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Functions of the Microbiota for the Physiology of Animal Metaorganisms

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Abstract
Animals are usually regarded as independent entities within their respective environments. However, within an organism, eukaryotes and prokaryotes interact dynamically to form the so-called metaorganism or holobiont, where each partner fulfils its versatile and crucial role. This review focuses on the interplay between microorganisms and multicellular eukaryotes in the context of host physiology, in particular aging and mucus-associated crosstalk. In addition to the interactions between bacteria and the host, we highlight the importance of viruses and nonmodel organisms. Moreover, we discuss current culturing and computational methodologies that allow a deeper understanding of underlying mechanisms controlling the physiology of metaorganisms.

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Introduction

Virtually all multicellular organisms are characterized by synergism with microbes and eukaryotic species. Already in 1877, the importance of these relationships was made evident by Karl Möbius by acknowledging that organisms form a unit with surrounding species in the habitat, which he termed "biocenosis" or "living community." Nowadays, the close interactions between a host and its associated microbial community are increasingly recognized as one functional unit defined as metaorganism or holobiont, a biocenosis on the individual level [1]. Investigating this interdependence became increasingly important. Thus, the collaborative research center (CRC) 1182 "Origin and Function of Metaorganisms" was funded in 2016 by the German Research Foundation. In 2018 the CRC 1182 organized the Young Investigator Research Day conference to discuss the current state of this research field. The Young Investigator Research Day motivated us to summarize recent advances in metaorganism research in this review.

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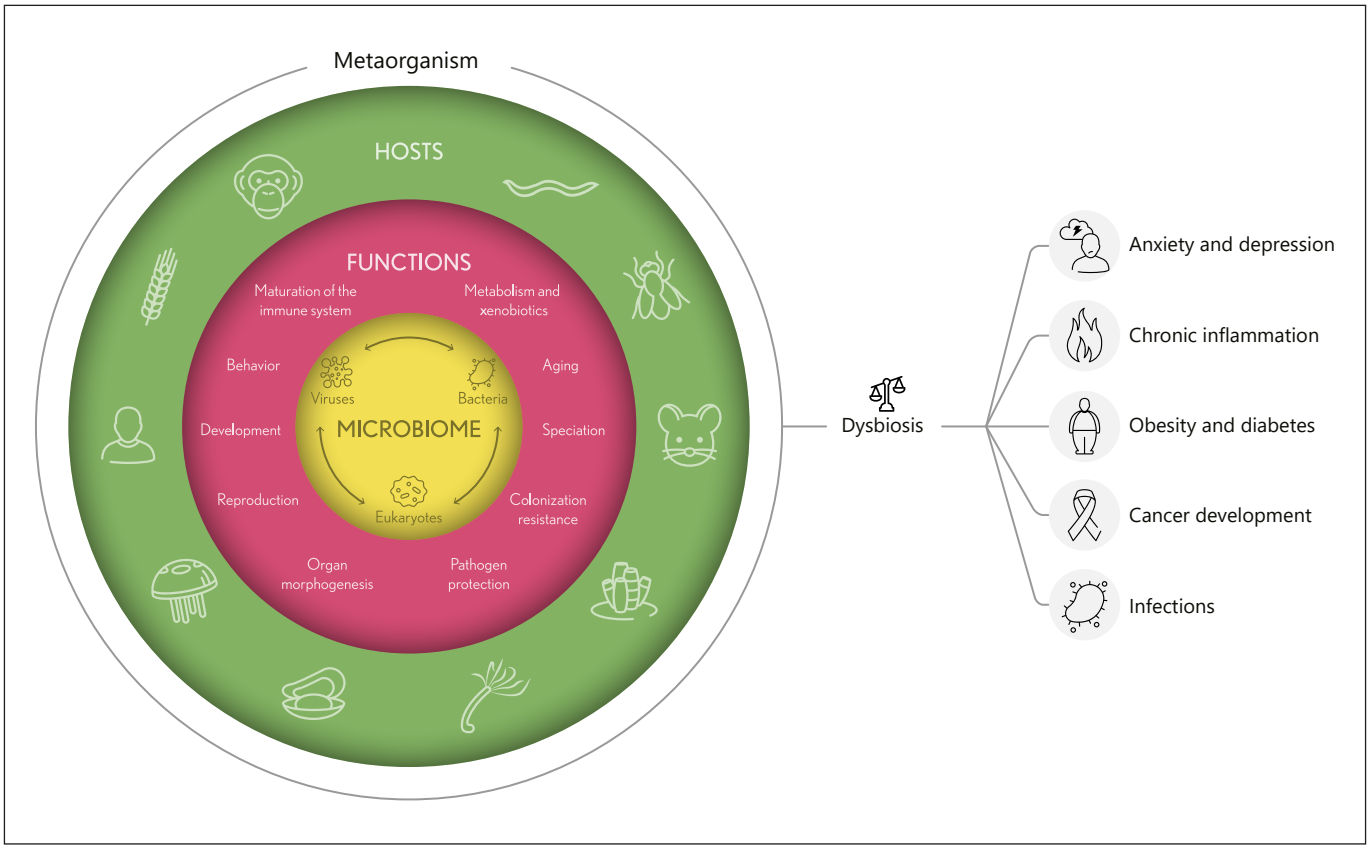

Fig. 1. Functional interactions in metaorganisms. All eukaryotic organisms live in a close and interdependent relationship with their microbiome, including bacteria, viruses, and other small eukaryotes, and are therefore regarded as metaorganisms. Members of the microbiome have various functions within the metaorganism. Microorganisms contribute to host development, organ morphogenesis, metabolism, aging, behavior, colonization resistance, pathogen protection, and maturation of the immune system. Dysbiosis or imbalances in these homeostatic host-microbiome interactions are associated with various diseases including anxiety, depression, diabetes, cancer, obesity, and chronic inflammation.

