Published in final edited form as:

Adv Drug Deliv Rev. 2016 October 1; 105(Pt A): 44–54. doi:10.1016/j.addr.2016.04.032.

Microbiome Therapeutics – Advances and Challenges

Mark Mimee[1,2], **Robert J. Citorik**[1,2], and **Timothy K. Lu**[1,2,3,4]

[1]MIT Microbiology Program, 77 Massachusetts Avenue, Cambridge, MA, USA

[2]MIT Synthetic Biology Center, 500 Technology Square, Cambridge, MA, USA

[3]Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA

[4]Department of Electrical Engineering and Computer Science, Massachusetts Institute of Technology, Cambridge, MA, USA

Abstract

The microbial community that lives on and in the human body exerts a major impact on human health, from metabolism to immunity. In order to leverage the close associations between microbes and their host, development of therapeutics targeting the microbiota has surged in recent years. Here, we discuss current additive and subtractive strategies to manipulate the microbiota, focusing on bacteria engineered to produce therapeutic payloads, consortia of natural organisms and selective antimicrobials. Further, we present challenges faced by the community in the development of microbiome therapeutics, including designing microbial therapies that are adapted for specific geographies in the body, stable colonization with microbial therapies, discovery of clinically relevant biosensors, robustness of engineered synthetic gene circuits and addressing safety and biocontainment concerns. Moving forward, collaboration between basic and applied researchers and clinicians to address these challenges will poise the field to herald an age of next-generation, cellular therapies that draw on novel findings in basic research to inform directed augmentation of the human microbiota.

Keywords

microbiome; host-bacteria interactions; microbial ecology; synthetic biology; bacteriophage

1. Introduction: Host-Associated Microbial Communities

The human body is home to a diverse community of symbiotic, commensal and pathogenic microorganisms, collectively known as the microbiota. These bacterial, viral and eukaryotic communities exist on all environmentally exposed sites in the body, including the skin, nasopharynx, oral cavity, respiratory tract, gastrointestinal tract and female reproductive tract. Community composition is largely phylogenetically conserved across healthy individuals for a given body site [1]. The intimate association between man and microbe over the course of a lifetime has profound implications on human health, including metabolism, immunity and the gut-brain axis. Recent technological advances in next-generation sequencing technology has enabled elucidation of the pleiotropic effects of microorganisms on the human host. These beneficial associations make the microbiota an

attractive avenue for engineered cell-based therapeutics that can interface with the human body. For example, communities of bacteria could be assembled to counter pathogenic invasion or subtractive technologies such as phages could be used to delete specific undesirable members of human-associated microbiota. In addition, with the advancement of synthetic biology and developments in precise and robust manipulation of living cells, the vision of engineered microbes that can sense and remedy disease by producing therapeutic molecules or degrading toxic metabolites *in situ* is on the horizon.

2. Effects of the Gut Microbiota on the Host

A major focus of human microbiome research has been studying the bacteria in the gut, which represent the largest community both in terms of abundance and diversity [1]. Initial colonization occurs at birth and the mode of delivery (i.e. vaginal or Caesarian section) influences the founding community [2]. Early-life events, such as transitions in diet [3] and antibiotic use [4], shape the volatile infant microbiome, which stabilizes with age. The composition of the adult human gut microbiota is generally dominated by strict anaerobes, members of the Firmicutes and Bacteroidetes phyla, followed by Proteobacteria and Actinobacteria [1]. The constellations of microbes that make up an individual's microbiome are highly unique, with only up to 30% conservation of strains shared among unrelated individuals [5].

The adult gut microbiota is highly resilient to minor perturbations. For instance, the individual gut microbiome was determined to be 60% conserved at even the strain level over the course of five years, with members of the phyla Bacteroidetes and Actinobacteria being the most stable [5]. This longitudinal stability, in combination with the great interpersonal diversity of the microbiome, permits identification of >80% of individuals by their unique 'microbial fingerprint' [6]. Despite its stability, large insults, such as antibiotic treatment [7] or the onset of disease [8], can lead to dramatic changes in the composition of the gut flora. Additionally, host genetics, diet and environment are major contributing factors shaping the community [9,10]. Studies in humans [11] and mice [12–14] have shown that the microbiota responds to major changes in diet that reflect nutritional intake. Moreover, the importance of host genetics is reflected by the higher concordance in microbiome composition between monozygotic versus dizygotic twins [15], though mouse studies have suggested these host genetics effects can be eclipsed by diet [13].

Defining the ecological rules that govern colonization and succession in the microbiota will be paramount in designing microbiota-based therapeutics. Moreover, microbiota-based therapeutics that are robust to the great interpersonal diversity and plasticity of an individual's microbiome will be a major challenge moving forward. Approaches to tackle this problem may include identifying and engineering bacteria that are stable and dominant in the microbiota, assembling cocktails of bacteria that maintain a desired function despite variations in underlying constituent species, or building distinct sets of bacterial therapies customized for different microbiota profiles for which patients can be screened.

2.1 Metabolism

At the community level, the collection of microbes that inhabit the gut plays a key role in human metabolism. Early observations recognized the relative proportions of Bacteroidetes to Firmicutes as a microbial signature of obesity in mice, where an obese-associated microbiota displayed an increased capacity for energy harvest [16,17]. Notably the increase in total body and fat mass, as well as the associated metabolic phenotypes, was transferrable by transplantation of obese-associated flora into germ-free mice devoid of any microbial inhabitants [17,18]. Similarly, studies on the gut microbiota of twins discordant for kwashiorkor, a severe form of acute malnutrition, demonstrated that transplantation of a kwashiorkor-associated microbiota into germ-free mice leads to a rapid decrease in body weight and metabolic phenotypes associated with malnutrition [19]. The microbial community that inhabits the gut is therefore an additional factor that can be modified to affect metabolism and the interested reader is referred to thorough reviews on this topic [20–22].

At the molecular level, microbially derived metabolites have been shown to directly or indirectly modulate host metabolism. A major metabolic role of the gut flora is the conversion of ingested dietary fiber and host mucosal glycans into short-chain fatty acids (SCFAs), which include acetate, propionate and butyrate [20]. SCFAs mediate a multitude of beneficial effects on the host. Butyrate serves as a primary energy source to colonic epithelial cells [23,24] and increases gut barrier integrity through stabilization of hypoxia-inducible transcription factor [25]. Moreover, SCFAs can modulate host gene expression in mice through inhibition of histone deacetylases [26] or through G-protein-coupled cell surface receptor signaling [27,28]. This signaling modulates gut motility [28,29] and stimulates intestinal gluconeogenesis [30], secretion of glucagon-like peptide 1, leptin, and peptide YY [28,31]. In addition to SCFAs, microbial synthesis of vitamins, including vitamin B12 [32], as well as metabolism of bile salts [14] can impact host nutrition and physiology. Furthermore, the gut microbiota, in concert with hepatic enzymatic activity, participates in the conversion of dietary phosphatidylcholine and L-carnitine into trimethylamine N-oxide (TMAO), a molecule strongly correlated with atherosclerotic heart disease in humans [33,34]. TMAO production in mice is dependent on an intact microbiota [33] and transferrable by microbiota transplantation [35]. Inhibition of microbial enzymes involved in TMAO production has been shown to be an effective means of treating atherosclerosis in mice, suggesting that inhibitors of microbial enzymatic activity could be potential therapeutics for host diseases [36]. In the light of the important metabolic role of the microbiome, microbes could potentially be engineered to better regulate energy balance, to synthesize vitamins and other beneficial molecules, and to inhibit the production of or detoxify toxic by-products.

Just as the gut microbiota metabolizes carbohydrates, proteins and lipids ingested by the host, bacteria in the gut interact with compounds foreign to living organisms, known as xenobiotics [7]. While these interactions often only result in mild changes in gene expression in gut microbes [7], microbial activity can disrupt the intended activity of host-targeted drugs. For example, *Eggerthella lenta*, a gut actinobacterium, can reduce the cardiac drug digoxin, thereby inhibiting its pharmacological activity [37]. While the presence of *E.*

lenta is not necessarily predictive of digoxin reduction *in vivo* [37], recent work has elucidated the operon responsible for drug inactivation, which can act as a better predictor [38]. Repression of this operon through supplementation of arginine or dietary protein can increase serum levels of active digoxin in mice [38]. Additionally, bacterial β-glucuronidases interfere with the efficacy of the colon cancer chemotherapeutic CPT-11 by reactivating the drug in the intestine, resulting in an increased toxicity that can be reversed through small molecule inhibition of β-glucuronidase activity [39]. The metabolic activity of microbes in the gastrointestinal tract can thus greatly affect the *in vivo* function of conventional therapeutics, suggesting that pharmacology should be viewed through both the lens of human and microbial metabolism. Synthetic biology and other approaches can provide technologies for removing specific bacterial species from microbial communities to study their metabolic function *in situ* as well as the ability to engineer new metabolic functions into microbial chassis to investigate potential impacts on the microbiota. Thus, characterization and control of microbial metabolic activity could be a means to predict drug efficacy and create better therapies.

2.2 Immunity

The homeostatic association of the human body with trillions of microbes demands a continuous dialogue between the host immune system and the microbial flora, which has been thoroughly reviewed elsewhere [40–42]. In both the small and large intestine, commensals are kept in check through a thick mucosal layer, gradients of antimicrobial peptides and mucosal IgA antibodies [40,43]. The immune system helps dictate the diversity and structure of host-associated microbial communities as deficits in immune function, such as HIV infection [44], B cell depletion [45] or chronic inflammation [8,46], alter the composition of the microbiota, which can, in turn, affect disease progression. The gut flora and their associated metabolites strongly influence the immune system in both health and disease. Inflammatory bowel disease (IBD) is thought to be caused by a combination of environmental, genetic and microbial factors [43]. Indeed, there is a strong association between constellations of microbial taxa in patients with IBD compared to healthy subjects [8,47]. Certain microbially derived products, such as polysaccharide A from *Bacteroides fragilis* [48,49] and butyrate from Clostridial spp. [50–52], have been shown to be protective in colitis through the induction and maintenance of peripheral regulatory T cells (T_{REG}). Similarly, protein antigens produced by segmented filamentous bacteria have been demonstrated to robustly induce T_{H17} responses [53,54]. While these microbe-host immune interactions are predominant in the gut, the effects can manifest systemically. For example, a depletion in certain microbial taxa in the infant gut, including *Lachnospira, Veillonella, Faecalibacterium,* and *Rothia*, is a risk factor for childhood asthma [55], while colonization of mice with spore-forming Clostridial species can protect against food allergy sensitization [56]. The interaction between microbe and host is a powerful determinant in the development of a healthy, balanced immune system and could thus be exploited as an avenue for immune therapies.

Bacteria in the gut can also indirectly modulate immune function through interactions with invading pathogens [57,58]. The capacity of the normal flora to defend against pathogenic invasion is referred to as colonization resistance and is founded on either competition for

limited resources or microbial warfare. Ablation of the gut microbiota through broad-spectrum antibiotics predisposes individuals to opportunistic pathogens, including *Clostridium difficile*, that are normally kept at bay by commensal microbes [59]. Endogenous and probiotic strains of *Escherichia coli* can protect against *Salmonella* Typhimurium infection in mice by scavenging iron necessary for a productive infection [60]. Furthermore, the presence of *Clostridium scindens* can defend against recurrent *C. difficile* infections through the synthesis of secondary bile acids toxic to *C. difficile* [61]. Bacteria also possess an arsenal of molecular weapons, such as bacteriocins, microcins and Type VI secretion systems [62] that can exclude competing invaders from a community [57]. While antagonistic interactions help prevent pathogens from entering the microbiota, some interactions have been shown to exacerbate infection. For example, in mono-colonized mice, *Bacteroides thetaiotaomicron* can forage sialic acid from mucosal glycans and generate succinate, which can then serve as a carbon source for *C. difficile* during infection [63,64]. Thus, bacteria-bacteria interactions can have consequences for the host in which they occur. Elucidating communities of microbes that are recalcitrant to pathogenic invasion could pave the way for the rational design of consortia that exclude pathogens and prevent infectious diseases [58,61].

