REVIEW

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Current understanding of the human microbiome

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Our understanding of the link between the human microbiome and disease, including obesity, inflammatory bowel disease, arthritis and autism, is rapidly expanding. Improvements in the throughput and accuracy of DNA sequencing of the genomes of microbial communities that are associated with human samples, complemented by analysis of transcriptomes, proteomes, metabolomes and immunomes and by mechanistic experiments in model systems, have vastly improved our ability to understand the structure and function of the microbiome in both diseased and healthy states. However, many challenges remain. In this review, we focus on studies in humans to describe these challenges and propose strategies that leverage existing knowledge to move rapidly from correlation to causation and ultimately to translation into therapies.

The microbial cells that colonize the human body, including mucosal and skin environments, are at least as abundant as our somatic cells[1] and certainly contain far more genes than our human genome (**Box 1**). An estimated 500–1,000 species of bacteria exist in the human body at any one time[2], although the number of unique genotypes (subspecies) could be orders of magnitude greater than this[3]. Each bacterial strain has a genome containing thousands of genes, offering substantially more genetic diversity, and hence more flexibility, than the human genome. However, different people harbor radically different collections of microbes with densities that vary substantially even among conserved taxa, and little is understood about what leads to variation and what regulates it. Importantly, we do not yet understand how the variation within a person over time or that between different people influences wellness, the preservation of health or the onset and progression of disease. However, we do know that changes in the microbiome, the microbial metabolome and their interaction with the immune, endocrine and nervous systems are correlated with a wide array of illnesses, ranging from inflammatory bowel disease[4–6] to cancer[7] to major depressive disorder[8,9].

Human microbiome investigations have now reached a critical inflection point. We are transitioning from description and investigation to understanding the mechanism of action and developing new clinical interventions on the basis of this understanding[10]. These advances have also created a surge in translational research, resulting in substantial private investment not only in academic research, but also in the private sector, including so-called 'big pharma'. This drive toward clinical microbiome studies is supported by a revolution in personalized medicine, in which, for example, the decline in the cost of cancer genome sequencing is allowing the rapid identification of the precise treatment regimen that will lead to a positive outcome in an individual patient with a disease, such as with colorectal cancer[11]. Our ability to rapidly and reproducibly characterize the microbiome offers an opportunity to develop new diagnostic biomarkers and therapeutics, for example in cancer treatment.

Here we present the current state of knowledge linking the microbiome to human disease. We have focused on human studies when possible, but we also highlight select mouse studies when human studies were not available. This is to provide a platform from which the future of applied clinical microbiome research can be explored.

We will strategize on how to progress from the correlative and biomarker studies toward studies that will reveal the underlying mechanisms and opportunities for new preventive and therapeutic modalities.

Factors influencing the human microbiome

To alter the microbiome deliberately for preventive or therapeutic purposes or to use it to understand a particular medical condition, the factors that influence its composition must first be understood.

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Box 1 How many microbial cells and genes colonize a human?

Although it is frequently reported that our microbes outnumber our own cells in a 10:1 ratio, this figure stems from a 1972 article that used a 'back-of-the-envelope calculation' to arrive at this number[84]. A more prosaic figure of between 5 and 724 × 10^{12} human cells and between 30 and 400 × 10^{12} bacterial cells was provided by Rosner[85]. More recently, a refined estimate based on experimental observation and extrapolation actually arrives at a ratio of 1.3 bacterial cells for every 1 human cell[1]. However, these estimates don't take into consideration the fungi, viruses and phages present in various body environments, which, in the case of viruses and phages, could equal bacterial estimates or, more likely, could outnumber them by at least an order of magnitude[86]. Although these estimates reduce the extent to which microbial cells outnumber human cells, they do not reduce the estimates associated with the diversity of microbial life associated with the human body. Bacteria and other microbes, including archaea, fungi, and arguably, viruses, are extremely diverse. A similarly rough estimate of 1,000 bacterial species in the gut with 2,000 genes per species yields an estimate of 2,000,000 genes, which is 100 times the commonly estimated figure of approximately 20,000 human genes[2]. This agrees well with the actual size of microbial gene catalogs obtained by MetaHIT[87] and the Human Microbiome Project[13].

We have reviewed many of these factors in detail recently[10,12], so we provide only a brief summary here.

