MICROBIOME

Assigning function to symbionts

The development of tools to accelerate identification of causal microorganisms is crucial, and advances in microbial culture, bioinformatics and animal experimentation are currently driving these discoveries.

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It is well recognized that the intestinal microbiome has broad and wide-ranging effects on systemic physiology and pathophysiology, both in mouse models and in human subjects[1]. Unbiased approaches that include high-throughput technologies such as 16S ribosomal RNA gene sequencing, shotgun sequencing of community metagenomes or metatranscriptomes, and whole-community metaproteomics and metabolomics have all been instrumental in characterizing the relationship between the gut microbiome and various host physiologic and disease states. However, defining the precise microbial mechanisms that drive these relationships is challenging and in many cases is still not well understood. Such high-throughput methods can provide important clues and tools towards understanding these mechanisms, but additional methods are needed to functionally assess the role of specific microorganisms in select phenotypes.

Reporting in *Nature*, Surana and Kasper utilize a method to aid identification of causal microorganisms, which they term 'microbe-phenotype triangulation'[2]. They initially tackled the microbial basis for the well-established finding that germ-free or antibiotic-treated mice administered the chemical toxin dextran sodium sulfate (DSS) do not maintain the distal colonic epithelial barrier. This is due to an arrest of epithelial stem and progenitor cell proliferation[3,4], and this acute barrier defect leads to profound weight loss and eventual death. The authors tested two consortia of microorganisms, one isolated from mice and a second from humans, in germ-free mice treated with DSS. These microorganisms had divergent effects on rescue of the DSS phenotype. Co-housing of mice previously colonized with either of these two consortia and subsequent evaluation of their gut microbiome and DSS phenotypes allowed the authors to find candidate protective microorganisms. They found that a Lachnospiraceae family member could rescue the detrimental DSS phenotype in germ-free mice and stimulate gene expression of *Reg3γ*, one of the transcripts regulated in this model[2]. Consequently, this method can be used to identify microorganisms that can be further studied for their role in host-microbial interactions and protection from extreme DSS-induced damage.

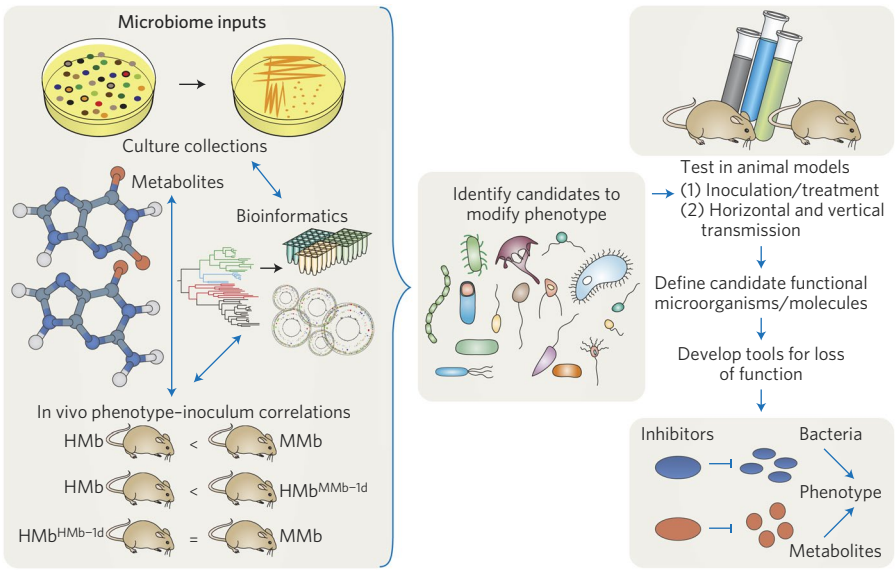

Fig. 1 | Examples of available approaches to identify functional symbionts. Combinations of inputs, such as cultured microorganisms and their metabolic products, together with bioinformatics data and mouse model systems (including in vivo phenotype–inoculum correlations using murine (MMb) and human (HMb) microbiota), can provide key microbiome inputs to identify microbial candidates that alter host phenotypes. Additional in vitro and in vivo evaluation will better define candidates and their molecular mechanisms, with the ultimate goal being loss-of-function studies.

Various methods are in development, in labs around the world, to tackle this issue (Fig. 1). One approach is to improve culture methods (for both aerobic and anaerobic bacteria) or use chloroform-resistant spores to provide material to introduce to antibiotic-treated, conventionally raised or germ-free mice. This can then be used to screen and functionally test for the role of candidate microorganisms in producing a given phenotype. These methods have been used to determine specific microorganisms or consortia that, for example, drive colitis in genetically susceptible hosts[5], stimulate differentiation of T-cell subsets[6] or promote colonization resistance[7]. These studies also rely on a multipronged microbial culture and bioinformatics approach to identify functional microorganisms for a given phenotype.

Other complementary methods have utilized combinations of bioinformatics, chemical biology and screening approaches in targeted cells to uncover key host–microorganism interactions. One functional approach that synthesizes the output of the microbiome is to analyse its metabolic products. Metabolite pools from the intestinal contents of mice and other hosts can now be determined by untargeted mass-spectroscopy methods. Several groups have successfully used targeted pools of metabolites to uncover specific and functional microbial products that control key physiological and pathophysiological situations, for example by promoting suppression of inflammation or protection against viral infection[8–11].

While identification of new bioactive microorganisms and microbial products is exciting, there are still some major challenges ahead for this burgeoning field. The literature suggests that many interactions are likely to be polymicrobial[7]. It is unclear if polymicrobial mechanisms of symbionts will be the norm or the exception here. To study these mechanisms, it will be critical to have a suite of robust culture methods, as well as the ability to screen for combinations of microorganisms, both in vitro and with complementary in vivo studies. Scenarios involving these polymicrobial interactions will prove challenging in demonstrations of Koch's postulates.

A key approach in the future will be loss-of-function experiments (selectively deleting microorganisms or inhibiting the production of microbial products). These experiments will be important in establishing that selected microorganisms are sufficient to cause a particular phenotype. This will be challenging and will require a deeper understanding of the microorganism, its functional product(s), and the specific effect that elicits a host phenotype. Uncovering these detailed molecular mechanisms is critical, so development of new tools to determine specific causal microorganisms is a step in the right direction. However, future work will need further tools to perform loss-of-function studies. Such studies will, hopefully, pave the way for microbial-based therapeutics. ❐

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Published online: 18 December 2017
https://doi.org/10.1038/s41564-017-0088-0

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Competing interests

The author declares no competing financial interests.