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Advancing Our Functional Understanding of Host–Microbiota Interactions: A Need for New Types of Studies

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1. Introduction

Multicellular life evolved in the presence of microorganisms and formed complex associations with their microbiota, the sum of all associated archaea, bacteria, fungi, and viruses. These associations greatly affect the health and life history of the host, which led to a new understanding of "self" and establishment of the "metaorganism" concept.[1] The Collaborative Research Centre (CRC) 1182 aims at elucidating the evolution and function of metaorganisms. Its annual conference, the Young Investigator Research Day (YIRD), serves as a platform for scientists of various disciplines to share novel findings on host–microbiota interactions, thereby providing a comprehensive overview of recent developments and new directions in metaorganism research. Even though we have gained tremendous insights into the composition and dynamics of host-associated microbial communities and their correlations with host health and disease, it also became evident that moving from correlative toward functional studies is needed to examine the underlying mechanisms of interactions within the metaorganism. Non-classical model organisms in particular possess significant potential to functionally address many open questions in metaorganism research. Here, we suggest and introduce a roadmap moving from correlation toward a functional understanding of host–microbiota interactions and highlight its potential in emerging ecological, agricultural, and translational medical applications.

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2. Approaches toward a Functional Understanding of Metaorganisms

Upon identification of a potential host–microbiota interaction, functional profiling must address who is doing what, how, when, and where. "Who" refers to the precise identification of both host and microbial partners. "What" denotes the phenotypic impact of microorganisms on their associated host and vice versa. "How" refers to the process and the involved molecules. "When" and "where" denote the spatial and temporal dynamics. Metaorganism function can be analyzed in three scales: potential, active, and realized function.[2] Potential function is assessed by analyzing genomes. Active function studies exploit transcriptomes, proteomes, and metabolomes. Realized function is measured by examining the phenotype. To foster an integrative view of metaorganism function, future efforts should address all three scales (**Figure 1**). Gnotobiotic model systems, in which all associated entities of a metaorganism are known and accounted for, lay the foundation of functional studies in host–microbiota interactions, together with the isolation and cultivation of microorganisms.