Human genetics and immune interactions in early development

The composition of the human microbiome is unique in each individual, and the differences among individuals are larger than the typical biochemical differences that arise within a person over time[13,14]. Identical twins are barely more similar to one another in microbial composition and structure than are nonidentical twins[15], at least over the range of environmental factors captured in studies to date. This suggests that the effect of the human genome is limited and that most of the assembly of the microbial community may be determined through environmental factors. Early underpowered studies suggested that monozygotic twins were no more similar in terms of their overall gut microbiota than dizygotic twins[16–18], although larger sizes of twin cohorts show a small but statistically significant effect of genetics on microbiome composition, in which certain taxa were identified as highly heritable, such as *Christensenella*[15]. However, one way to rationalize this is that the number of species that are able to successfully colonize humans is limited. Colonizing initially germ-free mice with diverse environmental samples demonstrates that very few bacteria present in the environment can survive in the mouse gut, and those that do are rapidly displaced upon introduction of human- or mouse-derived bacteria[19]. Furthermore, human immune responses shape responses to changes in the microbiome and are involved in shaping the microbiome itself[20].

Most human immunological studies regrettably still lack a microbiome component, which will be essential for untangling the relationship between the immune response and microbial colonization and stability. The mammalian immune system has a complex and dynamic bidirectional relationship with the microbiome. Although recent human cohort studies suggest that most of the variability in human immune response to stimulation is derived from the genome, at least 10% of the variability in immune response is derived directly from interactions associated with the microbiome[21].

The large majority of microbiome colonization occurs in the early years of life. This topic has been reviewed extensively[22,23]. During and shortly after birth, newborns are exposed to maternal and environmental microbes initiating gut microbiome establishment[24]. Within the first year of life, an estimated 10^{13} to 10^{14} microbes, comprising 500–1,000 species colonize the gastrointestinal tract[25]. After weaning, the gut microbiota becomes firmly established, leading to a lifelong microbiome signature in healthy individuals[26].

Body site

When the microbiomes at a given body site of large cohorts of people are compared, individuals fit on a continuum of microbial diversity within a human population rather than clustering into discrete groups[27,28]. During human development, the human microbiome follows body site–specific trajectories such that each body site develops a specific biogeography (**Fig. 1**). The skin, for example, shows dramatic variation in microbiome composition and structure across different sites[29]. The physical and topographical characteristics of skin play a substantial role in shaping the microbial community similarity between sites[30]. These factors also play a role in shaping the individuality of the microbiome so that each person develops a unique microbial signature on their skin irrespective of the differences between skin sites[31]. Similarly, although prolonged physical oral interaction between humans influences microbial community composition over time[32], the oral microbiome still maintains a relatively unique composition in each person[33]. Longitudinal characterization of the human gut microbiome has shown that the microbiota in an adult remain relatively stable and are unique to each person, which stands in contrast with the drastic change over the first three years of life[31,34].

However, the microbiome is a living ecosystem, and each of its constituents consequently undergoes fluctuations in growth rate and survival. For example, changes in diet can profoundly impact the gut microbial community structure[35,36], and vigorous cleaning can temporarily alter the skin microbiome. However, in both cases, the original microbiota and structure re-emerge when the original conditions resume[37]. The transit time of food through the gut also influences the types of microbes that proliferate within the gut; a rapid transit time selects for functions associated with biofilm formation or rapid cell division[38,39]. Defining a microbiota on the basis of the relative abundance of its members may therefore provide only a limited view of the microbial assemblage, and integrating more information about the function of each gene and genome in the context of the ecosystem and the host will provide increasingly important insights. Human microbiome variability makes blanket stratification difficult for particular disease states, although it is possible to identify biomarkers for some conditions (**Box 2**).

The vaginal microbiome has a similar degree of stability to that of the skin microbiome, and unlike the gut, classifying the vaginal microbiome into discrete states during disease has been possible. The vaginal microbiota of asymptomatic women tends to be dominated by individual species of *Lactobacillus* and diverse additional anaerobic taxa[40]. The Lactobacilli are believed to benefit the host by lowering vaginal pH through fermentation end products, thereby reducing the likelihood of allochthonous microbial colonization or pathogen invasion. Microbial variation within an individual woman does occur over days to weeks[41], although menstruation and pregnancy appear