2.3 Gut-Brain Axis

Strikingly, the impact of the gut flora on the host reaches beyond the gastrointestinal tract to the brain, where microbially derived metabolites can influence neuroendocrine function and behaviour [65–67]. As mentioned above, in mice, butyrate produced by the microbiota modulates levels of neuropeptides that govern satiety, such as leptin and peptide YY [28]. The metabolic activity of mouse-associated bacteria can also influence circulating levels of neurotransmitters, such as serotonin [68], and bacterial enzymes are capable of directly synthesizing the neurotransmitter tryptamine [69]. Together, these endocrine effects can manifest in changes in behaviour. Germ-free mice display higher levels of anxiety-related behaviours compared to conventional mice, which is attributed to higher levels of circulating ACTH and corticosterone [70]. Moreover, in mouse models of autism spectrum disorders, correction of a dysbiotic microbiota through administration of *B. fragilis* can lead to a reversal in behavioural abnormalities [71].

3. Efforts to Harness and Engineer the Microbiota

As a decade of research has solidified the importance of the microbiome on human health and disease, recent efforts have sought to capitalize on this interaction to create microbiome-based therapeutics. These approaches can be classified into three major paradigms: additive, subtractive or modulatory therapies. Additive therapy includes the supplementation of the host microbiota with either individual strains (Fig. 1a) or a consortium of natural or engineered microorganisms (Fig. 1b). Subtractive therapy refers to the specific elimination of deleterious members of the microbiome to cure disease (Fig. 1c). Finally, modulatory therapies involve administration of non-living agents, or prebiotics, to modulate the composition or activity of the endogenous microbiome. Probiotics and prebiotics constitute the first generation of microbiome therapies and have been reviewed extensively elsewhere [72–76]. Here, we will focus on the next-generation of microbiome therapeutics using

recombinant probiotics, designed microbial consortia and selective antimicrobials. Thus far, therapeutic bacterial consortia have been primarily composed of natural strains, whereas genetically engineered bacteria have mainly been delivered as monotherapies. However, moving forward, genetically modified communities may be developed that can incorporate the diversity and robustness of microbial consortia with the added efficacy and controllability of synthetic gene circuits. We will first discuss recent advances in the development of microbiome therapeutics and later the outstanding challenges faced in translating the potential of the microbiota to real-world therapies.

3.1 Genetically Engineered Probiotics

Probiotic therapies are founded on the rationale that naturally occurring human-associated microbes provide a myriad of health benefits. Indeed, oral ingestion of *Lactobacillus spp.*, *E. coli* and *Bifidobacterium spp.* has been clinically shown to remedy a wide variety of diseases [77–80]. Recent efforts seek to augment the natural benefits of probiotics through recombinant expression of therapeutic biomolecules. Implementation of cells as vehicles of drug delivery could enable continuous *in situ* production of biotherapeutics that could overcome issues such as bioavailability and drug inactivation associated with oral administration. Synthesis of protein therapeutics could be conditioned upon the detection and integration of specific environmental cues related to disease. This conditional, on-demand drug release is a particularly attractive advantage of cell-based therapeutics that could enable entirely new pharmacological paradigms. If the therapeutic organism can stably colonize the host, the engineered microbe could dynamically correct perturbations caused by disease to restore homeostasis in the host. Realization of this vision of fully autonomous ‘smart’ cell-based therapeutics is still forthcoming. However, recent efforts have demonstrated the efficacy of cell-based bacterial therapeutics in preventing infection, resolving inflammation and treating metabolic disorders.

Natural colonization resistance conferred by native members of the normal flora has been augmented through cellular engineering. Duan *et al.* explored the prophylactic use of probiotic *E. coli* Nissle 1917 engineered to inhibit virulence of *Vibrio cholerae* in infant mouse models [81]. Virulence of *V. cholerae* is in part coordinated by extracellular quorum sensing molecules, which modulate gene expression in a density-dependent manner. When bacterial numbers are low, *V. cholerae* expresses virulence factors necessary for the establishment of infection; when numbers are high, virulence factor expression is repressed to allow escape from the host. *E. coli* was engineered to crosstalk with quorum sensing and thereby inhibit productive infection. Administration of therapeutic cells resulted in an increase in survival and a concomitant decrease in bacterial burden and cholera toxin expression. Similarly, *Lactobacillus jensenii* was genetically modified to prevent transmission of chimeric simian/human immunodeficiency virus (SHIV) in a rhesus macaque model (Fig. 2a) [82]. Bacteria were engineered to produce the antiviral cyanovirin-N. Prophylactic treatment of macaques decreased both the occurrence of SHIV following multiple challenges as well as the peak viral load. Engineered bacteria could thus serve as treatments for both bacterial and viral infections.

IBD has drawn particular attention as a compelling candidate for cell-based therapeutics, due to the implications of the gut microbiota in disease and the lack of long-term, cost-effective treatments [43]. Early work explored the use of *Lactococcus lactis* engineered to constitutively secrete recombinant interleukin-10 (IL-10), a potent anti-inflammatory cytokine depleted in IBD (Fig. 2b) [83]. Using both chemically and genetically induced mouse models of colitis, Steidler *et al.* demonstrated that recombinant microorganisms could be implemented to reduce pathology and suppress pro-inflammatory cytokine secretion [83]. Phase I clinical trials also showed that recombinant *L. lactis* treatment was well-tolerated in a small Crohn's disease cohort, although efficacy was modest [84]. IL-10-secreting *L. lactis* were further modified to produce either the auto-antigen proinsulin [85] or glutamic acid decarboxylase-65 [86] for treatment of autoimmune diabetes. When administered in conjunction with anti-CD3 therapy, both recombinant organisms were able to induce tolerance, increase numbers of regulatory T cells and reverse hyperglycemia in mice [85,86]. Microbial expression of other anti-inflammatory cytokines, such as transforming growth factor-β1 [87] and anti-tumor necrosis factor α Nanobodies [88], as well as the tissue repair factor keratinocyte growth factor-2 [89], have been shown to be protective against colitis in murine models of IBD. In addition, production of the protease inhibitor Elafin from lactic acid bacteria was shown to restore proteolytic homeostasis in mouse models of colitis and protect against inflammation [90]. Recombinant bacteria have also been implemented for treating oral mucositis, a condition involving ulcerative lesions that is a common side effect of chemotherapy. Topical application of *L. lactis* engineered to secrete trefoil factor-1 was effective at treating oral mucositis in hamster models [91] and early clinical trial data indicate that treatment is well-tolerated and could be effective at reducing incidence [92]. Together, these studies suggest that recombinant cellular therapies could be viable therapeutic agents to treat inflammatory diseases.

Integration of recombinant microbes into the host microbiome has also seen success in treating metabolic diseases, such as obesity and diabetes. Daily feeding of probiotic *E. coli* engineered to synthesize precursors of anorexigenic lipids reduced obesity, adiposity and food intake in mice fed a high-fat diet (Fig. 2c) [93]. These protective effects were sustained weeks after cessation of bacterial treatment [93]. Moreover, *Lactobacillus gasseri* was used as a vehicle for delivery of GLP-1, a protein able to induce conversion of intestinal epithelial cells into insulin-producing cells [94]. Administration of the engineered probiotic increased the numbers of intestinal insulin-producing cells and decreased hyperglycemia in a rat model [94].

3.2 Engineered Consortia

Engineering the microbial community as a whole is an alternative strategy that has seen tremendous clinical success for treatment of recurrent *C. difficile* infections. Infusion of stool from healthy donors to diseased patients, termed a fecal microbiota transplant, boasts greater than 90% efficacy in resolving recurrent infections [95] and is more than twice as effective as antibiotic treatment alone [96]. Despite their clinical success, fecal microbiota transplants draw safety concerns for fear of introducing pathogens or opportunists that could exacerbate disease [97]. A regulatory framework and strict screening guidelines for donors has been established [97], but deciphering the minimal subset of therapeutic microbes has

been a focus for mitigating safety concerns and increasing the reliability of treatments [98]. Aside from recurrent *C. difficile* infections, many believe fecal microbiota transplants hold promise in treating IBD [99] and early trials showed modest success [100,101]. However, due to the more complex nature of disease and a higher incidence of adverse events, more trials are necessary to establish stool transplants or infusions of defined microbial communities as a viable treatment option for IBD [101]. The identification and tailoring of microbial communities that can address the complexities of human disease and the diversity of human-associated microbiota will remain an ongoing challenge in the development of microbiota-based therapeutics.

In mouse models, microbiota reconstitution has proved successful in altering community-wide metabolic activity of urea (Fig. 2d) [102]. In the gut, urea produced by the liver is converted to ammonia and carbon dioxide by bacterial ureases. Accumulation of systemic ammonia is associated with neurotoxicity and encephalopathy in patients with liver deficiency. Following depletion of the endogenous microbiota using antibiotics and polyethylene glycol as a purgative, Shen *et al.* could transplant a defined microbial community with low urease activity that remained stable for months [102]. In a hepatic injury model, the redefined microbiota increased survival and protected against cognitive defects associated with hyperammonemia. This study demonstrates the feasibility in rationally sculpting a host-associated microbial community to protect against metabolic diseases.

3.3 Subtractive Approaches

Subtractive therapies may employ chemicals, peptides, or even replicating entities to remove bacteria from the gut with varying degrees of specificity. In medicine, this is currently accomplished through the use of antibiotics, which tend to be broad-spectrum in nature, exhibiting activity towards many different bacteria. As a result, treatment of a patient aimed to remove an infectious pathogen also leads to the unintended reduction of other members of the microbiota. This community shifting may cause the patient to become susceptible to other temporary or chronic conditions to which they are normally protected, including antibiotic-associated infections with *C. difficile*. The development of targeted antimicrobials, such as bacteriocins and bacteriophages, could yield more effective subtractive therapies.

Bacteria that occupy overlapping niches need to compete for the same resources. Bacteriocins are ribosomally synthesized antimicrobial peptides that are produced by bacteria and may help gain an edge over competitors by exhibiting toxic activity towards susceptible cells. A producer cell encodes both toxic and immunity functions, killing neighbouring cells while protecting itself and its progeny from the effects of the bacteriocin. Recent studies have used metagenomics to identify bacteriocins [103,104], shown potential fitness advantages of producers *in vivo* [105], and observed protective effects of producers against pathogens [106,107]. As these molecules seem to play a role in modulating microbial populations within a host, they may prove useful tools for subtractive-type therapies.

Bacteriophage therapy is a highly specific method of killing bacteria through the use of natural or engineered viral parasites. Bacteriophages, or phages, are viruses that infect

bacteria and use cellular resources to produce progeny, generally killing the bacterial host in the process. Discovered approximately 100 years ago [108,109], the application of phages as antimicrobials has seen a renewed interest with the growing threat of antibiotic-resistant pathogens [110,111]. Though early phage therapies targeted intestinal pathogens [112], clearance issues have recently been reported wherein bacterial and phage populations stably coexist in the murine gut [113,114]. Knowledge attained from research into the ecology of phages in the gut may be pivotal in determining factors that lead to successful therapies in this complex ecosystem.