Why Does Metaorganism Research Matter?

Metaorganisms are outstanding when one considers that even human individuals consist of roughly the same amount of bacteria and host cells [2]. The highest microbial density can be found in the gastrointestinal tract followed by the skin [2]. Especially in the intestine many metabolic interactions between the host and its microbiota are closely intertwined. These interactions potentially have long-term effects, as for example the bacterial colonization during infancy seems to play a pivotal role for the structure of the microbial community and therefore the health status throughout life [3]. Thus, the microbial community influences the host's immune system and contributes to many aspects of host physiology (Fig. 1), including aging and diseases [4].

Taking into account the importance of this crosstalk, researchers have developed multiple approaches to study them. Based on the outcome of wet lab experiments, computational methodologies have become determinative for the advancement of the biomedical field. Systems biology has the potential to predict exchanged metabolites and thus identify key players in host-microbiome interactions. These predictions require metabolic networks and pathways, which are for example annotated in the Virtual Metabolic Human database [5].

Viruses are mostly neglected in metaorganism studies, but possibly just as important as bacteria. Healthy humans are constantly infected with several different viral species at any given time, and while viruses are still mostly considered pathogens, recent studies show that they can also act as mutualistic symbionts. Those symbionts

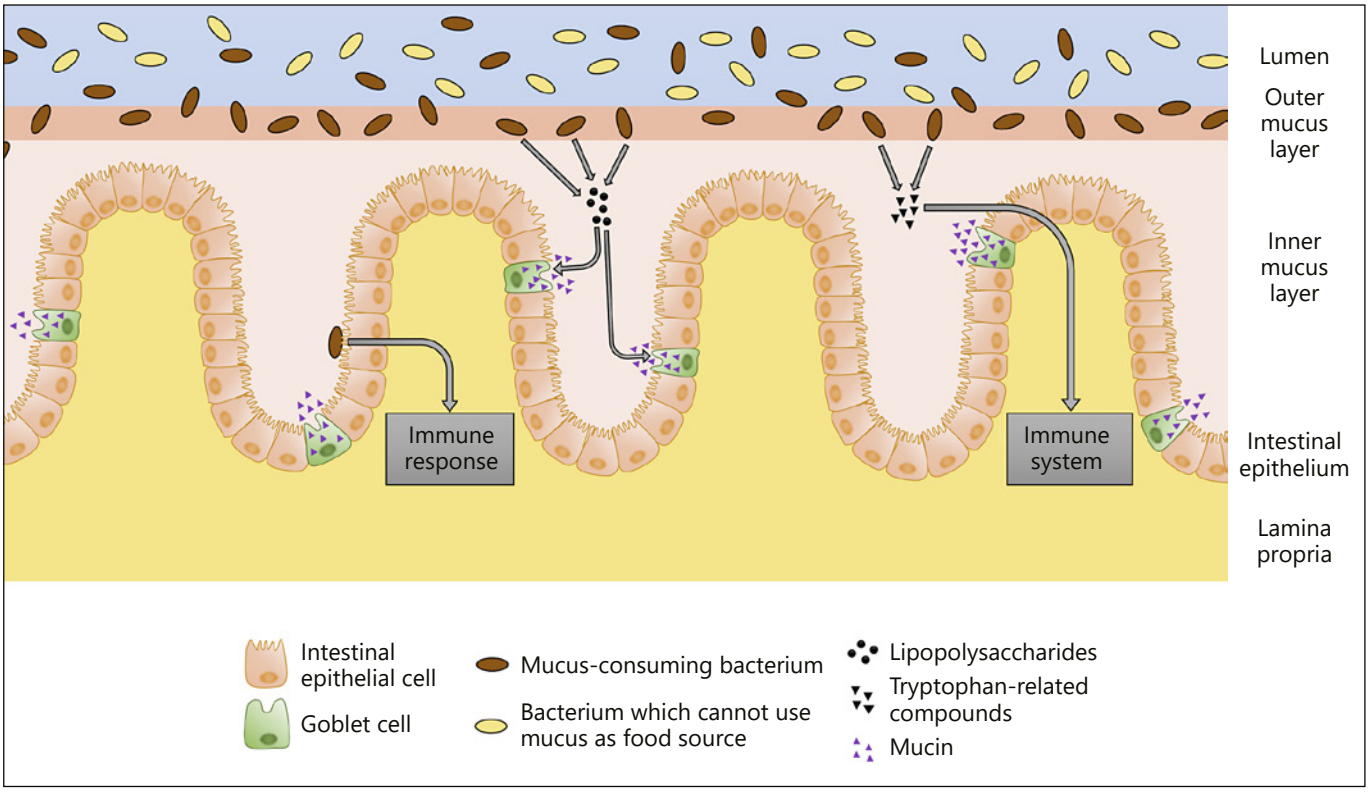

Fig. 2. Interactions between the intestinal mucus and the microbiome. The intestinal mucus layer serves as an interface of the host with the microbiota and also represents a specific niche. The species composition differs between intestinal lumen and mucus. Mucus-consuming bacteria colonize the mucus layer and use mucins as an energy source. Products of these metabolic activities, such as tryptophan or its metabolites, are then provided to the host and widely affect its physiology. Microorganisms can also influence mucus production or host immune responses and thereby shape the intestinal habitat.