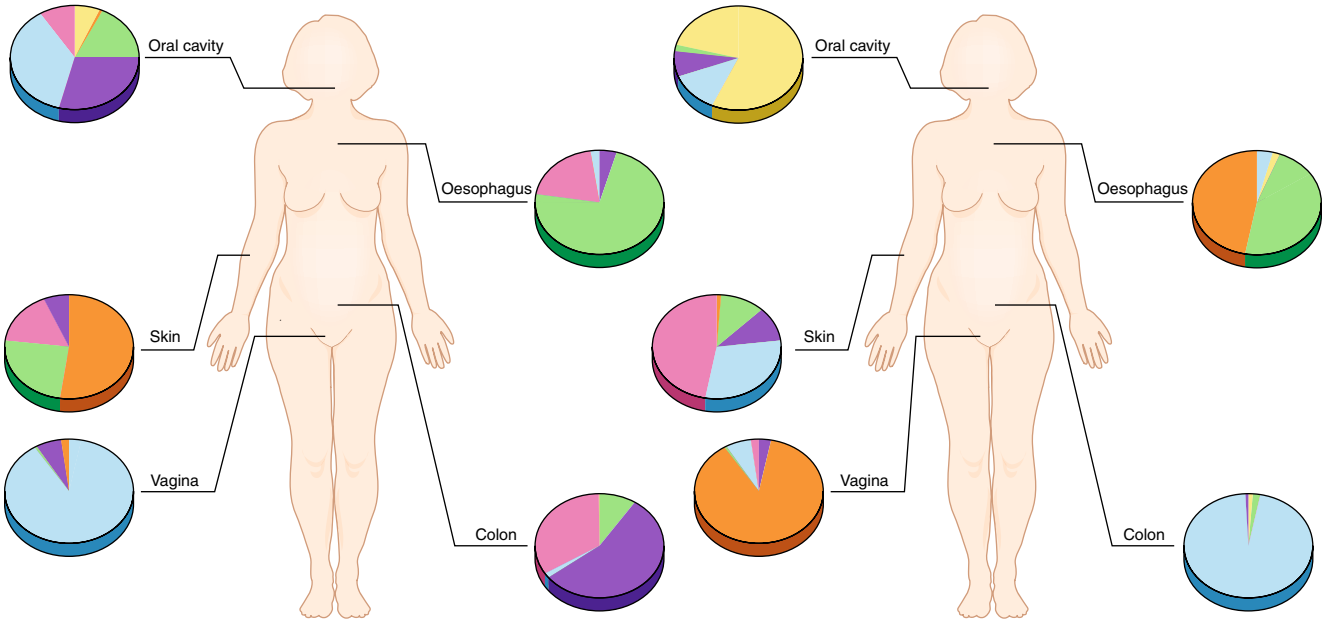

Figure 1 The human microbiome is highly personalized. Understanding the relevance of the differing microbiota between individuals is confounded by the uniqueness of an individual's microbiome. The different colors in the pie charts represent different kinds of bacteria.

to result in a similar microbiome in different groups of women[42]. Diseases, such as bacterial vaginosis, not only result in disruption of the 'normal' vaginal ecosystem function, but also result in a highly similar microbial profile between women, thereby providing a generalized biomarker of disease[43].

Diet

Diet has been extensively studied in relation to the gut microbiome[44] but less so in respect to other microbiomes at other sites of the human body. Modulating diet is an ideal opportunity for culturally and psychologically acceptable low-risk intervention to change the microbiome. Therefore, this avenue of research could yield new therapeutic strategies for conditions for which the gut microbiome and its metabolic products have been shown to be disease-causative. Evidence to date suggests that long-term diet has very large effects on gut microbiome composition[45], although a sufficiently extreme short-term dietary change can cause the gut microbiomes of different people to resemble one another within days[35]. Fascinatingly, the effects of the same dietary ingredient on blood glucose measurements can vary in different people, an effect mediated by the microbiome[46]. Although we know that the microbiome can influence the leptin concentration in humans and hence can influence appetite[47], an open question is whether the microbiome can influence dietary preferences, which could lead to positive feedback loops when these dietary changes in turn alter the microbiome.

Antibiotics

The effect of antibiotics on all microbiomes is expected to be large relative to that of other factors, and preliminary studies have been performed to determine its impact[48]. The gut microbiome in adults appears not to be resilient to repeated antibiotic administration[49]. The same antibiotic appears to affect particular microbes differently depending on the rest of the microbiome[50]; this may be due to different growth phases, metabolic states or the contextual microbial network in which the microorganisms exist. The increasing evidence that antibiotics taken early in life have a profound effect on the gut microbiome that can result in later development of obesity[51], asthma, inflammatory bowel disease and other disorders is especially interesting.

Lifestyle

Lifestyle is also thought to have a strong influence on microbiome composition. Cohabitation with pets, such as dogs, has a statistically significant association with microbiome composition. In one study, the skin microbiome of couples living together had a closer resemblance if the couple had a dog, but intriguingly, living with a small child did not produce the same trend, so couples with a child but no dog were not significantly more similar to one another than couples without a child[52]. Pet ownership and exposure to livestock have been associated with a decreased risk of asthma[53]. Interrupting this exposure in infants from human populations with a known ancestral history of interaction with animals has been shown to lead to a substantial increase in atopy, especially asthma[54]. If it turns out that these results are caused by the microbiome, rather than simply correlated with it, they suggest potential new therapeutic strategies for disease intervention could come from microbial exposure focused on immune activation.