A newer focus of the field is to examine the natural role of these viruses in shaping host-associated bacterial populations [115]. Metagenomic research of the human microbiome has described the fecal virome of both healthy and diseased donors. These studies include measuring phage diversity, variability, and stability [116], and analyzing changes associated with diet [117] or IBD [118] in humans, or antibiotic treatment in mice [119]. For example, a study of the viromes of monozygotic twins and their mothers revealed high interpersonal variation of virome composition, but low intrapersonal diversity that was dominated by temperate phages, or those that can exist in a silenced life cycle within bacteria [116] Diet has been identified as one factor that affects the viral community, as putting human subjects on a controlled diet altered community structure and resulted in a level of convergence for individuals on the same diet [117]. Interestingly, whereas IBD correlates with a reduced level of bacterial diversity, multiple cohorts have revealed a concomitant increase in bacteriophage richness, specifically those belonging to *Caudovirales* [118]. To explore phage-bacterial host dynamics, gnotobiotic mice were seeded with a defined, 15-member human commensal community and challenged orally with virus-like particles (VLPs) purified from healthy human donors. In this study, the authors made several interesting observations, including an increase in specific phages correlating with a transient decrease in specific bacteria, different phages and bacteria showing non-simultaneous temporal population dynamics, and evidence of phage resistance likely due to ecological, rather than genetic, factors [120]. These types of comparison and challenge studies should continue to yield important information useful for designing phage-based strategies for targeting pathogens or altering microbial communities in the gut, particularly in regards to determining the factors that lead to transient versus stable community rearrangements.

Engineered phages, or those modified to carry additional or alternative functions to those naturally occurring, may prove useful for therapeutics as design can be informed by new knowledge. Recently, it was found that certain phages possess Ig-like protein domains on their capsids that enhance association with mucus [121]. This or alternative localization domains may be useful for improving residence time in the gut or helping concentrate phages to relevant biogeographies. Phage engineering efforts have included altering host adsorption factors to change host range [122] or encoding a dispersal enzyme to help break up bacteria in protective biofilms [123]. Phages have also been used as DNA delivery agents to reverse antibiotic resistance [124,125] or to exert broad-spectrum [126–128] or sequence-specific [129,130] antimicrobial activity. Additionally, genome engineering and tools such as CRISPR-Cas [131] and assembly methods including Gibson [132] and yeast [122] assembly should prove useful for the development of new phages with augmented capabilities in modulating microbial communities. We believe that the use of natural or engineered phages

as therapeutics for microbiota-related diseases has been understudied relative to the complementary modality of introducing natural or engineered microbes, and thus represents a fascinating area of investigation.

4. Challenges and Outlook in the Development of Microbiota-Based Therapeutics

Advances in synthetic biology and the understanding of the ecology of host-associated microbial communities have accelerated the development of microbiota-based therapeutics. However, in order to effectively translate this early work into the clinic, we must address numerous challenges. Many of the advances in the development of microbiome therapeutics have been demonstrated in rodent models and their generalizability to humans, due to the fundamentally different nature of their respective microbiomes, has yet to be tested in a comprehensive fashion. Fundamental understanding of the forces that shape host-associated microbial communities and mediate host-bacterial interactions is essential for the rational design of microbiome therapeutics. The development of clinically relevant biosensors and stable, robust genetic circuits would help realize the vision of fully autonomous cellular therapies. Finally, cognisance of regulatory, biocontainment and safety issues during research of novel therapeutics are needed to help hasten translation of basic research into the clinic.

4.1 Stable Engraftment

A comprehensive understanding of the rules which govern invasion, resilience and succession in a host-associated microbial ecosystem is crucial for the development of long-term cell-based therapies (Fig. 3a). Indeed, for some applications, stable colonization of recombinant microbes or microbial communities may not be necessary for treatment of disease if cells can exert their intended function during transit through the intestine. For instance, many current efforts for recombinant therapies have employed *L. lactis*, a bacterium that does not colonize the mammalian intestine, as a chassis for therapeutic protein production [84–86,88,92]. Likewise, the common chassis, *E. coli* Nissle 1917, shows greatly variable colonization capacity in different healthy individuals following daily probiotic treatment; fewer than 50% of volunteers became decolonized two weeks after cessation of treatment, whereas the probiotic was detectable by PCR in the stool of only 17.5% of volunteers after 6 months [133]. However, stable engraftment of cell-based therapies into the endogenous microbiota may increase the efficacy of treatment and enable the development of long-term, fully autonomous, disease-responsive therapies.

Further studies into the ecological principles that underpin the human microbiome are necessary for rational manipulation of microbial communities. Seedorf *et al.* explored successive colonization of germ-free mice with microbial communities from diverse habitats to elucidate patterns of invasion in pre-established communities [134]. Pooled screens for identifying the genetic determinants of colonization and resilience in *Bacteroides spp.* have revealed the importance of carbohydrate [135–137] and cofactor [138] metabolism as well as resistance to antimicrobial peptides [139]. Importantly, pairing additive approaches with subtractive or modulatory ones could yield more reliable and stable engraftment into the

microbiome. Targeted antimicrobials, such as bacteriophages, or dietary supplementation with prebiotics could open niches for therapeutic microbes to occupy. As the gut microbiota is a complex ecological community, assimilating these biological findings into predictive systems biology models [140,141] will greatly facilitate the creation of microbiota-based therapeutics.

Different therapeutic applications should demand different chassis organisms for cell-based therapies (Fig. 3b). To date, *E. coli* Nissle 1917 [81,93], *L. lactis* [84–86,88,92], *Lactobacillus spp.* [82,90,94] and *Bacteroides spp*. [87,89,142] have served as vectors for cell-based therapies. While *L. lactis* cannot colonize the intestine, *E. coli* and *Lactobacillus spp.* tend to be enriched in the small intestine, whereas *Bacteroides spp*. reside in the cecum and colon [143]. Some species tend to colonize the mucosal layer, whereas others prefer the intestinal lumen [144–146]. The biogeography of disease should thus dictate the best-suited organism for therapy. The natural health benefits of bacteria can also serve as an adjuvant for engineered therapeutics. For example, *B. fragilis, Faecalibacterium prausnitzii* and those from *Clostridium* clusters IV and XIVa naturally protect against inflammatory disease [48,50,147] and may serve as prime candidates for anti-inflammatory therapies compared to *E. coli*, which are enriched in an inflamed gut [148]. Finally, organisms that are naturally abundant and resilient to environmental perturbations should be preferred for long-term therapies. To this end, the choice of microbial chassis should be paired with thorough characterization of its relative benefits for the target disease. The development of methods for engineering currently intractable organisms would also enable additional possibilities for cell-based therapies. While ample genetic tools exist for *E. coli* and lactic acid bacteria and recent work has extended these to *Bacteroides spp.* [142], new and efficient genetic techniques for the manipulation of intractable group IV and XIVa Clostridia and *F. prausnitzii* are needed to accelerate the development of future therapies that utilize these organisms.

4.2 Development of Clinically Relevant Sensors

The creation of fully autonomous cell-based therapies demands a well-characterized library of biosensors that would allow dynamic responses to environmental perturbations (Fig. 3c). Synthetic biology has engendered a plethora of genetic parts for sensing and integrating environmental signals into changes in gene expression. Luminescent, fluorescent or colorimetric readouts are common outputs of biosensors and can be designed to be transient if transcriptionally regulated or permanent if coupled to genomic alterations [142,149–151] or a bistable toggle switch [152,153]. Danino *et al.* recently developed a non-invasive biosensor for cancer metastasis, taking advantage of the fact that metastasis leads to the natural translocation of probiotic *E. coli* to the liver. Bacterial enzymatic activity on chromogenic substrates allowed for excretion of compounds that could readily be detected in the urine [154]. Additionally, genetic circuits have also been implemented *in vivo* for sensing a small-molecule inducer [153] and dietary carbohydrates [142]. To date, the development of biosensors has relied on genome mining and previous descriptions of systems in scientific literature. Directed evolution has been successfully applied to change substrate specificity of enzymes [155] and promoter specificity of RNA polymerases [156] and similar strategies could be applied for the development of novel biosensors. Moreover,

hybrid transcription factors composed of distinct DNA-binding and ligand-binding domains has expanded the collection of available sensors [157–159]. A generalized approach for *de novo* discovery of sensors of clinically relevant molecules is greatly needed, as well as fluid dialogue between synthetic biologist and clinicians to identify relevant candidate disease biomarkers. By pairing metabolomic studies with streamlined strategies for biosensor discovery, engineered microbes could offer a new class of diagnostics by assaying concentrations of biomarkers inside the body instead of in *ex vivo* samples. These sensors could be used to trigger the expression of therapeutic molecules to enable on-demand and localized production of medicines only during active disease.

4.3 Robustness and Evolutionary Stability of Genetic Circuits

The robustness of engineered functions to time and changing environments is a major challenge to long-term cellular therapies (Fig. 3d). Currently, prototyping the complex genetic circuits required for sense-and-respond cellular therapeutics most often occurs in optimal *in vitro* growth conditions that may not accurately replicate the intended environment. As work in synthetic biology progresses towards animal models and clinical applications, new, more sophisticated *in vitro* systems should be developed to better emulate the variable conditions faced by the endogenous microbiota. Both single [160,161] and multi-stage [162] chemostats have been used to support culture of fecal samples and could provide a testbed to understand how interbacterial interactions impact functionality of genetic circuits. Additionally, organoid [163], 3-D intestinal scaffolds [164] and gut-on-a-chip [165] models have also been employed to predict interactions between probiotics and the host.

Genetic circuits described in synthetic biology often fail to report the evolutionary stability of engineered functions. Assessment of gene circuit function is generally made on short timescales (<24 hours). However, cellular therapies may need to retain function for weeks or months to achieve significant efficacy. The numerous genetic parts required to maintain a complex gene circuit are energetically taxing on cells [166]. *In vitro* evolution experiments revealed this strong fitness cost can lead to a rapid loss of function in engineered bacteriophages [167,168]. Efforts to quantify and minimize the burden placed on recombinant cellular therapies [166] should help maintain long-term function of therapeutics in the competitive context of the microbiota.

4.4 Regulation, Safety and Biocontainment

As cell-based therapies constitute a novel paradigm in drug development, a regulatory framework that addresses safety and biocontainment issues should be established to minimize adverse events and environmental release of engineered organisms (Fig. 3e). Many of the bacterial chassis used for recombinant therapies are generally recognized as safe (GRAS) organisms as listed by the U.S. Food and Drug Administration. These organisms are largely probiotics or bacteria employed in the production of food products. However, as natural commensals, such as clostridial or *Bacteroides* species, may be prime candidates for microbiota-based therapies, the safety of these organisms should be evaluated. The ability of these organisms to stably colonize their target environments may enable greater therapeutic efficacy, but may raise questions about the pharmacology and control of such therapies. The

spread of genetically modified DNA from recombinant organisms to endogenous members of the microbiota may also be a concern as natural horizontal gene transfer is prevalent in the human microbiome [169]. The escape of engineered organisms into the environment that may lead to unintentional colonization of others may be of similar concern, even though most genetically modified organisms developed in the lab seem to be less fit than wild-type [166]. Conditional kill switches that eliminate engineered microbes [158,170] or destroy genetic circuits [171] have been created for biocontainment. Generating auxotrophic microbes that cannot replicate in or outside of the gut has also proved a successful strategy in limiting the spread of recombinant cells [172]. Indeed, auxotrophy was employed as the sole biocontainment strategy in early clinical trials involving recombinant microbes [84,92]. Moving forward, a dialogue between regulators and researchers should help shape the creation of technologies necessary for the safe implementation of cellular therapies.