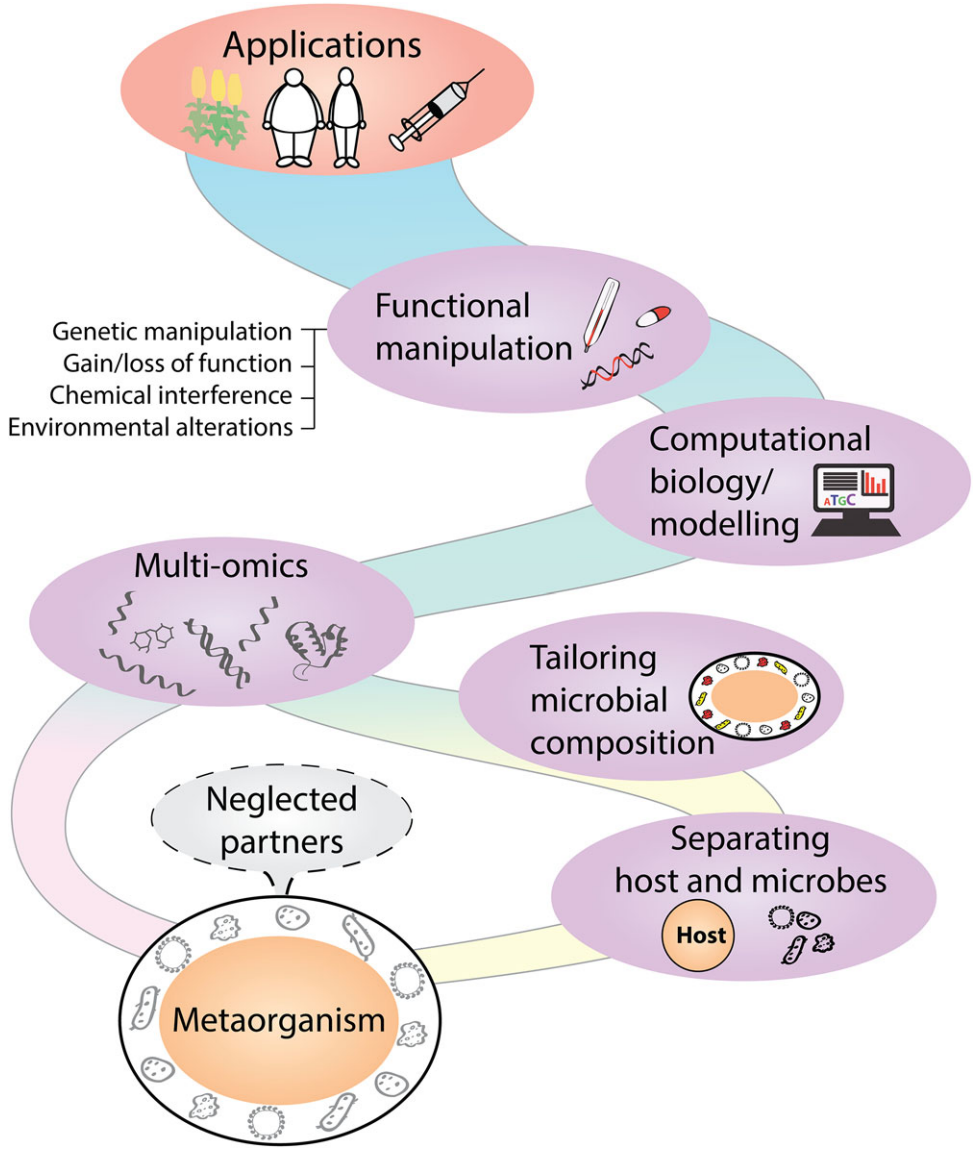

Figure 1. Roadmap toward a functional understanding of metaorganisms. At the organismal scale, the individual host and microbial partners may be separated to study them individually and to create custom microbiota assemblies to understand the contribution of the particular entities. At the molecular scale, several "omics" methodologies including genomics, proteomics, or metabolomics may be employed. These approaches are especially crucial for model systems, where interacting partners cannot be separated yet. Through bioinformatic analysis and mathematical modeling, potential candidates can be identified and new hypotheses can be developed. Ultimately, metaorganisms may be studied functionally using genetic, environmental, or pharmaco-chemical manipulations. Extending our understanding of the metaorganism will aid in improving agricultural and medical practices. Since most studies this far focused only on bacteria, there is a vast knowledge gap in the understanding of neglected partners within the microbiota such as algae, archaea, fungi, protists, and viruses.

Germ-free hosts, that is, plants or animals without any associated microorganisms, represent the most reduced form of a gnotobiotic system enabling to assess the functional capacity of the host in the absence of a microbiota. Subsequent exposure of germ-free individuals to a single microbial strain or a chosen set of microorganisms provides a platform where the impact of each microbial component on the host can be studied separately. These chosen sets of microorganisms can be developed as standardized minimal reference microbiota,[3] that not only reduces the complexity compared to a full regular microbiota and thereby promote functional analyses, but also facilitate comparisons across experiments and laboratories or animal facilities. Therefore, efforts should be undertaken to develop newly emerging animal and plant model systems for metaorganism research as gnotobiotic along with suitable standardized minimal microbiota, akin to the "Oligo-Mouse-Microbiota".[3] However, one has to bear in mind that host–microbiota interactions are far more complex than direct links between isolated partners. Thus,

intact metaorganism functionality may be compromised in these minimal communities. Experiments with animals harboring "wild" microbiota demonstrated significantly altered physiological responses compared to standardized lab microbiota,[4] demonstrating the importance of these types of reconstituted models. To further characterize the molecular mechanisms of host–microbiota interactions, gnotobiotic models ideally are supplemented by genetic manipulations of both the host and its microbiota, for example, using genome editing or RNA-interference. With the advent of genome editing technologies like CRISPR-Cas9 we anticipate that in the upcoming years many more model systems will be genetically modifiable. Meanwhile in genetically inaccessible systems, pharmacochemical interference methods may be used to target the candidate genes or pathways instead.

