator of healthy microbiomes

It is well established that interactions between the microbiome and the immune system play vital roles for host health. The lining of the gut is the largest surface area of human body that

comes in direct contact with the foreign material. The immune cells of the mucosal immune system are positioned throughout the length of the gut and are in active cross-talk with the rest of the immune system through local lymph nodes. The gut-associated immune system needs both to tolerate the microbiota, preventing harmful systemic immune responses and at the same time control the microbiota, preventing growth of microorganisms and translocation outside the gut. The microbiome plays a fundamental role in the development and training of the host immune system in early life (Sommer and Bäckhed, 2013).

Microbes and the molecules they produce are constantly sensed by the immune system, resulting in immune responses that have effects far beyond the gut, and that can promote or inhibit pathological conditions. Immune imbalances are likely mechanisms explaining the link between the microbiome and many diseases by causing inflammation. A healthy microbiome thus might be a microbiome that promotes healthy and appropriate immune responses, in contrast to inappropriate immunity causing pathology. With age a negative spiral of dysbiosis of the microbiome and age-related dysfunction of the immune system contributes to sterile, chronic inflammation and age-related diseases (Figure 2) (Nagpal *et al.*, 2018).

What is a healthy microbiome?

Microbiome researchers have sought to answer this question, and several hypotheses have been formulated. An early suggestion was that a healthy microbiome consists of a core of microbial taxa which are universally present in healthy individuals, and that an absence of these core microbes would result in dysbiosis and disease. But studies of microbiome diversity, including The Human Microbiome Project, an initiative funded by the National Institute of Health to improve our understanding of microbiome effects on health, revealed an unexpected amount of variation in microbiome composition in healthy individuals (Turnbaugh *et al.*, 2007). These studies suggested that there are many different types of healthy microbiomes of different compositions, and that characterising a "healthy" microbiome as an ideal set of specific microbes is therefore not a practical definition (Lloyd-Price, Abu-Ali and Huttenhower, 2016).

A core of microbial functions required for host health

An alternative hypothesis is that it is not the exact identity of microbes in the microbiome that matters, but rather their metabolic functions. Gut microorganisms carry their own genomes and perform a set of biochemical and metabolic functions, and there are many examples of microbial species that perform functions with positive effects on host physiology. Each of these functions does not necessarily always need to come from the same microbial species, and

possibly a healthy microbiome consists of a core of functional reactions, rather than a core of microbial taxa or species (Shafquat *et al.*, 2014). It is becoming clear that healthy microbiomes come in many different shapes and forms and that what determines if a microbiome is healthy is far more complicated than simply its microbial composition (Lloyd-Price, Abu-Ali and Huttenhower, 2016).

Robustness and resilience: Important determinants of healthy microbiomes

Another important characteristic of a healthy microbiome may be its behaviour over time, in particular its resilience to internal and external changes and stresses. Perturbation of the composition of the microbiome, dysbiosis, is associated with a long list of diseases. Dysbiosis contributes to inflammation, providing an explanation of how the microbiota might affect diseases. It also explains links between the microbiome and ageing (López-Otín *et al.*, 2013). Age-related inflammation is common among the elderly, and differs from the acute inflammation caused by pathogens, in that it is a chronic inflammatory response involving activation of the immune system in the absence of infection. One of the factors triggering age-related inflammation is dysbiosis. Our microbiome becomes less diverse with age and it has been proposed that a negative cycle of decreased gut function, suppressed immune function and imbalance of the microbiome results in gut dysfunction and increased gut permeability. This allows microbial metabolites to cross the intestinal barrier, resulting in additional inflammatory responses, including inflammation of the nervous system (Vaiserman, Koliada and Marotta, 2017; Nagpal *et al.*, 2018) (Figure 2), and other processes central to ageing, such as the accumulation of misfolded proteins and mitochondrial dysfunction (Biagi *et al.*, 2010; Kim and Jazwinski, 2018; Nagpal *et al.*, 2018). Thus an essential aspect of healthy microbiotas is relative stability in terms of resistance to perturbations and the ability to recover from perturbations (Lloyd-Price, Abu-Ali and Huttenhower, 2016).