can be eukaryotic viruses or bacteriophages, which are viruses that infect and kill eukaryotes or bacteria, respectively. Both types play an important role in metaorganism homeostasis by enhancing the host's immune system or shaping the bacterial community composition [6].

Most host-microbiome research is carried out in model organisms because methods and protocols are established. Yet, to gain a complete picture including environmental factors and other natural influences as well, it is also important to include nonmodel organisms. These include organisms from marine environments such as sponges or corals, which are at risk due to dramatic environmental perturbations associated with climate change such as global warming. Microorganisms play an important role in health and disease in these holobionts. Thus, understanding the underlying mechanisms of coral-microbe interactions can be crucial to prevent the worldwide destruction of coral reefs [7].

Taken together, metaorganism or holobiont research is an uprising and rapidly developing research field. Here, we summarize recent studies of bacteria-host interaction or symbiosis and link them to the new emerging fields like the role of mucus, aging, mutualistic viruses, nonmodel organisms, and computational modeling approaches. The microbiota are crucial for many physiological processes metaorganisms, and for the functional analysis of host-microbiome interactions different systems and in particular nonmodel organisms are required. With this review, we therefore aim to provide a comprehensive overview of the metaorganism concept by covering a range of metaorganisms and by providing examples why these are useful model systems for host-microbiome research.

Mucus: A Special Home of Our Microbes

The gastrointestinal tract is a special environment as microorganisms and host interact closely and are in addition constantly exposed to varying environmental and nutritional perturbations. The mammalian intestinal mucosa is a renowned model for studying such interactions, and numerous human or rodent samples have been explored with numerous techniques to this end [8]. A mucus layer covers the intestinal epithelium. The main components of the mucus are mucins – glycoproteins secreted by intestinal goblet cells [8, 9]. These mucins form a mucus sheath that separates the luminal contents of the tract, including the microorganisms, from the epithelium, while simultaneously acting as a lubricant and protecting the host from damage [8, 9]. The colonic mucus is considered to be comprised of two layers, an inner layer adherent to the epithelium and an outer loose layer [9]. However, this "layer" structure has been questioned recently [8]. Irrespective of the actual structure of the mucus layers, this interface seems to be crucial for both the microorganisms and the host (Fig. 2). On the one hand, bacteria may trigger host immunity if they are in too close proximity to the mucosa. Therefore, the majority of bacteria reside in the lumen or outer mucus layer [8, 9], and structure and metabolic function differ greatly between bacteria residing in the lumen and the outer layer, which is rich in mucus-related sugars [9, 10]. On the other hand, the production of mucus is stimulated by the microbiome, as various microbial molecules (e.g., lipopolysaccharides) trigger mucus production [8, 9]. Additionally, tryptophan-related compounds, which are derived from the utilization of mucus by microorganisms, influence the immunological profile of the host. Disbalances in this microorganism-mucus-host crosstalk are therefore associated with disease, for example inflammatory bowel disease (IBD) [10]. Taken together, the mucus promotes interactions between host and microbiome and thus serves as a connecting interface not only in the mammalian gastrointestinal tract, but also in the lung and other organisms such as basal metazoans. This host-microbiome axis therefore plays a major role in health and disease of metaorganisms [10].

The Role of the Microbiome in Aging and Health of Metaorganisms

The gut microbiota constantly develops throughout a host's lifespan (Fig. 3). In mammals, clear differences in the structure and composition of the microbiome are apparent between infants, adults, and elderly. The microbiomes of newborns are characterized by a high interindividual variation but a low diversity within one organism. Maternal contact and other environmental factors have a strong influence on the microbial composition at that early stage of life. In this period, neonatal priming takes place, which contributes to the microbiota composition for a whole lifetime [3], and several studies reported that this period is tightly time-restricted during development (window of opportunity). For example, only during this time window can exposure to environmental microorganisms correctly instruct the host immune system to prevent the development of allergies [11]. During childhood, the microbiome diversifies and stabilizes in adulthood. In elderly individuals, the overall diversity further increases until the centenarian stage, but it becomes less resilient and is characterized by changed species composition and microbiome function. For example, microbiome functions such as DNA repair or cobalamin and biotin biosynthesis decrease with age [12, 13]. Furthermore, the production of β-glucuronidases, which trigger drug-induced epithelial cell toxicity, is reduced in the microbiome of older individuals. In contrast, the ability to degrade creatine, which is associated with muscle wasting, and the ratio between utilization of monosaccharide compared to di-, oligo-, and polysaccharides are increased in old mice [12].