Other lifestyle traits have been shown to correlate with the composition of the microbiota. For example, exercise appears to influence the structure of the microbiome through reducing inflammation, which produces subtle changes in the microbial community composition that are correlated with changes in the cytokine profile[55]. Sleep deprivation correlates with changes in the gut microbiome: there is a greater ratio of Firmicutes to Bacteroidetes and elevated abundance of Coriobacteriaceae and Erysipelotrichaceae associated with sleep loss[56]. Stress increases intestinal permeability and is correlated with changes in Bacteroidetes and Actinobacteria and corresponding shifts in metabolite concentrations and inflammatory markers[57].

Box 2 The microbiome provides novel biomarkers for disease

For many diseases, there is extensive evidence that the microbiome can be used to explain a substantially greater percentage of variance in the relevant phenotypes for a given condition within a population than can human genetic factors. For example, in individuals with *Clostridium difficile* infection (CDI), the aberrant stool microbiome looks nothing like a healthy stool but rather more like the microbiome of a completely different body site. Fecal microbiota transplant is able to cure CDI, and restoration of the stool microbiome to a community that matches that of the healthy state is both rapid and visible following transplantation[88]. CDI has a much larger impact on the stool microbiome composition than does any human genetic variation observed to date, which may explain the high efficacy of stool transplant relative to standard antibiotic treatments for *C. difficile*[89].

Obesity provides an example in which human genetics has failed to explain the obesity epidemic; in contrast, the gut microbiome can classify individuals as lean or obese with over 90% accuracy within the context of a given case-control study[90], although this result is dependent on using the correct methods[91,92]. Conversely, the abundance of *Christensenella* within the human gut is negatively correlated with BMI and can induce weight loss when experimentally fed to mice[15].

Autism spectrum disorder has a complex presentation of symptoms and is difficult to attribute entirely to host genetics mainly owing to the number of confounding influences and variables[93]. Yet environmental interaction, and potentially the microbiome, plays a substantial role in shaping the etiology of the disease[94,95]. Animal models have been used to uncover the ability of bacterial metabolites to mediate autism-like behaviors[96], and fecal microbiota transplant in humans has been associated with improvement in behavioral and gastrointestinal symptoms of autism[97]. In further work, the link between host genetics, behavior and the gut microbiome has been partially elucidated, identifying a strong association between *Lactobacillus* and memory formation[98].

A host of allergic and immune diseases has increased in frequency in parallel to the above metabolic and cognitive diseases. These include childhood-onset asthma and allergies, including food and cutaneous allergies. Similarly, inflammatory bowel disease and type 1 diabetes (T1D) have been increasing globally, and this cannot be explained by differences in either host genetics or assessment practices. A growing body of evidence links these conditions with the altered microbiota composition, especially loss of diversity, seen in patients with inflammatory bowel disease[99,100] and children at risk for T1D[101]. One hypothesis is that this might be linked to a general microbiome perturbation rather than the acquisition or loss of specific microbes that modify phenotype[102]. Perturbation of the microbiome during early life might be particularly important, because that is when immunity, metabolism and cognition are under development.

Three independent birth cohort studies have now shown that gut microbiome perturbation in early life is associated with development of allergic sensitization and/or asthma in childhood[103,104]. Early-life depletion of certain bacterial taxa and metabolic dysfunction were characteristic of children who went on to develop disease in childhood. Moreover, the products of these perturbed early-life gut microbiomes have been shown to induce allergic inflammation *in vitro*[105], suggesting that the foundation for allergic disease development occurs in early life and is mediated at least in part by gut microbiome dysbiosis.

Occupation has primarily been assumed to influence the microbiome via exposure to different environments and place of residence. For example, farmers have a different microbiome than city workers[58]. However, very few microbiome studies have isolated occupation as a variable influencing composition. For example, the oral microbiota of sailors is significantly altered by their occupational activities—after 120 days at sea, they show a fivefold reduction in alpha diversity and an increase *Streptococcus*[59]. Similarly, sexual intercourse between heterosexual partners leads to an increased similarity of the penile and vaginal microbiota, which could potentially alter the sexual disease ecology of the participants; there is emerging evidence that microbiome differences might affect transmission of sexually transmitted infections (STIs)[60]. Finally, couples who physically interact have a more similar microbiota than people who share the same living quarters but do not physically interact[14], indicating that physical interaction influences microbial sharing and hence microbiome similarity, highlighting the effects of social interaction on the microbiome.

Dynamics of the human microbiome

Human interaction with the environment, including with other people, creates the potential for specific microbial taxa either to act as an immune stimulant that influences the microbiome through, for example, inflammation, or as a source for bacteria, fungi and viruses that can colonize the human body. The identification of bacterial taxa in the gut that alter animal hormonal regulation, leading to obesity in mice[61], suggests that such events may alter our physiology. The composition of the gut microbiome itself is influenced by circadian rhythm, which also then affects host circadian cycles (**Fig. 2**). Disruption of the microbial diurnal cycle can lead to disruption in host circadian rhythms, which can specifically alter hormone regulation in mice[62]. The human microbiome demonstrates enormous plasticity while also being extremely robust over a long timescale and in response to many types of variation[31,34,35], but experiments in mouse models have shown some of the ways in which the microbiome can be reshaped.