4.5 Conclusions

The profound impact that microbial communities have on human health is providing new diagnostic and therapeutic avenues for treating disease. However, existing therapeutic approaches for modulating microbiomes in the clinic remain relatively crude. Exciting research into additive, subtractive or modulatory strategies for affecting the human microbiota and, in turn, human health, are progressing towards the clinic, powered by advancements in synthetic biology and microbial ecology. While prototypical examples of these approaches have been described, additional basic research to elucidate the function of host-associated microbial communities and host-microbe interactions and progression into clinical trials is needed to guide the creation of more effective therapies. Furthermore, enhanced engineering approaches to enable the modification of a wide range of bacterial hosts, to create disease-relevant sensors that can drive the conditional production of heterologous therapeutics, and to achieve robust yet controllable function of these sense-and-respond diagnostics *in vivo* will ultimately improve the translation of microbiome therapeutics into real-world use.

Acknowledgments

This work was supported by the National Institutes of Health (DP2 OD008435, P50 GM098792, R01 EB017755), the Office of Naval Research (N00014-13-1-0424), the National Science Foundation (MCB-1350625), the Center for Microbiome Informatics and Therapeutics, the Defense Threat Reduction Agency (HDTRA1-14-1-0007, HDTRA1-15-1-0050) and the Defense Advanced Research Projects Agency. M.M. is a Howard Hughes Medical Institute Student Research fellow.

References

1. Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. Nature. 2012; 486:207–214. DOI: 10.1038/nature11234 [PubMed: 22699609]
2. Dominguez-Bello MG, Costello EK, Contreras M, Magris M, Hidalgo G, Fierer N, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proc Natl Acad Sci. 2010; 107:11971–11975. DOI: 10.1073/pnas.1002601107 [PubMed: 20566857]
3. Bergstrom A, Skov TH, Bahl MI, Roager HM, Christensen LB, Ejlerskov KT, et al. Establishment of Intestinal Microbiota during Early Life: a Longitudinal, Explorative Study of a Large Cohort of Danish Infants. Appl Environ Microbiol. 2014; 80:2889–2900. DOI: 10.1128/AEM.00342-14 [PubMed: 24584251]

4. Cho I, Yamanishi S, Cox L, Methé Ba, Zavadil J, Li K, et al. Antibiotics in early life alter the murine colonic microbiome and adiposity. Nature. 2012; 488:621–626. DOI: 10.1038/nature11400 [PubMed: 22914093]
5. Faith JJ, Guruge JL, Charbonneau M, Subramanian S, Seedorf H, Goodman AL, et al. The long-term stability of the human gut microbiota. Science. 2013; 341:1237439.doi: 10.1126/science.1237439 [PubMed: 23828941]
6. Franzosa EA, Huang K, Meadow JF, Gevers D, Lemon KP, Bohannan BJM, et al. Identifying personal microbiomes using metagenomic codes. Proc Natl Acad Sci. 2015; 112:E2930–E2938. DOI: 10.1073/pnas.1423854112 [PubMed: 25964341]
7. Maurice CF, Haiser HJ, Turnbaugh PJ. Xenobiotics shape the physiology and gene expression of the active human gut microbiome. Cell. 2013; 152:39–50. DOI: 10.1016/j.cell.2012.10.052 [PubMed: 23332745]
8. Morgan XC, Tickle TL, Sokol H, Gevers D, Devaney KL, Ward DV, et al. Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. Genome Biol. 2012; 13:R79.doi: 10.1186/gb-2012-13-9-r79 [PubMed: 23013615]
9. Voreades N, Kozil A, Weir TL. Diet and the development of the human intestinal microbiome. Front Microbiol. 2014; 5:1–9. DOI: 10.3389/fmicb.2014.00494 [PubMed: 24478763]
10. Lozupone, Ca; Stombaugh, JI.; Gordon, JI.; Jansson, JK.; Knight, R. Diversity, stability and resilience of the human gut microbiota. Nature. 2012; 489:220–230. DOI: 10.1038/nature11550 [PubMed: 22972295]
11. David, La; Maurice, CF.; Carmody, RN.; Gootenberg, DB.; Button, JE.; Wolfe, BE., et al. Diet rapidly and reproducibly alters the human gut microbiome. Nature. 2014; 505:559–63. DOI: 10.1038/nature12820 [PubMed: 24336217]
12. Zhang C, Zhang M, Pang X, Zhao Y, Wang L, Zhao L. Structural resilience of the gut microbiota in adult mice under high-fat dietary perturbations. ISME J. 2012; 6:1848–1857. DOI: 10.1038/ismej.2012.27 [PubMed: 22495068]
13. Carmody RN, Gerber GK, Luevano JM, Gatti DM, Somes L, Svenson KL, et al. Diet Dominates Host Genotype in Shaping the Murine Gut Microbiota. Cell Host Microbe. 2015; 17:72–84. DOI: 10.1016/j.chom.2014.11.010 [PubMed: 25532804]
14. Dey N, Wagner VE, Blanton LV, Haque R, Ahmed T, Gordon JI, et al. Regulators of Gut Motility Revealed by a Gnotobiotic Model of Diet-Microbiome Interactions Related to Article Regulators of Gut Motility Revealed by a Gnotobiotic Model of Diet-Microbiome Interactions Related to Travel. Cell. 2015; 163:95–107. DOI: 10.1016/j.cell.2015.08.059 [PubMed: 26406373]
15. Goodrich JK, Waters JL, Poole AC, Sutter JL, Koren O, Blekhman R, et al. Human Genetics Shape the Gut Microbiome. Cell. 2014; 159:789–799. DOI: 10.1016/j.cell.2014.09.053 [PubMed: 25417156]
16. Ley RE, Bäckhed F, Turnbaugh P, Lozupone Ca, Knight RD, Gordon JI. Obesity alters gut microbial ecology. Proc Natl Acad Sci. 2005; 102:11070–11075. DOI: 10.1073/pnas.0504978102 [PubMed: 16033867]
17. Turnbaugh PJ, Ley RE, Mahowald Ma, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 2006; 444:1027–1031. DOI: 10.1038/nature05414 [PubMed: 17183312]
18. Ridaura VK, Faith JJ, Rey FE, Cheng J, Duncan AE, Kau AL, et al. Gut Microbiota from Twins Discordant for Obesity Modulate Metabolism in Mice. Science. 2013; 341:1241214.doi: 10.1126/science.1241214 [PubMed: 24009397]
19. Smith MI, Yatsunenko T, Manary MJ, Trehan I, Mkakosya R, Cheng J, et al. Gut Microbiomes of Malawian Twin Pairs Discordant for Kwashiorkor. Science. 2013; 339:548–554. DOI: 10.1126/science.1229000 [PubMed: 23363771]
20. Canfora EE, Jocken JW, Blaak EE. Short-chain fatty acids in control of body weight and insulin sensitivity. Nat Rev Endocrinol. 2015; 11:577–591. DOI: 10.1038/nrendo.2015.128 [PubMed: 26260141]
21. Fischbach, Ma; Sonnenburg, JL. Eating for two: how metabolism establishes interspecies interactions in the gut. Cell Host Microbe. 2011; 10:336–47. DOI: 10.1016/j.chom.2011.10.002 [PubMed: 22018234]

22. Krishnan S, Alden N, Lee K. Pathways and functions of gut microbiota metabolism impacting host physiology. Curr Opin Biotechnol. 2015; 36:137–145. DOI: 10.1016/j.copbio.2015.08.015 [PubMed: 26340103]

23. Donohoe DR, Garge N, Zhang X, Sun W, O'Connell TM, Bunger MK, et al. The Microbiome and Butyrate Regulate Energy Metabolism and Autophagy in the Mammalian Colon. Cell Metab. 2011; 13:517–526. DOI: 10.1016/j.cmet.2011.02.018 [PubMed: 21531334]

24. Blouin JM, Penot G, Collinet M, Nacfer M, Forest C, Laurent-Puig P, et al. Butyrate elicits a metabolic switch in human colon cancer cells by targeting the pyruvate dehydrogenase complex. Int J Cancer. 2011; 128:2591–2601. DOI: 10.1002/ijc.25599 [PubMed: 20715114]

25. Kelly CJ, Zheng L, Campbell EL, Saeedi B, Scholz CC, Bayless AJ, et al. Crosstalk between Microbiota-Derived Short-Chain Fatty Acids and Intestinal Epithelial HIF Augments Tissue Barrier Function. Cell Host Microbe. 2015; 17:662–671. DOI: 10.1016/j.chom.2015.03.005 [PubMed: 25865369]

26. Davie JR. Inhibition of histone deacetylase activity by butyrate. J Nutr. 2003; 133:2485S–2493S. http://www.ncbi.nlm.nih.gov/pubmed/12840228. [PubMed: 12840228]

27. Brown AJ, Goldsworthy SM, Barnes AA, Eilert MM, Tcheang L, Daniels D, et al. The Orphan G protein-coupled receptors GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids. J Biol Chem. 2003; 278:11312–9. DOI: 10.1074/jbc.M211609200 [PubMed: 12496283]

28. Samuel BS, Shaito A, Motoike T, Rey FE, Backhed F, Manchester JK, et al. Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, Gpr41. Proc Natl Acad Sci. 2008; 105:16767–16772. DOI: 10.1073/pnas.0808567105 [PubMed: 18931303]

29. Soret R, Chevalier J, De Coppet P, Poupeau G, Derkinderen P, Segain JP, et al. Short-Chain Fatty Acids Regulate the Enteric Neurons and Control Gastrointestinal Motility in Rats. Gastroenterology. 2010; 138:1772–1782. DOI: 10.1053/j.gastro.2010.01.053 [PubMed: 20152836]

30. De Vadder F, Kovatcheva-Datchary P, Goncalves D, Vinera J, Zitoun C, Duchampt A, et al. Microbiota-generated metabolites promote metabolic benefits via gut-brain neural circuits. Cell. 2014; 156:84–96. DOI: 10.1016/j.cell.2013.12.016 [PubMed: 24412651]

31. Tolhurst G, Heffron H, Lam YS, Parker HE, Habib AM, Diakogiannaki E, et al. Short-Chain Fatty Acids Stimulate Glucagon-Like Peptide-1 Secretion via the G-Protein-Coupled Receptor FFAR2. Diabetes. 2012; 61:364–371. DOI: 10.2337/db11-1019 [PubMed: 22190648]

32. Degnan PH, Taga ME, Goodman AL. Vitamin B12 as a Modulator of Gut Microbial Ecology. Cell Metab. 2014; 20:769–778. DOI: 10.1016/j.cmet.2014.10.002 [PubMed: 25440056]

33. Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, Dugar B, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nature. 2011; 472:57–63. DOI: 10.1038/nature09922 [PubMed: 21475195]

34. Koeth RA, Levison BS, Culley MK, Buffa JA, Wang Z, Gregory JC, et al. γ-Butyrobetaine Is a Proatherogenic Intermediate in Gut Microbial Metabolism of L-Carnitine to TMAO. Cell Metab. 2014; 20:799–812. DOI: 10.1016/j.cmet.2014.10.006 [PubMed: 25440057]

35. Gregory JC, Buffa JA, Org E, Wang Z, Levison BS, Zhu W, et al. Transmission of Atherosclerosis Susceptibility with Gut Microbial Transplantation. J Biol Chem. 2015; 290:5647–5660. DOI: 10.1074/jbc.M114.618249 [PubMed: 25550161]