Changes in the microbiome due to environmental factors such as lifestyle and diet can destroy the gut homeostasis and thus influence the host's immune system and its disease susceptibility [3, 12]. In particular, microbial dysbiosis, an abnormal microbiome state typically associated with disease, in elderly humans correlates with overall poorer health. The susceptibility of a host to frailty correlates with its microbiome diversity, which in turn is shaped by dietary habits [14]. The microbiome composition was significantly correlated with inflammation, the ability of independent living, sarcopenia, as well as geriatric depression. On a metabolic level the microbiota of less frail and more independent individuals produced higher levels of short-chain fatty acids (SCFAs) [14]. In conclusion, our microbiome impacts not only life expectancy but also health status, especially late in life.

Several studies investigated the effect of microbiome manipulation on health or lifespan in a variety of metaorganisms. Sonowal et al. [15] observed that indoles, which are molecules produced by the commensal microbiome, cannot extent the lifespan of worms, flies, and mice, but improve health with a longer reproductive span and increased fertility. The African turquoise killifish is a

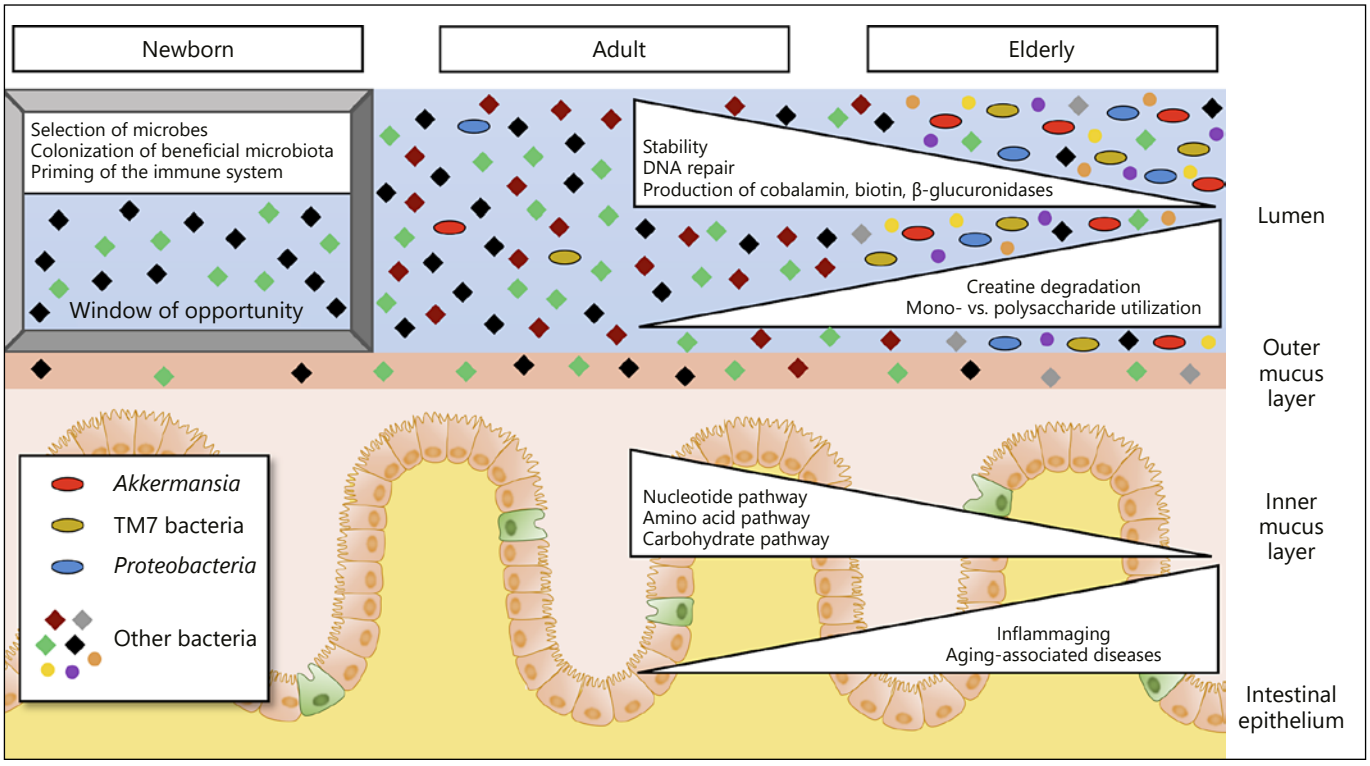

Fig. 3. Molecular changes of intestinal host-microbiome interactions during aging. Upon birth the newborn is colonized by environmental microorganisms. Microbial diversity increases and stabilizes until adulthood. In the elderly, microbiome diversity increases further, presumably due to loss of regulatory processes. Moreover, bacterial composition and microbiome functions change in the elderly. The relative abundance of bacteria that trigger inflammatory responses increases, whereas functional processes involved in DNA repair as well as production of cobalamin, biotin, and β-glucuronidases decrease in the elderly microbiome. In contrast, bacteria which are involved in creatine degradation and polysaccharide utilization increase in the elderly. These changes in the microbiome over the course of an individual's life therefore impact on metabolism and inflammatory processes, which in turn affect disease susceptibility and development.

very short-lived organism with an approximately ten times more diverse microbiome than that of invertebrate model organisms. Aged killifish harbor a microbiome with reduced diversity, which is linked to loss of metabolic function in carbohydrate, amino acid, and nucleotide pathways. This demonstrates that not only the species composition, but also the metabolic capabilities of the microbiome contribute to the host's phenotype. Similar findings were reported for aging in mouse and human. In a microbiome transfer experiment from young fish to middle-aged individuals, a lifespan-extending effect was observed. Importantly, the fish also aged more healthily, which was observed by increased motility, a sign of being healthy [16].