At first glance, this apparent dichotomy between dynamism and the robustness of the microbiome seems difficult to resolve until the ecological dynamics of the system are considered. All ecosystems undergo variation in species population density and assemblage diversity, but this occurs with differing magnitudes at different temporal scales. This variation includes competition among microbial taxa and shifting metabolic relationships, which are compounded and influenced by the state of the immune system, a changing dietary pattern and a constant exposure to microbes from other individuals and the environment. Longitudinal characterization of the host microbiome and its sources is therefore essential to capture dynamic variance within an individual and to determine the degree to which the system demonstrates predictable successional traits[63].

The plasticity versus stability dichotomy of the human microbiome is evident over a period of days, as was illustrated in the first dense time-series analysis of the human microbiome[31] and confirmed in later analyses[34]. In that study, two subjects provided daily samples of their oral, skin and fecal microbiota. One subject provided samples for six months, and the other did so for fifteen months. The results

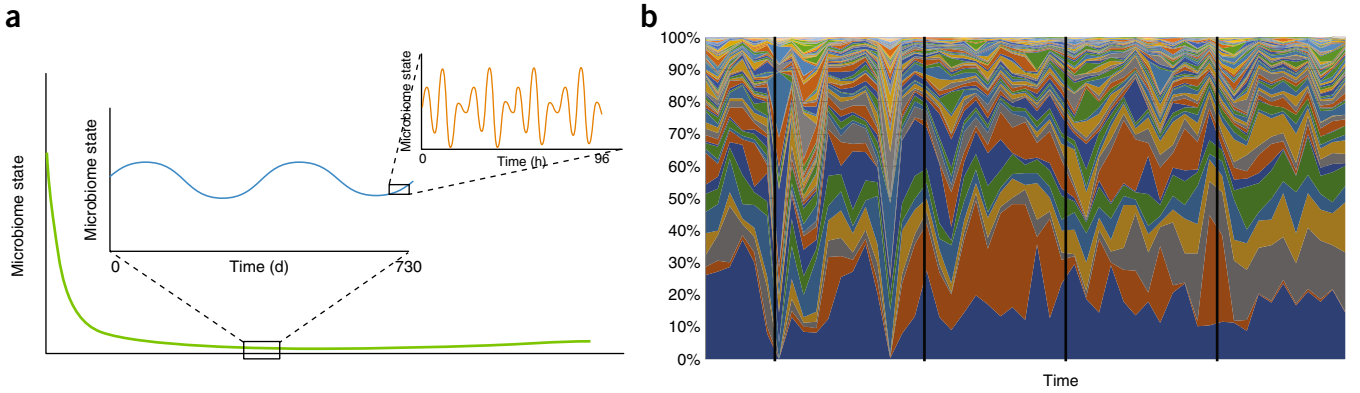

Figure 2 The dynamics of the human microbiome. The human microbiome has been shown to be highly dynamic. (**a**) Taking a 'representative' sample of a human microbiome at any given site is challenging because, although the microbiome is known to settle after birth (green line), the composition can vary both over short-term and long-term timescales (orange line and blue line, respectively). (**b**) The effect of the rate of change of the varying species on the ability to take a representative sample, as indicated by the black line, is shown.

illustrate that, at the sequencing depth studied, only a tiny fraction of bacterial taxa were found to be consistently present across all (or even most) samples in an individual host. For the skin sites (the left and right palm) there were no species detected in all samples, whereas in the gut and the mouth, about 5% of the species were defined as belonging to a core microbiome that is stable over time. Yet each person still maintained a personalized microbiome. The degree of personalization of the human microbiome vastly exceeds the host genome, which is over 99.5% identical between individuals, suggesting that only 0.5% of the genome is unique to an individual. However, based on current observations, two individuals can show no overlap in the microbial species of their microbiome. This degree of personalization is so high that it may even have forensic applications[64].