36. Wang Z, Roberts AB, Buffa JA, Levison BS, Zhu W, Org E, et al. Non-lethal Inhibition of Gut Microbial Trimethylamine Production for the Treatment of Atherosclerosis. Cell. 2015; 163:1585–1595. DOI: 10.1016/j.cell.2015.11.055 [PubMed: 26687352]

37. Dobkin JF, Saha JR, Butler VP, Neu HC, Lindenbaum J. Digoxin-inactivating bacteria: identification in human gut flora. Science. 1983; 220:325–7. http://www.ncbi.nlm.nih.gov/pubmed/6836275. [PubMed: 6836275]

38. Haiser HJ, Gootenberg DB, Chatman K, Sirasani G, Balskus EP, Turnbaugh PJ. Predicting and Manipulating Cardiac Drug Inactivation by the Human Gut Bacterium *Eggerthella lenta*. Science. 2013; 341:295–298. DOI: 10.1126/science.1235872 [PubMed: 23869020]

39. Wallace BD, Wang H, Lane KT, Scott JE, Orans J, Koo JS, et al. Alleviating cancer drug toxicity by inhibiting a bacterial enzyme. Science. 2010; 330:831–5. DOI: 10.1126/science.1191175 [PubMed: 21051639]
40. Belkaid Y, Hand TW. Role of the Microbiota in Immunity and Inflammation. Cell. 2014; 157:121–141. DOI: 10.1016/j.cell.2014.03.011 [PubMed: 24679531]
41. Kabat AM, Srinivasan N, Maloy KJ. Modulation of immune development and function by intestinal microbiota. Trends Immunol. 2014; 35:507–517. DOI: 10.1016/j.it.2014.07.010 [PubMed: 25172617]
42. Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. Nat Rev Immunol. 2009; 9:313–323. DOI: 10.1038/nri2614 [PubMed: 19343057]
43. Wlodarska M, Kostic AD, Xavier RJ. An Integrative View of Microbiome-Host Interactions in Inflammatory Bowel Diseases. Cell Host Microbe. 2015; 17:577–591. DOI: 10.1016/j.chom.2015.04.008 [PubMed: 25974300]
44. Vujkovic-Cvijin I, Dunham RM, Iwai S, Maher MC, Albright RG, Broadhurst MJ, et al. Dysbiosis of the gut microbiota is associated with HIV disease progression and tryptophan catabolism. Sci Transl Med. 2013; 5:193ra91.doi: 10.1126/scitranslmed.3006438
45. Shulzhenko N, Morgun A, Hsiao W, Battle M, Yao M, Gavrilova O, et al. Crosstalk between B lymphocytes, microbiota and the intestinal epithelium governs immunity versus metabolism in the gut. Nat Med. 2011; 17:1585–1593. DOI: 10.1038/nm.2505 [PubMed: 22101768]
46. Garrett WS, Gallini CA, Yatsunenko T, Michaud M, Dubois A, Delaney ML, et al. Enterobacteriaceae act in concert with the gut microbiota to induce spontaneous and maternally transmitted colitis. Cell Host Microbe. 2010; 8:292–300. DOI: 10.1016/j.chom.2010.08.004 [PubMed: 20833380]
47. Haberman Y, Tickle TL, Dexheimer PJ, Kim M, Tang D, Karns R, et al. Pediatric Crohn disease patients exhibit specific ileal transcriptome and microbiome signature. J Clin Invest. 2014; 124:3617–33. DOI: 10.1172/JCI75436 [PubMed: 25003194]
48. Mazmanian SK, Round JL, Kasper DL. A microbial symbiosis factor prevents intestinal inflammatory disease. Nature. 2008; 453:620–5. DOI: 10.1038/nature07008 [PubMed: 18509436]
49. Round JL, Mazmanian SK. Inducible Foxp3+ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. Proc Natl Acad Sci. 2010; 107:12204–9. DOI: 10.1073/pnas.0909122107 [PubMed: 20566854]
50. Atarashi K, Tanoue T, Shima T, Imaoka A, Kuwahara T, Momose Y, et al. Induction of Colonic Regulatory T Cells by Indigenous *Clostridium* Species. Science. 2011; 331:337–341. DOI: 10.1126/science.1198469 [PubMed: 21205640]
51. Furusawa Y, Obata Y, Fukuda S, Endo Ta, Nakato G, Takahashi D, et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. Nature. 2013; 504:446–50. DOI: 10.1038/nature12721 [PubMed: 24226770]
52. Arpaia N, Campbell C, Fan X, Dikiy S, van der Veeken J, deRoos P, et al. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. Nature. 2013; 504:451–5. DOI: 10.1038/nature12726 [PubMed: 24226773]
53. Yang Y, Torchinsky MB, Gobert M, Xiong H, Xu M, Linehan JL, et al. Focused specificity of intestinal Th17 cells towards commensal bacterial antigens. Nature. 2014; 510:152–6. DOI: 10.1038/nature13279 [PubMed: 24739972]
54. Ivanov II, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, et al. Induction of intestinal Th17 cells by segmented filamentous bacteria. Cell. 2009; 139:485–98. DOI: 10.1016/j.cell.2009.09.033 [PubMed: 19836068]
55. Arrieta MC, Stiemsma LT, Dimitriu PA, Thorson L, Russell S, Yurist-Doutsch S, et al. Early infancy microbial and metabolic alterations affect risk of childhood asthma. Sci Transl Med. 2015; 7:307ra152–307ra152. DOI: 10.1126/scitranslmed.aab2271
56. Stefka AT, Feehley T, Tripathi P, Qiu J, McCoy K, Mazmanian SK, et al. Commensal bacteria protect against food allergen sensitization. Proc Natl Acad Sci. 2014; 111:13145–13150. DOI: 10.1073/pnas.1412008111 [PubMed: 25157157]

57. Sassone-Corsi M, Raffatellu M. No Vacancy: How Beneficial Microbes Cooperate with Immunity To Provide Colonization Resistance to Pathogens. J Immunol. 2015; 194:4081–4087. DOI: 10.4049/jimmunol.1403169 [PubMed: 25888704]
58. Buffie CG, Pamer EG. Microbiota-mediated colonization resistance against intestinal pathogens. Nat Rev Immunol. 2013; 13:790–801. DOI: 10.1038/nri3535 [PubMed: 24096337]
59. Rupnik M, Wilcox MH, Gerding DN. *Clostridium difficile* infection: new developments in epidemiology and pathogenesis. Nat Rev Microbiol. 2009; 7:526–536. DOI: 10.1038/nrmicro2164 [PubMed: 19528959]
60. Deriu E, Liu JZ, Pezeshki M, Edwards RA, Ochoa RJ, Contreras H, et al. Probiotic Bacteria Reduce *Salmonella* Typhimurium Intestinal Colonization by Competing for Iron. Cell Host Microbe. 2013; 14:26–37. doi:http://dx.doi.org/10.1016/j.chom.2013.06.007. [PubMed: 23870311]
61. Buffie CG, Bucci V, Stein RR, McKenney PT, Ling L, Gobourne A, et al. Precision microbiome reconstitution restores bile acid mediated resistance to *Clostridium difficile*. Nature. 2014; doi: 10.1038/nature13828
62. Russell AB, Wexler AG, Harding BN, Whitney JC, Bohn AJ, Goo YA, et al. A Type VI Secretion-Related Pathway in Bacteroidetes Mediates Interbacterial Antagonism. Cell Host Microbe. 2014; : 1–10. DOI: 10.1016/j.chom.2014.07.007
63. Ng KM, Ferreyra JA, Higginbottom SK, Lynch JB, Kashyap PC, Gopinath S, et al. Microbiota-liberated host sugars facilitate post-antibiotic expansion of enteric pathogens. Nature. 2013; 502:96–99. DOI: 10.1038/nature12503 [PubMed: 23995682]
64. Ferreyra JA, Wu KJ, Hryckowian AJ, Bouley DM, Weimer BC, Sonnenburg JL. Gut Microbiota-Produced Succinate Promotes *C. difficile* Infection after Antibiotic Treatment or Motility Disturbance. Cell Host Microbe. 2014; 16:770–777. DOI: 10.1016/j.chom.2014.11.003 [PubMed: 25498344]
65. Lyte, M.; Cryan, JF. Microbial Endocrinology_: The Microbiota- Gut-Brain Axis in Health and Disease. Springer; 2014.
66. Sharon G, Garg N, Debelius J, Knight R, Dorrestein PC, Mazmanian SK. Perspective Specialized Metabolites from the Microbiome in Health and Disease. Cell Metab. 2014; 20:719–730. DOI: 10.1016/j.cmet.2014.10.016 [PubMed: 25440054]
67. Sampson TR, Mazmanian SK. Control of Brain Development, Function, and Behavior by the Microbiome. Cell Host Microbe. 2015; 17:565–576. DOI: 10.1016/j.chom.2015.04.011 [PubMed: 25974299]
68. Yano JM, Yu K, Donaldson GP, Shastri GG, Ann P, Ma L, et al. Indigenous Bacteria from the Gut Microbiota Regulate Host Serotonin Biosynthesis. Cell. 2015; 161:264–276. DOI: 10.1016/j.cell.2015.02.047 [PubMed: 25860609]
69. Williams BB, Van Benschoten AH, Cimermancic P, Donia MS, Zimmermann M, Taketani M, et al. Discovery and Characterization of Gut Microbiota Decarboxylases that Can Produce the Neurotransmitter Tryptamine. Cell Host Microbe. 2014; :1–9. DOI: 10.1016/j.chom.2014.09.001
70. Sudo N, Chida Y, Aiba Y, Sonoda J, Oyama N, Yu XN, et al. Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. J Physiol. 2004; 558:263–275. DOI: 10.1113/jphysiol.2004.063388 [PubMed: 15133062]
71. Hsiao EY, McBride SW, Hsien S, Sharon G, Hyde ER, McCue T, et al. Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. Cell. 2013; 155:1451–63. DOI: 10.1016/j.cell.2013.11.024 [PubMed: 24315484]
72. Derrien M, van Hylckama Vlieg JET. Fate, activity, and impact of ingested bacteria within the human gut microbiota. Trends Microbiol. 2015; 23:354–366. DOI: 10.1016/j.tim.2015.03.002 [PubMed: 25840765]
73. Marchesi JR, Adams DH, Fava F, Da Hermes G, Hirschfield GM, Hold G, et al. The gut microbiota and host health: a new clinical frontier. Gut. 2015; :1–10. DOI: 10.1136/gutjnl-2015-309990
74. Frei R, Akdis M, O'Mahony L. Prebiotics, probiotics, synbiotics, and the immune system. Curr Opin Gastroenterol. 2015; 31:153–158. DOI: 10.1097/MOG.0000000000000151 [PubMed: 25594887]