In mammals, age-related changes in the microbial composition can also affect inflammaging (basal inflammation in elderlies) and thus contribute to aging-associated diseases [17]. Transferring the microbiome of old mice to young germ-free mice led to upregulation of the proinflammatory cytokine tumor necrosis factor alpha (TNF-α), dysregulation of pathways involved in the immune response such as T cell differentiation and B cell development, and recognition of microbes by pattern recognition receptors. Additionally, levels of *Akkermansia*, TM7 bacteria, and *Proteobacteria*, all of which support inflammaging, were increased after microbiome transfer [16]. Even cohousing of germ-free with old mice resulted in an increased level of proinflammatory cytokines in the blood [18]. In line with these data, many indicators for inflammaging, such as circulating proinflammatory cytokines, are not detectable in germ-free mice [18]. Interestingly, the immune-related dysbiosis of the microbiome of old mice can be antagonized by the application of anti-TNF [18]. However, although mice colonized with old or

young microbiota were clearly distinguishable after a short time period, they became more similar over time [17].

In summary, the microbiome of a metaorganism constantly evolves and reshapes throughout the course of life. The microbiome largely influences host physiology, lifespan, as well as the extent of healthy aging and diseases, including age-related disorders of the host. In return, the host shapes its microbiome through diet and lifestyle choices as well as immune system functions.

Commensal Bacteria and Their Positive Influence on Health

The human body harbors a diverse and dynamic population of microorganisms, composed of bacteria, archaea, fungi, unicellular eukaryotes, and viruses [4]. In this section we will highlight selected core species that play central roles in the complex network of interactions in the human gut and provide specific benefits for host health. Further, we will discuss up-to-date culture-based approaches to functionally study the physiology and interactions of those microbes with the host and other members of the gut ecosystem.

Bacteria belonging to the genus *Bacteroides* (phylum: Bacteroidetes) are among the most dominant commensals because they are characterized by a high flexibility to nutritional conditions and can thus quickly adapt to changes in the intestinal environment [19]. They play central roles in modulating host health, for example by providing important metabolites such as SCFAs, which improve glucose and lipid metabolism and tone down inflammatory reactions [20]. Dysbiosis including changes in abundance of *Bacteroides* is linked to an altered metabolite profile and illness. For example, lower levels of *Bacteroides* are associated with IBD [21]. The most prominent and best-characterized *Bacteroides* species are *B. fragilis* and *B. thetaiotaomicron*. *B. fragilis* produces polysaccharide A, a symbiosis factor which modulates the innate immune system by inducing regulatory T cell growth and cytokine expression, ultimately protecting against colitis and inducing host-microbe symbiosis [22]. A recent study reported that the abundance of *B. thetaiotaomicron* is inversely correlated with obesity in Chinese subjects [23]. Importantly, in a mouse obesity model, supplementation of *B. thetaiotaomicron* improved metabolic parameters, thus demonstrating that *B. thetaiotaomicron* is functionally involved in tuning host metabolism in a beneficial manner.

The genus *Faecalibacterium* of the phylum Firmicutes has only one known representative: *Faecalibacterium prausnitzii* [24]. In addition to *Bacteroides*, it is a highly abundant gut commensal with a variety of beneficial functions for host health. *F. prausnitzii* is one of the main SCFA producers, mainly butyrate, by fermentation of dietary fiber. Butyrate has anti-inflammatory functions and contributes to a healthy intestine by providing energy to the epithelial cells and functioning as a signal molecule. A community shift characterized by a loss of butyrate producers, in particular loss of *F. prausnitzii*, is linked to IBD [25]. Thus, *F. prausnitzii* is being developed as a probiotic to maintain gut health [24].

Segmented filamentous bacteria (SFB) or *Candidatus* Savagella are prominent members of the gut microbiome of many vertebrates and most extensively studied in the mouse [26]. SFB have potent immune-stimulatory effects. They induce the maturation of B and T cell compartments and drive Th17 cell inflammatory responses, which are protective against pathogens [26]. SFB being potent immune modulators, their abundance has to be tightly regulated. Increased SFB levels are associated with enhanced disease severity in a number of autoimmune disorders. SFB are also found in humans, but only in an age-dependent manner [27] and in low abundance. In humans, SFB enhance immune responses, including sIgA production and Th17 induction, and activate T and B cell signaling. This indicates that SFB play an important role in modulating the immune system in early life [27].