While we are now used to thinking about the composition of the human microbiome as unique, it has also been shown that the rate of change of the human microbiome composition is personalized[65]. In that study, over an approximately three-month period, 85 adults aged 17–21 years donated weekly microbiome samples from gut, skin and oral sites. Over this timeframe, the microbiome composition remained almost constant in some individuals, while in other individuals, bacterial abundances changed rapidly. These differing rates of temporal variability were identified at all of the body sites that were profiled (the palm of the dominant hand, the forehead, the tongue and feces), and the rate of change was not correlated across the different sites. On average, skin sites changed the most rapidly, followed by gut and then oral sites (this pattern matches the relative sizes of the stable temporal core microbiome observed in the long-term survey mentioned above[31]). One potential reason for the high rate of change in skin is that there are many species present in low abundance. None of the information collected about the host correlated with the differing rates of change in the microbiome, so it was not possible to determine the cause underlying these differences. However, one interesting observation was that the microbiome of individuals who self-reported taking antibiotics during (or in the week preceding) the sampling period did not change more rapidly than that of subjects who did not report taking antibiotics. The absence of a difference may reflect that a one-week time frame does not fully capture the effects of recent or even lifetime antibiotic use. Nevertheless, on a per-individual basis in this study, reported antibiotic usage was typically associated with the largest change in an individual's microbiome overall.

Although most studies associate microbiome composition with the host's disease state and likelihood of response to a treatment, at least one recent study suggests that the rate of change of the microbiome may itself be a clinical feature[66]. A high rate of microbiome change due to periodic antibiotic administration was also associated with a higher incidence of type 1 diabetes in a model of juvenile mice[67]. The rate of change of the vaginal microbiome differed across a group of women with bacterial vaginosis and predicted the subtype of bacterial vaginosis affecting these women. That observation, paired with data indicating that the rate of change of gut, skin and oral microbiomes differs between individuals, suggests that characterizing temporal variability may be an important part of characterizing an individual's microbiome.

Understanding traits, such as variance, in microbiome dynamics in individuals and whether such traits relate to patterns of succession will simplify understanding of causal relationships between species and disease and the interpretation of correlations among taxonomic groups[68]. By prospectively assessing the microbiomes of patients undergoing different procedures, we can determine its rate of change and potentially its rate of recovery if it is altered by the procedure or by the disease state that led to the procedure. Doing this in a human population will provide the statistical power necessary to relate these measurements to remission of clinical symptoms. Examining the sources that shape the microbiome is key to determining this variance.

Bayesian statistics can also be used to map the relative contribution of a specific source to the human microbiome over time[69] or to create artificial neural networks of conditional dependencies that can be used to capture predictive characteristics of a microbial network[70,71]. Using these methods, the dynamic nature of the human microbiome or metabolome both within an individual and within a population of individuals can be captured. Once gathered, the data can be harnessed to provide a predictive signature or characteristic biomarker for a given physiological, immunological or neurological condition. The application of machine learning algorithms has also proven to be valuable in identifying predictive characteristics of a microbial signature, such as who in a group of individuals has inhabited a build environment, which is suggestive of the forensic potential of microbiome profiling[14].

Toward mechanistic studies of the microbiome

Mechanistic studies of the microbiome are typically difficult to perform in humans, in part because of tremendous genetic and lifestyle

heterogeneity and because of ethical issues associated with colonizing human subjects with microbes that are hypothesized to cause disease. Therefore, most of what is currently known stems from experiments in animal models. However, recent studies that use interventions in animal models to complement observations in humans have produced striking new insights into the microbial origins of disease that cannot be acquired from human studies alone.

The importance of strain-level resolution for microbiome studies

The field of host–pathogen interactions has long relied on culturing strains of pathogens, including clinical isolates, and transferring these pathogens to isolated cells, tissues or whole animals to perform intervention studies. Many components of the microbiome have been inaccessible to such techniques because the relevant organisms cannot be cultured, although recent advances have greatly expanded the repertoire of organisms that can be grown from the human gut[72], so this barrier may be temporary. However, the culturable component of the microbiome can still be extraordinarily useful, even if incomplete. For example, a recent study in which 53 strains of bacteria were isolated from the human gut and used to monocolonize previously germ-free mice revealed large differences in the immunomodulatory properties of these bacteria, including closely related strains that affected production of cytokines, such as IL-10, IL-17A, IL-22 and IFN-γ; some bacteria promoted production of cytokines and others inhibited it[73]. These results underscore the need to characterize microbial activity at the strain level, not just at the higher taxonomic levels that are typically assessed in amplicon profiling, and will probably reveal important links between the microbiome and disease when extended to more complex communities.

Identifying disease-relevant strains from population studies

Population-based microbiome studies complemented with mechanistic experimental work in mice can use microbial associations with phenotype in humans to identify bacteria or compounds that can then be tested in intervention studies to reveal causal pathways. For example, a study of heritability of different taxa within the gut microbiome in twins in the UK revealed that one specific taxon, *Christensenella*, was highly heritable and associated with low body mass index (BMI) in this population[15]. Strains from this genus were cultured in the lab and then were transplanted into germ-free mice, resulting in decreased weight gain in these mice when compared to transplantation from an obese human, which would normally induce weight gain (as described above).