75. de LeBlanc, A de M. Effect of probiotic administration on the intestinal microbiota, current knowledge and potential applications. World J Gastroenterol. 2014; 20:16518.doi: 10.3748/wjg.v20.i44.16518 [PubMed: 25469019]
76. Varankovich NV, Nickerson MT, Korber DR. Probiotic-based strategies for therapeutic and prophylactic use against multiple gastrointestinal diseases. Front Microbiol. 2015; 6:1–14. DOI: 10.3389/fmicb.2015.00685 [PubMed: 25653648]
77. Cuello-Garcia, Ca; Brożek, JL.; Fiocchi, A.; Pawankar, R.; Yepes-Nuñez, JJ.; Terracciano, L., et al. Probiotics for the prevention of allergy: A systematic review and meta-analysis of randomized controlled trials. J Allergy Clin Immunol. 2015; :1–10. DOI: 10.1016/j.jaci.2015.04.031
78. Ritchie ML, Romanuk TN. A Meta-Analysis of Probiotic Efficacy for Gastrointestinal Diseases. PLoS One. 2012; 7:e34938.doi: 10.1371/journal.pone.0034938 [PubMed: 22529959]
79. Zuccotti G, Meneghin F, Aceti A, Barone G, Callegari ML, Di Mauro A, et al. Probiotics for prevention of atopic diseases in infants: systematic review and meta-analysis. Allergy. 2015; 70 n/a–n/a. doi: 10.1111/all.12700
80. Fujiya M, Ueno N, Kohgo Y. Probiotic treatments for induction and maintenance of remission in inflammatory bowel diseases: a meta-analysis of randomized controlled trials. Clin J Gastroenterol. 2014; 7:1–13. DOI: 10.1007/s12328-013-0440-8 [PubMed: 26183502]
81. Duan F, March JC. Engineered bacterial communication prevents *Vibrio cholerae* virulence in an infant mouse model. Proc Natl Acad Sci. 2010; 107:11260–11264. DOI: 10.1073/pnas.1001294107 [PubMed: 20534565]
82. Lagenaur LA, Sanders-Beer BE, Brichacek B, Pal R, Liu X, Liu Y, et al. Prevention of vaginal SHIV transmission in macaques by a live recombinant *Lactobacillus*. Mucosal Immunol. 2011; 4:648–657. DOI: 10.1038/mi.2011.30 [PubMed: 21734653]
83. Steidler L, Hans W, Schotte L, Neirynck S, Obermeier F, Falk W, et al. Treatment of Murine Colitis by *Lactococcus lactis* Secreting Interleukin-10. Science. 2000; 289:1352–1355. DOI: 10.1126/science.289.5483.1352 [PubMed: 10958782]
84. Braat H, Rottiers P, Hommes DW, Huyghebaert N, Remaut E, Remon J, et al. A Phase I Trial With Transgenic Bacteria Expressing Interleukin-10 in Crohn's Disease. Clin Gastroenterol Hepatol. 2006; 4:754–759. http://dx.doi.org/10.1016/j.cgh.2006.03.028. [PubMed: 16716759]
85. Takiishi T, Korf H, Van Belle TL, Robert S, Grieco Fa, Caluwaerts S, et al. Reversal of autoimmune diabetes by restoration of antigen-specific tolerance using genetically modified *Lactococcus lactis* in mice. J Clin Invest. 2012; 122:1717–1725. DOI: 10.1172/JCI60530 [PubMed: 22484814]
86. Robert S, Gysemans C, Takiishi T, Korf H, Spagnuolo I, Sebastiani G, et al. Oral delivery of glutamic acid decarboxylase (GAD)-65 and IL10 by *Lactococcus lactis* reverses diabetes in recent-onset NOD mice. Diabetes. 2014; 63:2876–2887. DOI: 10.2337/db13-1236 [PubMed: 24677716]
87. Hamady ZZR, Scott N, Farrar MD, Wadhwa M, Dilger P, Whitehead TR, et al. Treatment of colitis with a commensal gut bacterium engineered to secrete human TGF-β1 under the control of dietary xylan. Inflamm Bowel Dis. 2011; 17:1925–1935. DOI: 10.1002/ibd.21565 [PubMed: 21830271]
88. Vandenbroucke K, de Haard H, Beirnaert E, Dreier T, Lauwereys M, Huyck L, et al. Orally administered *L. lactis* secreting an anti-TNF Nanobody demonstrate efficacy in chronic colitis. Mucosal Immunol. 2010; 3:49–56. DOI: 10.1038/mi.2009.116 [PubMed: 19794409]
89. Hamady ZZR, Scott N, Farrar MD, Lodge JPA, Holland KT, Whitehead T, et al. Xylan-regulated delivery of human keratinocyte growth factor-2 to the inflamed colon by the human anaerobic commensal bacterium *Bacteroides ovatus*. Gut. 2010; 59:461–9. DOI: 10.1136/gut.2008.176131 [PubMed: 19736360]
90. Motta JP, Bermudez-Humaran LG, Deraison C, Martin L, Rolland C, Rousset P, et al. Food-Grade Bacteria Expressing Elafin Protect Against Inflammation and Restore Colon Homeostasis. Sci Transl Med. 2012; 4:158ra144–158ra144. DOI: 10.1126/scitranslmed.3004212
91. Caluwaerts S, Vandenbroucke K, Steidler L, Neirynck S, Vanhoenacker P, Corveleyn S, et al. AG013, a mouth rinse formulation of *Lactococcus lactis* secreting human Trefoil Factor 1, provides a safe and efficacious therapeutic tool for treating oral mucositis. Oral Oncol. 2010; 46:564–70. DOI: 10.1016/j.oraloncology.2010.04.008 [PubMed: 20542722]

92. Limaye SA, Haddad RI, Cilli F, Sonis ST, Colevas aD, Brennan MT, et al. Phase 1b, multicenter, single blinded, placebo-controlled, sequential dose escalation study to assess the safety and tolerability of topically applied AG013 in subjects with locally advanced head and neck cancer receiving induction chemotherapy. Cancer. 2013; 119:4268–4276. DOI: 10.1002/cncr.28365 [PubMed: 24114811]

93. Chen Z, Guo L, Zhang Y, Walzem RL, Pendergast JS, Printz RL, et al. Incorporation of therapeutically modified bacteria into gut microbiota inhibits obesity. J Clin Invest. 2014; 119:1–16. DOI: 10.1172/JCI72517

94. Duan FF, Liu JH, March JC. Engineered Commensal Bacteria Reprogram Intestinal Cells Into Glucose-Responsive Insulin-Secreting Cells for the Treatment of Diabetes. Diabetes. 2015; doi: 10.2337/db14-0635

95. Kassam Z, Lee CH, Yuan Y, Hunt RH. Fecal Microbiota Transplantation for *Clostridium difficile* Infection: Systematic Review and Meta-Analysis. Am J Gastroenterol. 2013; 108:500–508. DOI: 10.1038/ajg.2013.59 [PubMed: 23511459]

96. van Nood E, Vrieze A, Nieuwdorp M, Fuentes S, Zoetendal EG, de Vos WM, et al. Duodenal Infusion of Donor Feces for Recurrent *Clostridium difficile*. N Engl J Med. 2013; 368:407–415. DOI: 10.1056/NEJMoa1205037 [PubMed: 23323867]

97. Smith MB, Kelly C, Alm EJ. How to regulate faecal transplants. Nature. 2014; 506:290–1. DOI: 10.1038/506290a [PubMed: 24558658]

98. Petrof E, Gloor G, Vanner S, Weese S, Carter D, Daigneault M, et al. Stool substitute transplant therapy for the eradication of *Clostridium difficile* infection: "RePOOPulating" the gut. Microbiome. 2013; 1:3. http://www.microbiomejournal.com/content/1/1/3. [PubMed: 24467987]

99. Ratner M. Microbial cocktails join fecal transplants in IBD treatment trials. Nat Biotechnol. 2015; 33:787–788. DOI: 10.1038/nbt0815-787 [PubMed: 26252119]

100. Moayyedi P, Surette MG, Kim PT, Libertucci J, Wolfe M, Onischi C, et al. Fecal Microbiota Transplantation Induces Remission in Patients With Active Ulcerative Colitis in a Randomized Controlled Trial. Gastroenterology. 2015; 149:102–109.e6. DOI: 10.1053/j.gastro.2015.04.001 [PubMed: 25857665]

101. Ianiro G, Bibbò S, Scaldaferri F, Gasbarrini A, Cammarota G. Fecal Microbiota Transplantation in Inflammatory Bowel Disease. Medicine (Baltimore). 2014; 93:e97.doi: 10.1097/MD.0000000000000097 [PubMed: 25340496]

102. Shen TD, Albenberg L, Bittinger K, Chehoud C, Chen Y, Judge Ca, et al. Engineering the gut microbiota to treat hyperammonemia. J Clin Invest. 2015; 125:2841–2850. DOI: 10.1172/JCI79214 [PubMed: 26098218]

103. Walsh CJ, Guinane CM, Hill C, Ross RP, O'Toole PW, Cotter PD. *In silico* identification of bacteriocin gene clusters in the gastrointestinal tract, based on the Human Microbiome Project's reference genome database. BMC Microbiol. 2015; 15:183.doi: 10.1186/s12866-015-0515-4 [PubMed: 26377179]

104. Zheng J, Gänzle MG, Lin XB, Ruan L, Sun M. Diversity and dynamics of bacteriocins from human microbiome. Environ Microbiol. 2015; 17:2133–2143. DOI: 10.1111/1462-2920.12662 [PubMed: 25346017]

105. Kommineni S, Bretl DJ, Lam V, Chakraborty R, Hayward M, Simpson P, et al. Bacteriocin production augments niche competition by enterococci in the mammalian gastrointestinal tract. Nature. 2015; doi: 10.1038/nature15524

106. Corr SC, Li Y, Riedel CU, O'Toole PW, Hill C, Gahan CGM. Bacteriocin production as a mechanism for the antiinfective activity of *Lactobacillus salivarius* UCC118. Proc Natl Acad Sci. 2007; 104:7617–7621. DOI: 10.1073/pnas.0700440104 [PubMed: 17456596]

107. Millette M, Cornut G, Dupont C, Shareck F, Archambault D, Lacroix M. Capacity of human nisin- and pediocin-producing lactic Acid bacteria to reduce intestinal colonization by vancomycin-resistant enterococci. Appl Environ Microbiol. 2008; 74:1997–2003. DOI: 10.1128/AEM.02150-07 [PubMed: 18245231]

108. Twort FW. An Investigation on the Nature of Ultra-Microscopic Viruses. Lancet. 1915; 186:1241–1243. http://dx.doi.org/10.1016/S0140-6736(01)20383-3.