Cultivation Methods Facilitate Functional Microbiome Analyses

Microbial cultivation bloomed in the 1960s and 1970s. Due to advances in sequencing technologies, molecular high-throughput methods are nowadays preferred to analyze complex microbial ecosystems [28]. These culture-independent sequencing approaches have many advantages, such as the identification of microbes that cannot be cultured and the generation of large datasets from entire ecosystems, thus revealing a broader picture of the whole network [28]. Thereby, currently underestimated but potentially important microorganisms may be identified. For example, archaea were for a long time mostly overlooked due to methodological restrictions, yet in the last years, using sequencing approaches it became evident that archaea are a crucial part of almost all microbiomes [29]. However, there are critical shortfalls that can only

be overcome using culturing approaches. Functional predictions based on sequencing data largely depend on the availability and quality of reference databases, which are based on pure isolates. Thus, downstream functional studies absolutely require culture of the candidate organism. Due to recent methodological advances, a large portion of the mammalian microbiome, including "difficult" microorganisms, can now be cultured, thus enabling functional tests. This even led to the introduction of *culturomics* to cultivate a huge number of microbes from the human gut. Briefly, various culture conditions are used in combination with mass spectrometry (MALDI-TOF MS) to identify enriched isolates [30]. Browne et al. [31] described a novel workflow based on targeted phenotypic culturing with additional whole-genome sequencing to culture novel intestinal bacteria, which were formerly considered to be uncultivable. Even demanding key members of the microbiome can now be cultured in vitro. Until recently, SFB could only be propagated using gnotobiotic mice, but in 2015 a coculture system was established which uses mouse and human cell lines to successfully culture and study SFB in vitro [32].

Taken together, an increasing percentage of the microbiome can now be cultured and is thus available for functional analyses, which will greatly advance our understanding of the host-microbiome interplay in health and disease.

Impact of Viruses on Host Physiology: More than Just Pathogens

Viruses have mostly been considered as pathogens or disregarded in metaorganism studies due to their size and their low percentage of the biomass [33]. However, metaorganisms are constantly reinfected with low-virulent viruses that can function as mutualistic symbionts, provide fitness advantages for their hosts [6], and even build a stable and species-specific virome [34]. There is evidence that eukaryotic virus infections can alter and enhance host immunity and make the host more resistant to pathogens and diseases. Chronic infections of mice with gamma-herpesvirus increase their resistance to pathogens such as *Listeria monocytogenes* and *Yersinia pestis* by triggering elevated levels of interferon-c and TNF-α [35]. Herpesvirus infection activates natural killer cells, which enhances tumor resistance [36]. In HIV-infected patients, coinfection with hepatitis G virus does not lead to hepatitis but a reduced HIV viral load, enhanced innate immune response, and reduced mortality [37].

Viral infections can also lead to a changed phenotype. Infection of aphid nymphs with the *Dysaphis plantaginea* densovirus promotes differentiation from a nonwinged to a winged form. This allows the aphid to colonize neighboring plants when food availability in its current habitat is low, but comes with the fitness cost of a reduced reproduction rate [38]. A similar phenomenon where virus infection can be both costly and beneficial can be observed in mammals. On the one hand, murine norovirus infection induces intestinal pathologies in a susceptible host [39], but on the other hand protects from pathogenic infection by boosting immune responses, in particular lymphocyte function [40].

Bacteriophages or phages are viruses that solely infect bacteria. Lytic phages attach to the bacterial cell and eventually kill their host. Since phages are often obligate killers of their bacterial hosts, they can shape bacterial communities. Following the "kill-the-winner" hypothesis, phages prey on the most abundant or most active population of bacteria and keep their abundance on a steady level [41]. Thus, phages ensure the coexistence of several prokaryotic species. Moreover, coevolution between lytic phages and bacteria drives genetic divergence and a diverse bacterial community composition [42], which positively affects health [43]. In conclusion, phages indirectly contribute to eukaryotic health by diversifying and shaping the bacterial community composition.

Lysogenic phages integrate their genomes (prophage) into the bacterial host genome and can alter the bacterial genotype and phenotype. These phages can also indirectly influence eukaryotes by contributing to bacterial population dynamics, where they serve as weapons against susceptible bacteria and benefit their host bacterium [44]. Prophages are also able to directly influence eukaryotes. In the aphid *Acyrthosiphon pisum*, the bacterial symbiont *Hamiltonella defensa* protects its host against its natural enemy *Aphidius ervi*, but only when *H. defensa* is associated with its prophage APSE-3. This indicates that important defense factors are encoded on the prophage chromosome [45]. Phages are also well-known transmitters of virulence genes. Several bacterial toxins are encoded by phages, for example the Shiga toxins of *E. coli*, the cholera toxin of *Vibrio cholerae*, or the toxins of *Pseudomonas aeruginosa* [46]. These toxins are the reason for the pathogenicity of their bacterial hosts and can cause serious diseases in eukaryotes [46]. Whether phages interact directly with eukaryotes is still highly discussed, but an indication stems from the phage adherence to mucus. Bacteriophages bind to mucin glycoproteins of eukaryotes via Ig-like protein domains presented on phage capsids.

Thereby, phages act as a non-host-derived immunity [47]. This is beneficial for the eukaryotic host because phages limit mucosal bacteria. In turn, it is also advantageous for the phage since it ensures frequent interactions with bacterial hosts (see Barr et al. [47] for a graphical summary).