Understanding how microbial species and products shape host health

The changes in microbiome composition that have been observed in different diseases range from changes in a single bacterial strain resulting in the production of a single bacterial metabolite to changes at the phylum and genus level, or in microbial communities in the gut (Gilbert *et al.*, 2016). How these microbial changes translate to altered health in the host is the major research question within the microbiome field – in order to understand what a healthy microbiome is we need to pinpoint the mechanisms underlying the links between microbiome composition and health. For example, is it the overall diversity of microbes that are beneficial,

or particular taxa, or specific communities? The biological functions of any organism are encoded by its genome, so it is likely that the genetic diversity or composition plays an important role, and that specific biochemical reactions and metabolic functions of the microbiome play a crucial part in shaping host health. Identifying the bacterial proteins and metabolites affecting human health and understanding how microbial species and communities act in terms of their biochemical functions will allow us to learn more about what a healthy microbiome looks like. So how can we drill down the mechanisms underlying host-microbiome interactions and their effects on human health, including microbial genes and functions and community effects?

Transition to an understanding of healthy microbiomes

The microbiome is enormously complex, consisting of thousands of species and millions of microbial genes. Typically, studies in humans yield correlations between microbiome composition and disease states, but determining causality and mechanisms requires performing experiments in laboratory conditions. Considering the technical and ethical limitations of human experimentation, laboratory animals are widely used. An important aspect of microbiome research is the ability to generate germ-free sterile animals which can be colonised with microbes of choice in order to generate comparisons between animals with and without microbiomes but also animals colonised with specific microbiomes, allowing direct testing of causative relationships (Fritz *et al.*, 2013; Kim and Jazwinski, 2018). Studies using these approaches in a variety of animal species show that that the microbiota is a direct cause of a number of different aspects of health, ranging from diseases and physiology to biological processes such as behaviour and ageing and longevity (Kim and Jazwinski, 2018).

Gathering evidence from natural host-microbiota interactions in a variety of animals

Eukaryotic organisms, from the first unicellular eukaryotes to complex multicellular animals, have repeatedly entered into symbiotic relationships with microorganisms, enabling them to exploit otherwise unavailable habitats and unsuitable diets. This means there are a variety of animals of a wide range of complexities, ranging from nematodes to humans, that can be used to study host-microbiome interactions. These include invertebrate and lower vertebrates associated with microbiomes of lower taxonomic diversity than in mammals (Douglas, 2018), and three traditional simple model organisms: the fruit fly *Drosophila melanogaster*, the zebrafish *Danio rerio* and the nematode worm *Caenorhabditis elegans*, which are very well-suited for laboratory studies. Compared to mammals, these simple systems enable

straightforward protocols to manipulate the microbiota and assign function to individual microbial taxa, allow for cost-effective experiments over short timescales, and enable complex experimental designs to investigate how host-microbiome interactions affect fundamental animal processes such as development, immunity and neurobiology.

For these reasons, in particular *C. elegans* and *Drosophila* are attracting increasing attention in microbiome research. Non-traditional models are also yielding insights into host-microbiome interactions. These include the honeybee *Apis mellifera*, the freshwater polyp *Hydra vulgaris*, the Hawaiian bob-tailed squid *Euprymna scolopes*, the wax moth *Galleria mellonella*, crustacean species belonging to the genus *Daphnia*, the medicinal leech *Hirudo medicinalis*, the sea anemone *Nematostella vectensis* and the turquoise killifish *Nothobranchius furzeri* (Smith *et al.*, 2017; Zhang *et al.*, 2017; Ezcurra, 2018; Douglas, 2019). Microbiome studies in this wide range of animals are yielding novel insights into the multiple ways in which individual microorganisms and microbial communities influence host physiology, illuminating the cellular and molecular processes underlying these interactions.

By studying natural host-microbiota interactions in a variety of animal species we are learning about general principles governing host-microbiota interactions. These interactions are largely biochemical. Microbial effects are generally mediated by the release of bioactive molecules (metabolites, proteins, lipids and small RNAs) from microbial cells, and acting on host cellular and molecular pathways to modulate host physiology.

For example, animals without a gut microbiome have a hyperactive movement pattern, with increased movement speed and longer periods of movement. This has been shown in mice, zebrafish and *Drosophila*. A study in *Drosophila* demonstrated the mechanisms underlying how the microbiome affects host behaviour. In flies, the gut bacterium *Lactobacillus brevis* produces a sugar-converting enzyme, xylose isomerase, resulting in reduced levels of the sugar trehalose and increased levels of another sugar, ribose. These changes in sugar levels interact with neurons within the central nervous system, thereby influencing activity of the animal (Schretter *et al.*, 2018; Douglas, 2019). Although this research is not directly translatable to humans, it provides a mechanistic understanding of how the microbiome can affect host physiology. Discoveries made with simple systems are important because they can be used to construct precise hypotheses of function in less tractable models such as rodents. The identification of interactions that are evolutionarily conserved can then be used to formulate hypotheses about interactions likely to also affect humans.