109. d'Herelle F. Sur un microbe invisible antagoniste des bacilles dysentériques. CR Acad Sci Paris. 1917; 165:373–375.
110. Reardon S. Phage therapy gets revitalized. Nature. 2014; 510:15–16. DOI: 10.1038/510015a [PubMed: 24899282]
111. Kingwell K. Bacteriophage therapies re-enter clinical trials. Nat Rev Drug Discov. 2015; 14:515–516. DOI: 10.1038/nrd4695 [PubMed: 26228748]
112. Sulakvelidze A, Alavidze Z, Morris JG. Bacteriophage Therapy. Antimicrob Agents Chemother. 2001; 45:649–659. DOI: 10.1128/AAC.45.3.649-659.2001 [PubMed: 11181338]
113. Weiss M, Denou E, Bruttin A, Serra-Moreno R, Dillmann ML, Brüssow H. *In vivo* replication of T4 and T7 bacteriophages in germ-free mice colonized with *Escherichia coli*. Virology. 2009; 393:16–23. DOI: 10.1016/j.virol.2009.07.020 [PubMed: 19699505]
114. Maura D, Morello E, du Merle L, Bomme P, Le Bouguénec C, Debarbieux L. Intestinal colonization by enteroaggregative *Escherichia coli* supports long-term bacteriophage replication in mice. Environ Microbiol. 2012; 14:1844–1854. DOI: 10.1111/j.1462-2920.2011.02644.x [PubMed: 22118225]
115. Mills S, Shanahan F, Stanton C, Hill C, Coffey A, Ross RP. Movers and shakers: Influence of bacteriophages in shaping the mammalian gut microbiota. Gut Microbes. 2013; 4:4–16. DOI: 10.4161/gmic.22371 [PubMed: 23022738]
116. Reyes A, Haynes M, Hanson N, Angly FE, Heath AC, Rohwer F, et al. Viruses in the faecal microbiota of monozygotic twins and their mothers. Nature. 2010; 466:334–8. DOI: 10.1038/nature09199 [PubMed: 20631792]
117. Minot S, Sinha R, Chen J, Li H, Keilbaugh SA, Wu GD, et al. The human gut virome: inter-individual variation and dynamic response to diet. Genome Res. 2011; 21:1616–25. DOI: 10.1101/gr.122705.111 [PubMed: 21880779]
118. Norman JM, Handley SA, Baldridge MT, Droit L, Liu CY, Keller BC, et al. Disease-Specific Alterations in the Enteric Virome in Inflammatory Bowel Disease. Cell. 2015; 160:447–460. DOI: 10.1016/j.cell.2015.01.002 [PubMed: 25619688]
119. Modi SR, Lee HH, Spina CS, Collins JJ. Antibiotic treatment expands the resistance reservoir and ecological network of the phage metagenome. Nature. 2013; 499:219–222. DOI: 10.1038/nature12212 [PubMed: 23748443]
120. Reyes A, Wu M, McNulty NP, Rohwer FL, Gordon JI. Gnotobiotic mouse model of phage-bacterial host dynamics in the human gut. Proc Natl Acad Sci. 2013; 110:20236–41. DOI: 10.1073/pnas.1319470110 [PubMed: 24259713]
121. Barr JJ, Auro R, Furlan M, Whiteson KL, Erb ML, Pogliano J, et al. Bacteriophage adhering to mucus provide a non-host-derived immunity. Proc Natl Acad Sci. 2013; 110:10771–6. DOI: 10.1073/pnas.1305923110 [PubMed: 23690590]
122. Ando H, Lemire S, Pires DP, Lu TK. Engineering Modular Viral Scaffolds for Targeted Bacterial Population Editing. Cell Syst. 2015; 1:187–196. DOI: 10.1016/j.cels.2015.08.013 [PubMed: 26973885]
123. Lu TK, Collins JJ. Dispersing biofilms with engineered enzymatic bacteriophage. Proc Natl Acad Sci. 2007; 104:11197–11202. http://www.pnas.org/content/104/27/11197.abstract. [PubMed: 17592147]
124. Lu TK, Collins JJ. Engineered bacteriophage targeting gene networks as adjuvants for antibiotic therapy. Proc Natl Acad Sci. 2009; 106:4629–4634. http://www.pnas.org/content/106/12/4629.abstract. [PubMed: 19255432]
125. Edgar R, Friedman N, Molshanski-Mor S, Qimron U. Reversing bacterial resistance to antibiotics by phage-mediated delivery of dominant sensitive genes. Appl Environ Microbiol. 2012; 78:744–51. DOI: 10.1128/AEM.05741-11 [PubMed: 22113912]
126. Westwater C, Kasman LM, Schofield DA, Werner PA, Dolan JW, Schmidt MG, et al. Use of Genetically Engineered Phage To Deliver Antimicrobial Agents to Bacteria: an Alternative Therapy for Treatment of Bacterial Infections. Antimicrob Agents Chemother. 2003; 47:1301–1307. DOI: 10.1128/AAC.47.4.1301 [PubMed: 12654662]

127. Hagens S, Habel A, von Ahsen U, von Gabain A, Bläsi U. Therapy of Experimental *Pseudomonas* Infections with a Nonreplicating Genetically Modified Phage. Antimicrob Agents Chemother. 2004; 48:3817–3822. DOI: 10.1128/AAC.48.10.3817-3822.2004 [PubMed: 15388440]
128. Krom RJ, Bhargava P, Lobritz Ma, Collins JJ. Engineered Phagemids for Nonlytic, Targeted Antibacterial Therapies. Nano Lett. 2015; 15:4808–4813. DOI: 10.1021/acs.nanolett.5b01943 [PubMed: 26044909]
129. Bikard D, Euler CW, Jiang W, Nussenzweig PM, Goldberg GW, Duportet X, et al. Exploiting CRISPR-Cas nucleases to produce sequence-specific antimicrobials. Nat Biotechnol. 2014; :1–6. DOI: 10.1038/nbt.3043 [PubMed: 24406907]
130. Citorik RJ, Mimee M, Lu TK. Sequence-specific antimicrobials using efficiently delivered RNA-guided nucleases. Nat Biotechnol. 2014; 32:1141–5. DOI: 10.1038/nbt.3011 [PubMed: 25240928]
131. Kiro R, Shitrit D, Qimron U. Efficient engineering of a bacteriophage genome using the type I-E CRISPR-Cas system. RNA Biol. 2014; 11:42–44. DOI: 10.4161/rna.27766 [PubMed: 24457913]
132. Gibson DG, Young L, Chuang R-Y, Venter JC, Hutchison CA, Smith HO. Enzymatic assembly of DNA molecules up to several hundred kilobases. Nat Meth. 2009; 6:343–345. http://dx.doi.org/10.1038/nmeth.1318.
133. Joeres-Nguyen-Xuan TH, Boehm SK, Joeres L, Schulze J, Kruis W. Survival of the probiotic *Escherichia coli* Nissle 1917 (EcN) in the gastrointestinal tract given in combination with oral mesalamine to healthy volunteers. Inflamm Bowel Dis. 2010; 16:256–62. DOI: 10.1002/ibd.21042 [PubMed: 19637333]
134. Seedorf H, Griffin NW, Ridaura VK, Reyes A, Cheng J, Rey FE, et al. Bacteria from Diverse Habitats Colonize and Compete in the Mouse Gut. Cell. 2014; 159:253–266. DOI: 10.1016/j.cell.2014.09.008 [PubMed: 25284151]
135. Wu M, McNulty NP, Rodionov Da, Khoroshkin MS, Griffin NW, Cheng J, et al. Genetic determinants of in vivo fitness and diet responsiveness in multiple human gut *Bacteroides*. Science. 2015; 350:aac5992–aac5992. DOI: 10.1126/science.aac5992 [PubMed: 26430127]
136. Sonnenburg ED, Zheng H, Joglekar P, Higginbottom SK, Firbank SJ, Bolam DN, et al. Specificity of polysaccharide use in intestinal *Bacteroides* species determines diet-induced microbiota alterations. Cell. 2010; 141:1241–52. DOI: 10.1016/j.cell.2010.05.005 [PubMed: 20603004]
137. Lee SM, Donaldson GP, Mikulski Z, Boyajian S, Ley K, Mazmanian SK. Bacterial colonization factors control specificity and stability of the gut microbiota. Nature. 2013; 501:426–9. DOI: 10.1038/nature12447 [PubMed: 23955152]
138. Goodman AL, McNulty NP, Zhao Y, Leip D, Mitra RD, Lozupone Ca, et al. Identifying genetic determinants needed to establish a human gut symbiont in its habitat. Cell Host Microbe. 2009; 6:279–89. DOI: 10.1016/j.chom.2009.08.003 [PubMed: 19748469]
139. Cullen TW, Schofield WB, Barry NA, Putnam EE, Rundell EA, Trent MS, et al. Antimicrobial peptide resistance mediates resilience of prominent gut commensals during inflammation. Science. 2015; 347:170–175. DOI: 10.1126/science.1260580 [PubMed: 25574022]
140. Levy R, Borenstein E. Metabolic modeling of species interaction in the human microbiome elucidates community-level assembly rules. Proc Natl Acad Sci. 2013; 110:12804–9. DOI: 10.1073/pnas.1300926110 [PubMed: 23858463]
141. Greenblum S, Chiu HC, Levy R, Carr R, Borenstein E. Towards a predictive systems-level model of the human microbiome: progress, challenges, and opportunities. Curr Opin Biotechnol. 2013; 24:810–820. DOI: 10.1016/j.copbio.2013.04.001 [PubMed: 23623295]
142. Mimee M, Tucker AC, Voigt CA, Lu TK. Programming a Human Commensal Bacterium, *Bacteroides thetaiotaomicron*, to Sense and Respond to Stimuli in the Murine Gut Microbiota. Cell Syst. 2015; :1–10. DOI: 10.1016/j.cels.2015.06.001 [PubMed: 27135681]
143. Donaldson GP, Lee SM, Mazmanian SK. Gut biogeography of the bacterial microbiota. Nat Rev Microbiol. 2015; 14:20–32. DOI: 10.1038/nrmicro3552 [PubMed: 26499895]
144. Li H, Limenitakis JP, Fuhrer T, Geuking MB, Lawson Ma, Wyss M, et al. The outer mucus layer hosts a distinct intestinal microbial niche. Nat Commun. 2015; doi: 10.1038/ncomms9292

145. Nava GM, Friedrichsen HJ, Stappenbeck TS. Spatial organization of intestinal microbiota in the mouse ascending colon. ISME J. 2011; 5:627–38. DOI: 10.1038/ismej.2010.161 [PubMed: 20981114]

146. Earle KA, Billings G, Sigal M, Lichtman JS, Hansson GC, Elias JE, et al. Quantitative Imaging of Gut Microbiota Spatial Organization. Cell Host Microbe. 2015; 18:478–488. DOI: 10.1016/j.chom.2015.09.002 [PubMed: 26439864]

147. Sokol H, Pigneur B, Watterlot L, Lakhdari O, Bermúdez-Humarán LG, Gratadoux JJ, et al. *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. Proc Natl Acad Sci. 2008; 105:16731–6. DOI: 10.1073/pnas.0804812105 [PubMed: 18936492]

148. Gevers D, Kugathasan S, Denson LA, Vázquez-Baeza Y, Van Treuren W, Ren B, et al. The Treatment-Naïve Microbiome in New-Onset Crohn's Disease. Cell Host Microbe. 2014; 15:382–392. DOI: 10.1016/j.chom.2014.02.005 [PubMed: 24629344]

149. Siuti P, Yazbek J, Lu TK. Synthetic circuits integrating logic and memory in living cells. Nat Biotechnol. 2013; 31:448–52. DOI: 10.1038/nbt.2510 [PubMed: 23396014]

150. Bonnet J, Yin P, Ortiz ME, Subsoontorn P, Endy D. Amplifying genetic logic gates. Science. 2013; 340:599–603. DOI: 10.1126/science.1232758 [PubMed: 23539178]

151. Farzadfard F, Lu TK. Genomically encoded analog memory with precise *in vivo* DNA writing in living cell populations. Science. 2014; 346:6211.doi: 10.1126/science.1256272

152. Gardner TS, Cantor CR, Collins JJ. Construction of a genetic toggle switch in Escherichia coli. Nature. 2000; 403:339–342. http://dx.doi.org/10.1038/35002131. [PubMed: 10659857]

153. Kotula JW, Kerns SJ, Shaket La, Siraj L, Collins JJ, Way JC, et al. Programmable bacteria detect and record an environmental signal in the mammalian gut. Proc Natl Acad Sci. 2014; 111:4838–43. DOI: 10.1073/pnas.1321321111 [PubMed: 24639514]

154. Danino T, Prindle A, Kwong GA, Skalak M, Li H, Allen K, et al. Programmable probiotics for detection of cancer in urine. Sci Transl Med. 2015; 7:289ra84–289ra84. DOI: 10.1126/scitranslmed.aaa3519

155. Ellefson JW, Meyer AJ, Hughes Ra, Cannon JR, Brodbelt JS, Ellington AD. Directed evolution of genetic parts and circuits by compartmentalized partnered replication. Nat Biotechnol. 2013; doi: 10.1038/nbt.2714