Computational Modeling Approaches

Transcriptomics, genomics, metabolomics, and proteomics, the so-called "omics" technologies, contributed to the elucidation of the functions and capabilities of microorganisms and host systems. However, the key to understanding host-microbe and microbe-microbe interactions requires shifting from descriptive or correlative to functional analyses and data-driven modeling. Current computational methods employing high-resolution predictions complement wet lab experiments and expand our current knowledge, which can be used to generate hypotheses for follow-up experiments [48]. Such systems biology approaches include modeling of ecological characteristics or the metabolism of cell communities [49]. In particular, functional pathways and modules of the microbiome can nowadays be annotated using sequence data from metagenomes or from the genomes of single microbial species [50]. For example, this approach was applied to create metabolic reconstructions and functional characterizations of the microbial communities of seven human body sites within the Human Microbiome Project [51] or of 773 human gut bacteria within the Virtual Metabolic Human database [5]. This knowledge can be used to model the metabolism, growth, and reproduction of microbes or cell communities, but also to predict the consumed and produced metabolites. Based on these predictions, it is possible to draw conclusions about interactions within the microbial community and between microbiome and host. Changes in this interplay can be associated with diseases and lifestyle factors of the host [4]. Vice versa, it is possible to simulate the effect of environmental factors such as nutrition or drugs on the host-microbiome crosstalk and thus on molecular features of the host. This can for example be applied to personalized medicine [48].

Cells or microorganisms are mostly members of communities. Under this prism, cells can be considered as independent entities that interact with their environment (e.g., nutrients) and other entities (e.g., other cells). This way of representation of community organization is called agent-based modeling and has been used together with the metabolic models [48]. For instance, BacArena is such an application that combines all these features to simulate communities in computational space and time. Researchers use BacArena to study biofilms and in the biomedical field [48]. For IBD in humans, this method allowed to not only associate the levels of microbe-derived SCFAs with the disease, but also to provide personalized dietary suggestions to restore SCFA levels [48].

Additionally, hosts can be viewed as ecosystems, and the resident microbial community has the potential to greatly affect the state of this ecosystem. Ecological theory has a long history in describing such complex ecosystems and is increasingly recognized as an important tool in understanding the human microbiome and that of other organisms. An important framework to describe networks of interacting species is based on generalized Lotka-Volterra equations. Coyte et al. [52] used this approach to explicitly address the stability of the microbiota as a function of the prevalence of different interaction types, e.g., competition and cooperation, within the interaction network. They found that an increase in the proportion of cooperative interactions, while usually deemed beneficial, has the tendency to destabilize the ecological community. Cooperation creates positive feedbacks between species and thus increases the likelihood that disturbances to one species propagate throughout the network. Competitive and exploitative interactions dampen such positive feedback loops and thereby increase microbiota stability. Such dampening interactions can either be internal, e.g., resource competition between microbes or exploitation by phages, or they can be externally imposed, e.g., by the host through immune regulation. In contrast to this view of networks of interacting species, ecological neutral theory takes a step back by proposing that communities assemble through purely random dispersal and population dynamics [53]. In particular, it does not invoke selection of or interactions between species and thus provides a null expectation of community structure against which microbiota composition data can be compared. A wide-ranging test of neutral predictions revealed that microbiota compositions from animal hosts across the tree of life are often surprisingly consistent with the neutral null expectation [53]. The hosts in this study included animals of very different complexity and with very different lifestyles, indicating that neutral processes are generally important in microbiota assembly. While this does not preclude a vital role of the microbiota in host physiology, it suggests that the specific species composition of the bulk of the microbial community may play a lesser role than previously thought for functional composition.

In summary, computational modeling is an important methodology to investigate the composition and function of a metaorganism complementing wet lab experiments. Besides interaction-directed approaches such as metabolic or agent-based modeling, these methods can also account for random dispersal and population dynamics. However, these approaches are still limited because they depend on so far limited information about, for example, molecular processes in bacteria. Another drawback is that most simulations and predictions of microbiomes are based on 16S rRNA or metagenome data, which do not reflect the actual process activities and only show a snapshot of species composition. Nevertheless, metabolic and ecological modeling are very promising methods which have already proved valuable in many studies. Further research and resources using this approach are required to improve our understanding of the function of metaorganisms.

Endosymbiosis: The Most Extreme Host-Microbiome Interaction

Host-microbiome interactions within the metaorganism range from parasitism, where the symbiont benefits at the cost of host fitness, to mutualism, where both of the partners mutually profit. Endosymbiosis imposes the strongest interdependence between host and symbiont. Evolution of the eukaryotic cell began when endosymbiotic proteobacteria or cyanobacteria were taken up, but instead of being digested evolved to form mitochondria or plastids, respectively. Extensive gene transfer accompanied organelle endosymbiosis over the course of evolution. Similar yet less extensive phenomena can be observed in other cases of endosymbiosis including *Aphid-Buchnera*, *Hydra-Chlorella*, and *Coral-Symbiodinium* symbiosis [54–56]. In all these cases, nitrogen and carbon are exchanged in the form of amino acids in one or both directions. For example, in case of the *Aphid-Buchnera* symbiosis, the symbiont *Buchnera* underwent severe genome reduction resulting in loss of capability to produce certain amino acids by itself. Therefore, *Buchnera* depends on the *Aphid* host. In turn, it provides other essential amino acids to the host, indicating complementarity and syntrophy [56]. In the case of *Hydra viridissima*, the endosymbiotic *Chlorella* algae lost essential genes for nitrate and ammonium fixation and assimilation, rendering them dependent on the host for nitrogen supply. This nitrogen is provided in the form of glutamine. In exchange for glutamine, the algae provide photosynthetically fixed carbon in the form of maltose [54].