The rapid development of high-throughput and high-resolution analytical techniques also fostered the functional profiling of host–microbiota interactions, especially in the absence of gnotobiotic model systems. Next-generation-sequencing-based amplicon (e.g., 16S, internal transcribed spacer (ITS)), metagenome and epigenome analyses allow the revelation of the identity of individual partners and their functional potentials. In contrast, metatranscriptomics and metaproteomics expose snapshots of cellular expression activities, whereas metabolomics reveals actual metabolites within metaorganisms. By using longitudinal multiomics studies, individual entities (e.g., strains, species, cell types of the host or microbiota) along with genes and molecules or chemical modifications can be identified that coordinate the host–microbiota interactions and phenotypes.[5] Systems biology and mathematical modeling of these multiomic data represent another important cornerstone of metaorganism research. Modeling-assisted prediction of host–microbiota interactions have the potential to guide experimental identification of metabolites and metabolic pathways or to generate new metaorganism concepts.[6] Study design is crucial to identify functional alterations in the metaorganism using multiomics. Longitudinal studies allow the identification of shifts in the microbiota due a treatment (e.g., therapy) or before disease onset, ruling out disease-dependent confounding or secondary effects. However, all microbial species, candidate genes, or molecules identified by "omics" technologies ultimately require validation by in vitro or in vivo assays, which, until recently, have largely been missing in metaorganism studies. As a side note, the spatial pattern of environmental factors, microorganisms and molecules are often neglected, but these are crucial drivers of metaorganism physiology.[7] Performing "omics" on intact specimen averages over the complete sample may mask important and functionally relevant differences. Thus, technologies that facilitate local, site-specific analyses may reveal novel insights into host–microbiota interactions and thus should be employed more frequently. For example, high-resolution imaging coupled to physical or chemical labeling such as matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry can be coupled with macroscale community dynamics on the host to better understand how microorganisms affect specific host functions. Additionally, in particular single-cell "omics" (e.g., scRNA-seq, scATAC-seq) has the potential to improve our understanding of metaorganisms at an unprecedented resolution.

3. Novel Model Metaorganisms Extend Our Functional Understanding of Host–Microbiota Interactions

Most research on host–microbiota interactions is being performed in established model organisms such as the plant *Arabidopsis*, mice, the fruit fly *Drosophila*, or the worm *Caenorhabditis*. However, their complexity or inability to assess certain physiological traits, for example, due to genetic redundancies or long generation times, limit their range of studying host–microbiota interactions. Novel model systems are capable of bridging these gaps, as demonstrated by recent studies comparing the microbiota of a wide range of different metaorganisms. First, *Bdellovibrio* and like organisms (BALO), a group of predatory bacteria, were identified in various metaorganisms as potential drivers of microbiota diversity, thereby alleviating dysbiosis and promoting host fitness.[8] Second, application of a neutral model to the microbiota composition in different host organisms revealed that microbiota community structure is generally consistent with neutral expectations and suggested that transient deviations from neutrality play a role in various diseases.[9] Third, a large scale metagenomics study revealed that the transition from aquatic to a terrestrial habitat marked a major event in the evolution of host-associated microbiota.[10] Finally, using basal metazoans as model systems enabled the recent elucidation of important conceptual and molecular advances for host–microbiota interactions. In the cnidarian *Hydra*, the importance of phages for maintaining a homeostatic equilibrium among different host-associated bacteria was uncovered, which was only possible due to the availability of gnotobiotic culture, the reduced complexity of its associated microbiota, isolation and culture of the members of the microbiota, and mathematical modeling approaches.[11] Taken together, these examples demonstrate the value of using a wide array of novel and well-suited metaorganism model systems to elucidate functional interactions between hosts and their associated microbiota.

4. A Deeper Functional Understanding of Metaorganisms Will Improve Agriculture and Disease Therapies

From plants to humans, one key function of the microbiota within the metaorganism is the protection against pathogens. In plants, the combinatorial action of two processes forms the first line of defense. First, the natural soil microbiota antagonizes pathogen colonization. Second, the plant releases organic compounds from its roots that actively recruit and enrich specific microorganisms. *Pantoea agglomerans* is a plant root-associated bacterium that forms a symbiotic relationship with its host. *Pantoea* promotes growth of its host plant through several mechanisms. These include the secretion of phytohormones, solubilization of otherwise inaccessible nutrients, and suppression of pathogen growth.[12] *Pantoea* colonizes a variety of plant hosts including the common domesticated wheat. Colonization of wheat plants with strains of *Pantoea* reduces susceptibility for infection by pathogenic fungi. Therefore, strains of *Pantoea* are now commercially utilized as biofertilizer in agriculture with the aim to improve plant growth and to reduce pathogenic infection rates.[12]

To prevent mass extinction similar efforts could be applied to other economically important plant species that are at risk due to pathogen epidemics (e.g., banana) facilitated by the vast monocultures in modern agriculture.