156. Esvelt KM, Carlson JC, Liu DR. A system for the continuous directed evolution of biomolecules. Nature. 2011; 472:499–503. DOI: 10.1038/nature09929 [PubMed: 21478873]

157. Shis DL, Hussain F, Meinhardt S, Swint-Kruse L, Bennett MR. Modular, Multi-Input Transcriptional Logic Gating with Orthogonal LacI/GalR Family Chimeras. ACS Synth Biol. 2014; 3:645–51. DOI: 10.1021/sb500262f [PubMed: 25035932]

158. Chan CTY, Lee JW, Cameron DE, Bashor CJ, Collins JJ. "Deadman" and "Passcode" microbial kill switches for bacterial containment. Nat Chem Biol. 2015; :1–7. DOI: 10.1038/nchembio.1979 [PubMed: 25517376]

159. Chou HH, Keasling JD. Programming adaptive control to evolve increased metabolite production. Nat Commun. 2013; 4:2595.doi: 10.1038/ncomms3595 [PubMed: 24131951]

160. Auchtung JM, Robinson CD, Britton RA. Cultivation of stable, reproducible microbial communities from different fecal donors using minibioreactor arrays (MBRAs). Microbiome. 2015; 3:42.doi: 10.1186/s40168-015-0106-5 [PubMed: 26419531]

161. McDonald JAK, Schroeter K, Fuentes S, Heikamp-deJong I, Khursigara CM, de Vos WM, et al. Evaluation of microbial community reproducibility, stability and composition in a human distal gut chemostat model. J Microbiol Methods. 2013; 95:167–174. DOI: 10.1016/j.mimet.2013.08.008 [PubMed: 23994646]

162. Van den Abbeele P, Belzer C, Goossens M, Kleerebezem M, De Vos WM, Thas O, et al. Butyrate-producing *Clostridium* cluster XIVa species specifically colonize mucins in an *in vitro* gut model. ISME J. 2013; 7:949–61. DOI: 10.1038/ismej.2012.158 [PubMed: 23235287]

163. Lukovac S, Belzer C, Pellis L, Keijser BJ, de Vos WM, Montijn RC, et al. Differential Modulation by *Akkermansia muciniphila* and *Faecalibacterium prausnitzii* of Host Peripheral Lipid Metabolism and Histone Acetylation in Mouse Gut Organoids. MBio. 2014; 5:e01438-14–e01438-14. DOI: 10.1128/mBio.01438-14 [PubMed: 25118238]

164. Costello CM, Sorna RM, Goh Y, Cengic I, Jain NK, March JC. 3D Intestinal Scaffolds for Evaluating the Therapeutic Potential of Probiotics. Mol Pharm. 2015:2030–2039.

165. Kim HJ, Li H, Collins JJ, Ingber DE. Contributions of microbiome and mechanical deformation to intestinal bacterial overgrowth and inflammation in a human gut-on-a-chip. Proc Natl Acad Sci. 2015; 201522193. doi: 10.1073/pnas.1522193112

166. Ceroni F, Algar R, Stan GB, Ellis T. Quantifying cellular capacity identifies gene expression designs with reduced burden. Nat Methods. 2015; 12:415–418. DOI: 10.1038/nmeth.3339 [PubMed: 25849635]

167. Springman R, Molineux IJ, Duong C, Bull RJ, Bull JJ. Evolutionary stability of a refactored phage genome. ACS Synth Biol. 2012; 1:425–30. DOI: 10.1021/sb300040v [PubMed: 23519680]

168. Gladstone EG, Molineux IJ, Bull JJ. Evolutionary principles and synthetic biology: avoiding a molecular tragedy of the commons with an engineered phage. J Biol Eng. 2012; 6:13.doi: 10.1186/1754-1611-6-13 [PubMed: 22947166]

169. Smillie CS, Smith MB, Friedman J, Cordero OX, David LA, Alm EJ. Ecology drives a global network of gene exchange connecting the human microbiome. Nature. 2011; 480:241–244. DOI: 10.1038/nature10571 [PubMed: 22037308]

170. Wright O, Delmans M, Stan G-B, Ellis T. GeneGuard: a Modular Plasmid System Designed for Biosafety. ACS Synth Biol. 2014; :140513123533005.doi: 10.1021/sb500234s

171. Caliando BJ, Voigt CA. Targeted DNA degradation using a CRISPR device stably carried in the host genome. Nat Commun. 2015; 6:6989.doi: 10.1038/ncomms7989 [PubMed: 25988366]

172. Steidler L, Neirynck S, Huyghebaert N, Snoeck V, Vermeire A, Goddeeris B, et al. Biological containment of genetically modified *Lactococcus lactis* for intestinal delivery of human interleukin 10. Nat Biotechnol. 2003; 21:785–789. DOI: 10.1038/nbt840 [PubMed: 12808464]

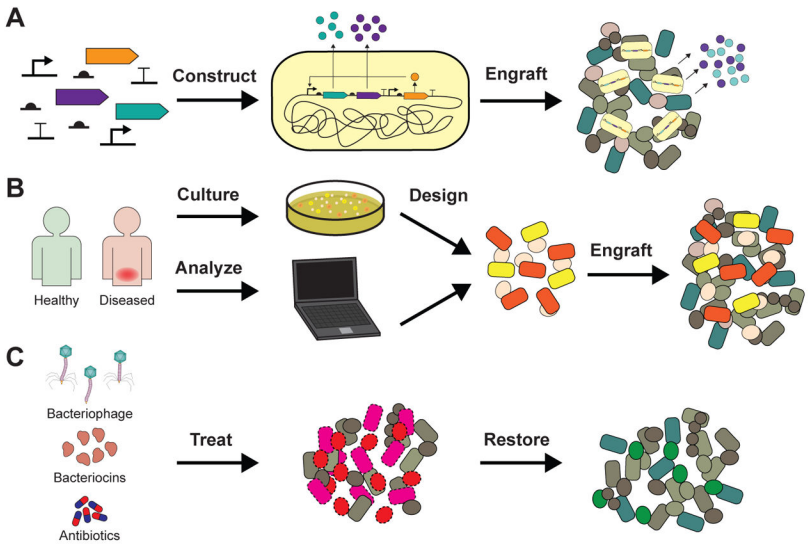

Fig. 1. Approaches in Microbiota-Based Therapeutics

(A) Engineered microbes have been one strategy for microbiota-based therapies. Gene circuits are constructed using libraries of genetic parts to enable microbial production of therapeutic proteins. Introduction of these microorganisms into the endogenous microbiota allows for *in situ* detection of disease biomarkers and/or drug production. (B) Designer microbial consortia are informed by community profiling studies of clinical samples from healthy and diseased patients. Clinical isolates from these patients can then be assembled into a defined mixture of microorganisms that can reprogram the microbial ecology within an individual. (C) Bacteriophages, bacteriocins and small molecule antibiotics can be used to selectively eliminate deleterious microbes from the microbiota. Consequently, the loss of specific taxa elicits a global shift in the microbial community as new constituents occupy the niches of the eliminated microbes. The addition of engineered bacteria together with selective elimination of targeted strains may provide enhanced therapeutic efficacy.

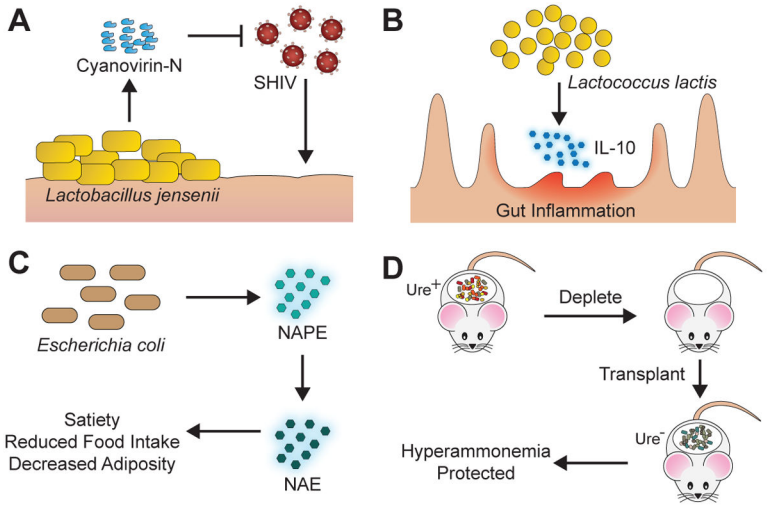

Fig. 2. Examples of Additive Microbiota Therapies

(A) The vaginal commensal *Lactobacillus jensenii* was engineered to produce the antiviral protein cyanovirin-N. Colonization of the vagina by recombinant bacteria inhibits host-infection by chimeric simian/human immunodeficiency virus (SHIV) in a rhesus macaque model. (B) *Lactococcus lactis* was genetically modified to produce the anti-inflammatory cytokine interleukin-10 (IL-10). When administered to mice afflicted with colitis, *L. lactis* transiting through the gut can alleviate intestinal inflammation. (C) Probiotic *Escherichia coli* were engineered to synthesize N-acyl-phosphatidylethanolamines (NAPEs). Host-mediated conversion of NAPEs to N-acylethanolamides (NAEs) can prevent obesity in mice by inducing satiety and reducing food intake. (D) The endogenous urease (Ure^{+}) activity of the mouse microbiota can exacerbate hyperammonemia caused by liver injury. Depletion of the native microbiota using antibiotics and polyethylene glycol and replacement with a urease-deficient (Ure^{-}) microbial consortium can protect mice from hyperammonemia and its associated neurotoxicity.

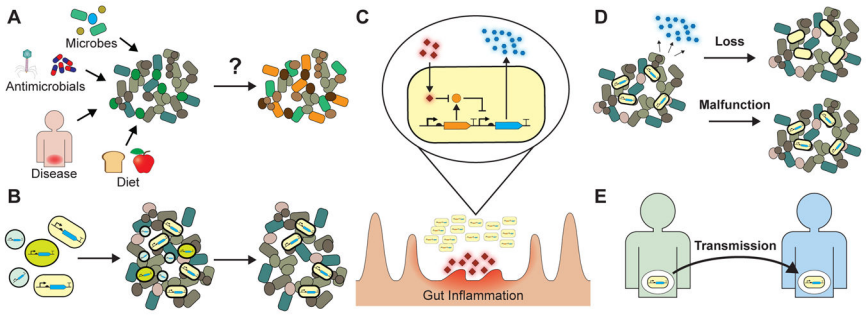

Fig. 3. Challenges in Microbiota-Based Therapeutics

(A) While it is well known that immigrant microbes, antimicrobials, disease and diet can alter the composition of a microbial community, a set of predictive rules that explain the consequences of these perturbations has yet to be elucidated. (B) Choosing the correct microbial chassis for a microbiota-based therapeutic is challenging, as it is difficult to predict which microbe is best suited for any given application. Consequently, a therapeutic microbe may fail to survive in the target environment and/or engraft in the endogenous microbiota. (C) The development of biosensors capable of detecting biomarkers associated with disease is necessary for the realization of fully autonomous microbial therapeutics. For example, biomarkers produced as a result of intestinal inflammation (red diamonds) are detected by recombinant bacteria and allow expression of therapeutic proteins (blue hexagons). (D) Engineered microbes must be both evolutionary and phenotypically robust to prevent the loss or dysfunction of recombinant genetic material. (E) Safety and biocontainment of microbiota-based therapies is a significant challenge for clinical translation of basic research. Strategies may be needed to prevent transmission of therapeutic microbes from a patient to other individuals.