Understanding microbial functions affecting human health

The major challenge in the microbiome field is pinning down host-microbiome interactions affecting human health. This will require gathering data from population-based microbiome studies and performing hypothesis-driven experiments in model organisms to investigate particular microbial species and functions to reveal causal relationships. These findings can then be used for intervention studies (Gilbert et al 2018). Transforming microbiome research from a descriptive to a causal and finally to a translational science will require researchers from different disciplines working together and combining data generated from population-based microbiome studies in humans, mechanistic studies in animal models and microbiological studies of the genes, pathways and functions of individual microorganisms and microbial communities.

It will be particularly important to identify bacterial *functions* and map how they relate to metagenomic studies in order to identify bacterial pathways that contribute to host health. The human microbiome encodes at least 100-fold more genes than their human hosts and produces an extraordinary array of structural components, cell surface molecules, and metabolic enzymes and by-products. Half of these microbial genes are unknown or have poorly characterised functions. It is estimated that at least 10% of all circulating metabolites in the human body are microbially derived and play a role in a variety of human functions (Joice *et al.*, 2014). An important challenge for microbiome research is therefore to determine the identity and function of these microbial products. Understanding the functions of the microbiome involves formulating hypotheses regarding their function and validating them by testing the bioactivity of microbial cells, microbial lysates and purified microbial compounds and proteins on model organisms, organoids or cell culture. Assessment of bioactivity can be e.g. enzymatic activity, immune cell activation, or physiology in animal models. Bioinformatic and statistical methods can be used to assess putative functions of microbial proteins (Joice *et al.*, 2014). The large number of unknown genes in our microbiome dramatically inhibits our understanding of healthy microbiomes and how the microbiome contributes to health and disease. But it is also a fantastic opportunity to identify microbial products involved in microbe-microbe and host-microbiome interactions affecting health.

Utilising the microbiome for human health – hype or hope?

Many human diseases have been suggested to be linked to the microbiome: inflammatory bowel disease, cancer, diabetes, obesity, atherosclerosis, fatty liver disease, malnutrition, autism, Alzheimer disease, depression, autoimmunity, asthma and more (Figure 2). The implication is that the microbiome could be affecting many, if not all, aspects of human health.

As a result, microbiome research has received a massive amount of attention in the media, with claims that there is a 'microbiota fix' for everything from gastrointestinal issues to mental health problems. Moreover, there are large numbers of microbiome-based services offering profiling of personal microbiomes, and a range of commercial microbiome-based therapeutics and products, including pro- and prebiotic products and DIY faecal transplants, with little to no scientific evidence backing up the claims of their health benefits. These developments have resulted in many microbiologists raising cautionary voices, warning both for hype within the microbiome field, and for the health risk associated with non-clinical use of e.g. faecal transplants (Hanage, 2014; Ma *et al.*, 2018)

Dietary interventions as means to improve the microbiome

The microbiome is becoming an increasingly attractive target for potential therapeutics and has a huge potential to improve health. It is possible to manipulate the microbiome through diet, prebiotics, probiotics and faecal transplants. Lifestyle, and *diet* in particular, has a significant and immediate effect on microbiome composition. Varied diets high in plants and fibre and low in processed foods promote diverse and resilient microbiomes (Sonnenburg and Sonnenburg, 2019).

One central function of the microbiome is to digest carbohydrates that cannot be digested by the host, most commonly dietary fibre. The beneficial role of dietary fibre on human health has been known for decades, but recent studies are suggesting that the missing mechanistic explanation for the beneficial effects of dietary fibre may be largely attributed to digestion by the microbiome. There is a vast number of carbohydrates of different chemical composition and with different properties, and different gut microbes specialise in breaking down specific types of carbohydrates. This means that carbohydrates act as selective agents, altering the composition of the microbiome, but also dictating functional and metabolic output. Multiple studies have shown that diet acts as a potent force in shaping the microbiome; quantity and type of carbohydrates alter the gut microbiome, promoting certain species and taxa. Fibre-rich diets promote microbes that produce short-chain fatty acids, which are important mediators of microbiome effects on physiology, and have beneficial effects on host health by, for example, regulating metabolism and reducing inflammation. As this is a very active research field, we will certainly be learning more about how specific foods and carbohydrates can be used to boost microbial metabolic activities and acquire healthy microbiomes (Sonnenburg and Sonnenburg, 2014).