Only few molecular symbiosis regulators have already been identified. Couzigou et al. [57] identified the miRNA mir171b, a member of mir171 family expressed specifically in root cells of *Panax quinquefolius*, that determines success of the mycorrhizal symbiosis. Moreover, primary mir171b and other primary miRNAs encode a regulatory micropeptide that positively regulates the expression of its own miRNA, thus stabilizing the symbiotic signature [58]. Taken together, symbiosis is tightly regulated by mechanisms acting in both the host and symbiont. Yet, the molecular framework of symbiosis largely remains elusive and thus requires further extensive research.

The Importance of Nonmodel Organisms in Metaorganism Research

The investigation of model organisms has led to major advances in our mechanistic understanding of microbiota-host interactions [59]. However, the study of nonmodel organisms revealed important discoveries as well. For example, the study of choanoflagellates, which are flagellated single-celled eukaryotic organisms and the closest living relatives to animals, revealed that bacterial exudates trigger aggregation behavior. Thus, microorganisms potentially contributed to the development of multicellularity [60]. In detail, morphogenesis of the choanoflagellate *Salpingoeca rosetta* is controlled by a sulfonolipid called rosette-inducing factor, which is produced by the bacterium *Algoriphagus machipongonensis*. At environmentally relevant concentrations the presence of the bacterium induces rosette colony development, indicating the importance of bacteria for life history transitions [60]. An additional example from *S. rosetta* suggests that bacteria regulate eukaryotic sexual reproduction. *Vibrio fischeri* secretes chondroitin lyase, which in *S. rosetta* initiates the switch from asexual to sexual reproduction by inducing swarming [61].

As life evolved in the oceans, basal marine metazoans are of primary importance to gain a holistic understanding about the evolution and functional relationships within metaorganisms. Ctenophores and true jellyfish of the phylum Cnidaria secrete large amounts of dissolved organic carbon and thereby enhance microbial activity. This process is regarded as a respiratory carbon sink for the food web, but it may also function to maintain a host-specific microbial community. Indeed, jellyfish harbor a species-specific microbial community that changes with developmental transitions [62]. Also in basal marine

metazoans, bacteria control major developmental transitions, for example settlement and metamorphosis in jellyfish [63] or settlement in corals via secreted tetrabromopyrroles by *Pseudoalteromonas* strains [64]. Corals and their interactions with microorganisms and the environment are of considerable scientific and public interest because of their susceptibility to climate change. Corals harbor algal endosymbionts, which are crucial for the health of the coral as they provide energy from photosynthesis to the coral. After prolonged heat stress, the coral expels its algal symbiont in a process termed bleaching. Bleached corals can survive, yet are energy-deprived and thus highly sensitive. The extent of coral heat tolerance depends at least in part on the microbiome, and both the coral and its microbiome can adapt to the thermal habitat [65]. Importantly, corals adapted to a temperature-variable environment bleach less and maintain a stable microbiome in contrast to corals from a moderately variable environment [65]. The heat stress tolerance can also be transmitted via microbiome transfer. These results therefore suggest that the environment and in particular changes in the coral microbiome contribute to heat tolerance [65]. Even though further experiments are needed to elucidate the molecular basis of these coral-microbiome interactions, this example highlights the importance of nonmodel organisms to translate metaorganism research onto a larger ecosystem perspective [65]. The interplay between host, symbionts, and its associated microbiota are crucial for ecosystem health and resilience to environmental changes. The complexity of metaorganisms highlights the need for further studies using nonmodel organisms to better understand and predict ecosystem responses to global change-induced pressures.

Summary and Conclusion

The concept that every organism cannot exist on its own but relies on several other organisms, such as microbes or other eukaryotes, was revolutionary. Our understanding of the mechanisms underlying this synergy is constantly developing but still not completely understood. So far, we know that the mucus of surface epithelia seems to be one of the most important habitats for host-associated bacteria. Therefore, the microorganism-mucus-host axis has to be explored further, especially if we consider that the host-related bacterial community constantly changes over lifetime and is associated with age-related diseases and lifespan. However, other potential key members of the microbial community, e.g., archaea, fungi, and viruses, may still be underestimated. Therefore, combining culture techniques with molecular tools will be essential to expand our view onto these overlooked microorganisms and to get a deeper functional understanding of the metaorganism. Moreover, computational modeling is an important tool to understand interactions within the metaorganism, especially when experiments are challenging or impossible due to technical limitations. However, these computational predictions should always be combined with and verified by wet lab experiments. In conclusion, we are still far away from fully understanding the complete structure and function of metaorganisms, especially concerning nonmodel metaorganisms, but our understanding of the interactions between host and microorganisms and their role and function within the metaorganisms have progressed remarkably in the last few years. Further efforts in this direction are therefore required but promise to be well worth it.

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Disclosure Statement

The authors declare no conflicts of interest.

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