In humans, a plethora of descriptive studies associated dysbiosis, which generally describes deviations in the commensal microbial community from the normal healthy conformation, with many diseases including inflammatory bowel disease, cancer, obesity, diabetes, and even mental health issues. Yet, only a small fraction of studies moved beyond correlations by employing microbiota-directed manipulations to test for their effects on metaorganism function. An elegant way to test the functional relevance of dysbiotic microbiota signatures is via fecal microbiota transplantation (FMT). It is the process of transplanting fecal bacteria from one individual into a recipient subject. One option is to transplant the dysbiotic microbiota of a diseased patient into a healthy recipient (animal model) to test whether the disease phenotype can be transferred along with the microbiota and to identify the disease-causing entity. This option should be used more frequently in upcoming studies to improve our functional understanding of the metaorganism. An alternative FMT option is to transplant the microbiota of a healthy subject into a diseased patient with the goal of overcoming dysbiosis and to abrogate disease symptoms. Both FMT options are now being increasingly used for functional microbiota characterization and therapy attempts in humans. The most common therapeutic application is to treat widespread infections with *Clostridium difficile*, which causes chronic diarrhea, potentially leading to death by dehydration and exhaustion. FMT successfully clears *C. difficile* infections with over 90% efficacy, yet also poses major challenges and limitations. So far we do not understand the molecular or ecological interactions between the donor and recipient microbiota to safely predict the FMT outcome. This is exemplified by a recent study,[13] where the transfer of a sterile stool filtrate alone successfully cleared *C. difficile* infection, suggesting that microbial metabolites or bacteriophages may play an important role in FMT outcomes. Furthermore, even with existing safety measures, FMT treatments have also already resulted in adverse effects for some patients. This is especially concerning since some members of the microbiota, which are normally harmless, have the potential to take on pathogenic traits under certain conditions, for example, upon transfer to a new host after FMT. However, which factors drive the pathogenic transformation of those pathobionts remains unclear. Thus, it is imperative to improve our functional understanding of the human gut microbiota and of the mechanisms maintaining healthy host–microbiota interactions to improve FMT safety and efficacy. Identifying those specific microorganisms capable of rescuing the disease phenotype could lead to the development of synthetic minimal microbiota to be used for safe and reproducible FMT treatments.

A large body of metaorganism studies in the last decade has changed our view of the individual host and provided important clues for microbial factors influencing health and disease. However, most of these studies were correlative, generating associations between entities of the microbiota and physiological processes of the metaorganism, and still require functional validation. We therefore call to move beyond correlation and focus on functional profiling of metaorganisms, which is now possible due to methodological advances such as longitudinal multiomics and novel metaorganism model systems. Finally, as most microbiota research focused on bacteria, we suggest moving other entities of the microbiota such as archaea, viruses and small eukaryotes, that potentially are equally important for metaorganism function, more into the spotlight. Improving our functional understanding of the complex host–microbiota interactions within metaorganisms will not only reveal deeper insights into the biology and ecology of life on Earth, but also promote the development of novel therapeutics against microbiota-associated diseases in a variety of organisms—from plants to humans.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

C.J. and F.S. are shared seniors authors. J.L., G.M., J.B., D.H., and R.S. are shared second authors.