Similarly, in a comparative study of different human populations in Finland, Russia and Estonia, which differ dramatically in the incidence of early-onset autoimmune diseases, *Bacteroides* sp. were especially common in the gut microbiomes of Finnish and Estonian children, in whom the incidence of the diseases were lowest, and were hypothesized to provide most of the lipopolysaccharide (LPS; a common marker of bacterial infection in the bloodstream) exposure in those populations. In contrast, the Russian children had high levels of *Escherichia coli.* in their microbiomes. Testing the effect of injections of LPS from *E. coli* and *Bacteroides dorei* showed that the former, but not the latter, protected mice with a genetic defect from developing autoantibodies and diabetes symptoms, providing a potential explanation for the consequences of the different early-life microbiomes on development of autoimmune disease in humans[74]. A similar strategy was used to explain differences in asthma development between Amish and Hutterite children in the United States. Dust extracts from houses from each population, shown to differ in their microbiome content, were tested in a mouse model of asthma development that examines sensitivity to ovalbumin. The tests indicated that the dust from Amish, but not Hutterite, homes protected against asthma development[54], which was attributed to differences in the bacterial content of the dust. These strategies are broadly applicable to many other situations in which differential exposure to environmental bacteria may play a role in disease etiology.

Identifying biomarkers in microbiome studies

Some studies are now performing such mechanistic experiments in humans directly. In one striking example, examining 500 individuals of European ancestry in the Netherlands, the authors tested the ability of the individual's blood to produce cytokines after several antigen challenges and then paired these with data on their gut metagenome. The data suggest that the yeast *Candida albicans* had an especially large influence on the host's TNF-α response[21]. This study also associated pathways active in bacteria, such as palmitoleic acid metabolism, with lower systemic inflammatory response; adding palmitoleic acid in challenge with *C. albicans* to an individual's blood resulted in a lower concentration of TNF-α, but the IFN-γ response was unchanged, as predicted from the association data. These types of studies are especially useful in conjunction with humans with naturally occurring genetic knockouts or variant alleles. These human genetic variants may enable microbially induced disease states that can be tested in mice with comparable null or variant genetic changes, as has been shown for Parkinson's Disease[73].

Characterizing microbial biomarkers has great potential for precision medicine and is therefore a relatively simple way of translating microbiome research into clinical practice. For example, from groundbreaking animal studies, we know that bacterial probiotics (live bacteria deliberately introduced to the animal to produce a therapeutic effect) can be used to enhance immune checkpoint blockade therapy for patients with melanoma[75]. Studying the microbiomes of patients with melanoma before immune checkpoint blockade therapy has identified microorganisms in the gut as biomarkers for diagnosis that can predict whether patients are at risk of developing checkpoint-blockade-induced colitis[76].

These prospective studies are extremely important for linking microbial community structure, function and metabolic products to health outcomes. Studies of the microbiome in infants are also key in this area, and many ongoing investigations, such as the National Institutes of Health Common Core program Environmental Influences on Child Health Outcomes (ECHO, https://www.nih.gov/echo/), now provide the infrastructure necessary to sequence participants who are healthy, susceptible or diseased to examine how lifestyle and environmental experiences shape the development of immune, endocrine and neurological conditions. Although cross-sectional single time point studies of birth cohorts provide intriguing statistical associations[77], longitudinal prospective studies complemented by mechanistic experiments in animal models are required to establish whether a certain microbiome causes disease.

Future studies: developing translational potential

There remains much that we do not understand about the human microbiome. The sources of bacteria that colonize an infant include the mother and other caregivers (even one-day-old preterm infants have unique microbiomes that differ from each other and from the mother but are possibly derived from their mothers[78]), and human genetics shapes microbiome–immune interaction. Given these observations, why do monozygotic twins growing up in the same household

develop microbiomes that are barely more similar than those of dizygotic twins? The role of exogenous immune stimulation in shaping the colonization efficiency of different strains must be investigated in more detail. Animal models have produced intriguing findings, but prospective longitudinal studies in human infants are required to better understand how human genetics influence the developing microbiome. These longitudinal investigations will also help provide an understanding of the implication of ecological dynamics of the microbiome in health and disease. Microbiome stability (resistance to change) and resilience (return to the initial state following perturbation) are essential but poorly understood ecological characteristics that can be quantified through longitudinal studies via serial collection of DNA-sequencing data from the microbiome, which can perhaps be complemented with metabolite and gene-expression profiling. For example, performing weekly microbiome profiling of participants before, during and after surgery could help identify whether (and which) microbiome ecological dynamics are linked to response to surgery, surgery complications and recovery. Similarly, understanding the resistance and resilience of the microbiome to antibiotics requires larger-scale longitudinal studies of diverse cohorts (**Fig. 3**). This is especially relevant in childhood, when the microbiome is in flux and may be less resistant, but more resilient, to these stresses.