Microbiome-based interventions as a route to health

In addition to diet, probiotics and prebiotics are increasingly popular. *Probiotics* aim at altering the microbial composition of the gut through exogenous administration of live microbes. *Probiotics* are widely used but there is little convincing evidence for their efficacy. *Prebiotics* are compounds that are consumed with the intention of affecting microbiome composition or function in a beneficial way. Prebiotics, like probiotics, are currently a relatively unspecific approach to microbiome-based interventions, and further studies are needed to fully characterise the effects of prebiotics on different bacterial species. As we learn more about the biology of the microbiome and host-microbiome interactions, the use of prebiotics will be evidence-based and more precise. *Faecal transplants* involve transplanting faeces containing microbes from healthy individuals to ill or old people to restore balance of the microbiome and is also gaining momentum. Grotesque as it might sound, there is increasing evidence in both animals and humans supporting this avenue to improve health. Currently faecal transplants are mainly being used as a complement to antibiotics to treat *C. difficile* infections, but future research is likely to identify other instances where transplants from healthy donors can safely be used to achieve benefits for health.

Bypassing the microbiome through secreted microbial metabolites

Alternatively, completely bypassing the microbiome and instead utilising microbial compounds and metabolites as pharmaceuticals offers a more controlled approach. Research over the past few years has revealed that the intestinal microbial community exerts much of its impact on host physiology through the secretion of small molecules that modulate cellular and organismal functions of the host, targeting host genes and proteins. These small molecules serve as an effective means of communication in host-microbe interactions. Rather than targeting the aberrant microbial composition, exogenous administration or inhibition of metabolites has the potential to counteract and correct the negative effects of imbalances of the microbiome.

Metabolite-based interventions are therapeutically attractive for several reasons. Metabolites are physiologically abundant at high concentrations, and thus the potential for toxicity is low. In contrast to the administration of live organisms, their dosage and routes of administration follow the principles of pharmacokinetics. Moreover, metabolites are present at most body sides and thus suitable for different routes of administration. Additionally, metabolites are generally stable in the systemic circulation and thus amenable for scalable modulation of their concentration. Metabolomics, that is the identification and characterisation

of all metabolites present in a sample, is becoming a routine approach in the microbiome field. Combined with other techniques, it will enable researchers to define functional signatures for disease states that have so far been associated only with compositional and metagenomic changes. Although it is early days for metabolite-based therapeutics, this strategy is highly promising, and concerted efforts over the coming few years may well result in the development of treatments for microbiome-associated diseases (Wong and Levy, 2019). In addition to the delivery of bacterial products, next generation microbiome-based interventions will involve using synthetic biology to engineer bacteria to produce certain compounds or proteins (Sonnenburg, 2015).

The potential of the microbiome for health

Among the general public there is increasing enthusiasm and interest in using products to modify one's individual microbiome to achieve a "healthy" microbiome, but we do not yet know what defines a healthy microbiome. Clinical translation of microbiota-based therapies has been slow, with one of the main obstacles being lack of a mechanistic understanding of the metabolic and ecological interactions between microorganisms within the microbiota and interactions with the host. Nonetheless, the microbiota holds huge potential to understand human health and as a source of novel therapeutics. The microbiome research field has now reached a critical inflection point and is transitioning from descriptive and associative studies towards understanding the underlying mechanisms of action and developing new evidence-based microbiome interventions (Gilbert *et al.*, 2018). This transition enables us to address critical questions regarding the microbiome and health and design interventions to establish and maintain healthy microbiomes.

Moving towards an understanding of healthy microbiomes

It is becoming clear that there is no such thing as a single healthy microbiome, and that there are huge variations in microbial composition between healthy individuals and between populations. Healthy microbiomes come in many different shapes and forms, and what defines a healthy microbiome is far more complicated than its microbial composition. Characterising a "healthy" microbiome as an ideal set of specific microbes is therefore not a practical definition. A better way may come from defining microbiome qualities. The qualities of healthy microbiomes include the *diversity* of microbial species, *robustness*, and *resilience* to internal and external stresses. These factors protect the host against imbalances in the microbiome which have been associated with a wide range of diseases.

It is likely that it is not the exact identity of microbes in the microbiome that matters, but rather their metabolic functions. Gut microorganisms carry their own genomes and perform a set of biochemical and metabolic functions, and there are many examples of microbial species that perform functions with positive effects on host physiology. Each of these functions does not necessarily always need to come from the same microbial species, and possibly a healthy microbiome consists of a core of biochemical and metabolic functions, rather than specific microbial taxa or species. Only by dissecting the genes and biochemical functions of our microbiomes and pinning down the molecular mechanisms underlying the interactions between our bodies and microbial functions, will we be able to move from association to causation and to establish a molecular understanding of host-microbiome interactions. Our current understanding of healthy microbiomes is quickly evolving, and during the coming years and decades we will develop a real understanding of healthy microbiomes.

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