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[1] T. Rees, T. Bosch, A. E. Douglas, *PLoS Biol.* **2018**, *16*, e2005358.
[2] A. T. Reese, S. M. Kearney, *Curr. Opin. Microbiol.* **2019**, *50*, 20.
[3] S. Brugiroux, M. Beutler, C. Pfann, D. Garzetti, H. J. Ruscheweyh, D. Ring, M. Diehl, S. Herp, Y. Lotscher, S. Hussain, B. Bunk, R. Pukall, D. H. Huson, P. C. Munch, A. C. McHardy, K. D. McCoy, A. J. Macpherson, A. Loy, T. Clavel, D. Berry, B. Stecher, *Nat. Microbiol.* **2017**, *2*, 16215.
[4] S. P. Rosshart, J. Herz, B. G. Vassallo, A. Hunter, M. K. Wall, J. H. Badger, J. A. McCulloch, D. G. Anastasakis, A. A. Sarshad, I. Leonardi, N. Collins, J. A. Blatter, S. J. Han, S. Tamoutounour, S. Potapova, M. B. Foster St Claire, W. Yuan, S. K. Sen, M. S. Dreier, B. Hild, M. Hafner, D. Wang, I. D. Iliev, Y. Belkaid, G. Trinchieri, B. Rehermann, *Science* **2019**, *365*, eaaw4361.

[5] W. Zhou, M. R. Sailani, K. Contrepois, Y. Zhou, S. Ahadi, S. R. Leopold, M. J. Zhang, V. Rao, M. Avina, T. Mishra, J. Johnson, B. Lee-McMullen, S. Chen, A. A. Metwally, T. D. B. Tran, H. Nguyen, X. Zhou, B. Albright, B. Y. Hong, L. Petersen, E. Bautista, B. Hanson, L. Chen, D. Spakowicz, A. Bahmani, D. Salins, B. Leopold, M. Ashland, O. Dagan-Rosenfeld, S. Rego, et al., *Nature* **2019**, *569*, 663.
[6] R. Pryor, P. Norvaisas, G. Marinos, L. Best, L. B. Thingholm, L. M. Quintaneiro, W. De Haes, D. Esser, S. Waschina, C. Lujan, R. L. Smith, T. A. Scott, D. Martinez-Martinez, O. Woodward, K. Bryson, M. Laudes, W. Lieb, R. H. Houtkooper, A. Franke, L. Temmerman, I. Bjedov, H. M. Cocheme, C. Kaleta, F. Cabreiro, *Cell* **2019**, *178*, 1299.
[7] F. Sommer, F. Bäckhed, *BioEssays* **2016**, *38*, 455.
[8] J. Johnke, S. Fraune, T. C. G. Bosch, U. Hentschel, H. Schulenburg, *Microb. Ecol.* **2019**, 1.
[9] M. Sieber, L. Pita, N. Weiland-Brauer, P. Dirksen, J. Wang, B. Mortzfeld, S. Franzenburg, R. A. Schmitz, J. F. Baines, S. Fraune, U. Hentschel, H. Schulenburg, T. C. G. Bosch, A. Traulsen, *PLoS Biol.* **2019**, *17*, e3000298.
[10] P. Rausch, M. Ruhlemann, B. M. Hermes, S. Doms, T. Dagan, K. Dierking, H. Domin, S. Fraune, J. von Frieling, U. Hentschel, F. A. Heinsen, M. Hoppner, M. T. Jahn, C. Jaspers, K. A. B. Kissoyan, D. Langfeldt, A. Rehman, T. B. H. Reusch, T. Roeder, R. A. Schmitz, H. Schulenburg, R. Soluch, F. Sommer, E. Stukenbrock, N. Weiland-Brauer, P. Rosenstiel, A. Franke, T. Bosch, J. F. Baines, *Microbiome* **2019**, *7*, 133.
[11] X. Y. Li, T. Lachnit, S. Fraune, T. C. G. Bosch, A. Traulsen, M. Sieber, *J. R. Soc., Interface* **2017**, *14*, 20170563.
[12] J. V. Shariati, M. A. Malboobi, Z. Tabrizi, E. Tavakol, P. Owilia, M. Safari, *Sci. Rep.* **2017**, *7*, 15610.
[13] S. J. Ott, G. H. Waetzig, A. Rehman, J. Moltzau-Anderson, R. Bharti, J. A. Grasis, L. Cassidy, A. Tholey, H. Fickenscher, D. Seegert, P. Rosenstiel, S. Schreiber, *Gastroenterology* **2016**, *152*, 799.