As we move forward with transforming microbiome research from a descriptive to a causal and finally to a translational science, the ability to define biomarkers that can stratify patient populations within a disease state represents 'low-hanging fruit' (**Box 2**). Of course, the effort required to take advantage of these biomarkers is considerable. Clinical studies that recruit large and representative patient populations to examine the response to a new drug or therapeutic intervention should definitely consider the opportunity to collect data on both immune function and the microbiome. These additional variables may lead to new noninvasive diagnostic platforms. In the future, it may be possible to request a stool or vaginal sample, or even a saliva sample (which has been shown to yield effective microbial biomarkers for diseases not centered on the mouth, such as rheumatoid arthritis[79] (**Box 2**)), from a patient before a surgical intervention. Then, along with their genome and medical history, scientists could make a more accurate prediction about the likelihood of successful outcome and/or of complications for each proposed intervention. This additional information, if presented in a sufficiently clear format, would substantially aid clinicians via providing new data layers that enrich the decision-making process. To realize this vision, we must better understand the factors that influence the microbiome of a healthy individual and how the microbiome is reshaped during different health and disease states.

Concluding remarks

Microbiome analysis and so-called microbiome-wide association studies (MWAS)[10] are revolutionizing clinical investigations through providing greater patient stratification and new biomarkers of disease. We are poised to make great advances in patient care over the next decade as we improve our ability to characterize and manipulate the microbiome and its metabolism. The omics tools available to perform this characterization have been developed independently, but now there is an ongoing concerted effort[80,81] to better standardize and integrate methods and data resources to improve our ability to understand microbial dynamics in human systems. Systems medicine approaches that incorporate the microbiome are rapidly finding their way into clinical investigations, and this is producing a need to integrate traditional clinical statistics and epidemiology with microbial ecological statistics and theory. Although these two concepts are not mutually exclusive, they are often treated as such; a new breed of data scientist is required as early-career clinician–scientists develop their new skills in this rapidly expanding field. This in turn increases the likelihood that patient cohort studies will be integrated with animal investigations that enable more accurate interpretation of observed host–microbiome traits.

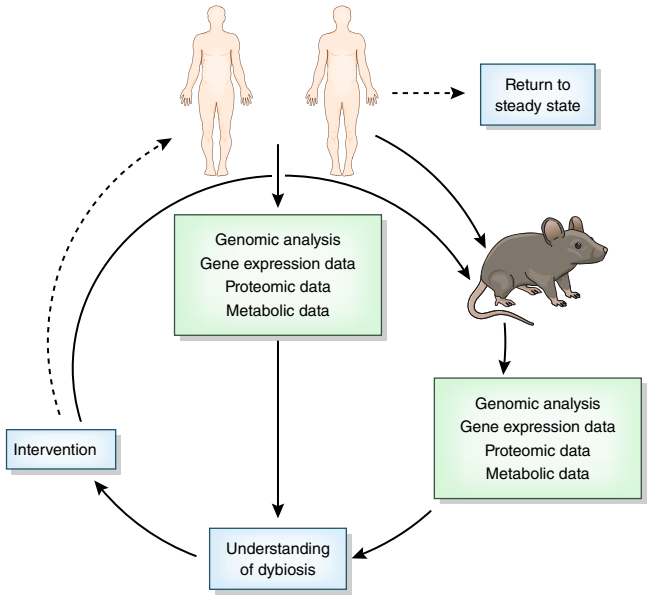

Figure 3 Iterative experiment and observation to understand and develop microbiome therapies. The iterative cycle of analysis, interpretation and translational intervention that facilitates moving microbiome research out of correlative observation and into therapeutic treatments is shown.

It is a brave new world, one where ecologists and data scientists are being integrated into clinical departments, but this paradigm shift is a necessary precondition to realize the potential of microbiome-informed and microbiome-based medicine. The societal need for improved medical interventions and preventive strategies is completely changing both the clinical and commercial world. The onus is on the basic and clinical translational research community to ensure that our experimental designs are robust and can deliver on the promises of this field. Just as important are the technical advances that must occur to ensure that we have the required tools to derive the data that are needed to test our hypotheses. The microbial ecology community came together in 2015–2016 to support the proposal for a National Microbiome Initiative, which was in turn supported by the United States President's Office of Science and Technology Policy[82]; one of the key outcomes of this effort was the identification of gaps in our technologies that would need to be filled to realize the full potential of microbiome science[83]. We have a long way to go, but with each new investigation, we are moving closer to the realization of more effective diagnosis, treatment and preventive modalities to improve human wellness and fight disease.

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