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Concepts and Consequences of a Core Gut Microbiota for Animal Growth and Development

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Abstract

Animal microbiomes are occasionally considered as an extension of host anatomy, physiology, and even their genomic architecture. Their compositions encompass variable and constant portions when examined across multiple hosts. The latter, termed the core microbiome, is viewed as more accommodated to its host environment and suggested to benefit host fitness. Nevertheless, discrepancies in its definitions, characteristics, and importance to its hosts exist across studies. We survey studies that characterize the core microbiome, detail its current definitions and available methods to identify it, and emphasize the crucial need to upgrade and standardize the methodologies among studies. We highlight ruminants as a case study and discuss

the link between the core microbiome and host physiology and genetics, as well as potential factors that shape it. We conclude with main directives of action to better understand the host–core microbiome axis and acquire the necessary insights into its controlled modulation.

ANIMAL-ASSOCIATED MICROBIOME: AN INTRODUCTION AND CURRENT STATE OF KNOWLEDGE

In many animals, the microbiome serves as an essential part of the host ecology and metabolic repertoire, making it a fundamental element for host survival and persistence across time and space. In particular, microbial gut communities often act as mutualistic symbionts and are important for many host functions, from digestive activity and immunity to behavior (1). The host genome together with the genes encoded by its symbiont microbes are referred to as a holobiont, which regards both interacting partners as a single unit of selection (2, 3). The nature of this symbiotic relationship varies from one host to another and has several degrees of dependency and fidelity. These degrees range from obligatory and consistent host–microbiome relationships to the total absence of a symbiont microbiome (**Figure 1**).

Aphids and additional sap-feeding insects serve as extreme examples of strict host dependency on microbial symbionts. Indeed, specific crucial metabolic pathways of these insect hosts depend on key functions encoded by their microbial gut symbiont genomes from the *Buchnera* genus. Such relationships reflect exceptional coordination, resembling a jigsaw puzzle of complementary gene expression between the host and its symbionts. Aphids' phloem sap diet is poor in essential amino acids; therefore, the insect hosts rely on amino acid synthesis (4). However, neither their genomes nor their microbial obligatory symbionts encode the complete set of genes necessary to synthesize these essential amino acids. Instead, most of the metabolic pathways are coded and expressed by the obligate *Buchnera*'s microbial symbiont genome, whereas the rest are coded and expressed by the aphid host genome (5). This metabolic dependency between the host and

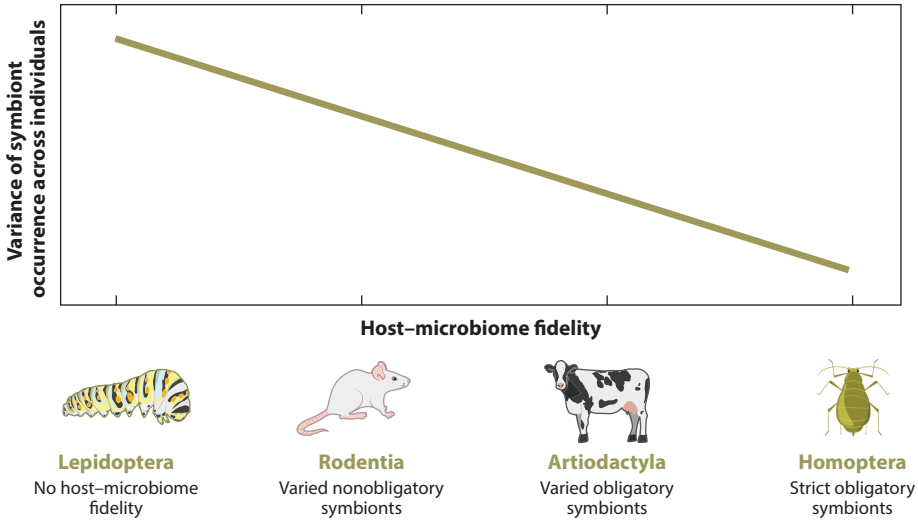

Figure 1

Examples of hosts' fidelity to their gut microbial symbionts across nature and the observed occurrence of specific symbionts.

its *Buchnera* symbionts could stem from genome streamlining, whereby the reduced genome content is linked to reproductive benefits (6). This strong and strict dependence is maintained by specialized, evolutionarily sculptured symbiotic organs called bacteriocytes, which house the *Buchnera* symbionts and ensure their persistence in the host as well as vertical transfer to offspring.

Another extreme obligatory relationship between the host and its microbiome appears in ruminant mammals. Ruminant animals have evolved to harbor microbial symbionts that carry out the function of the degradation and digestion of their plant fiber diets in a specific organ termed the reticulorumen, situated in their upper digestive tract (7, 8). The rumen serves as a supportive environment for the microbiota, providing constant carbon and nutrient supply, whereas the host gains plant-degrading functions that are essential to its survival (9, 10). By digesting and fermenting the plant cell wall that the host ingests, the microbial symbionts supply more than two-thirds of the host's energetic requirement through production of short-chain fatty acids (11, 12). Moreover, the host digests the microbial cells in its true stomach, termed the abomasum, where most of its protein needs are supplied. Excepting the reticulorumen adaptation, this host–microbiome dependency is supported by many other physiological characteristics the host has evolved, such as (*a*) rumination, which is the rechewing of cud to increase its surface area for the microbial symbionts to adhere, hydrolyze, and ferment the plant fiber, and (*b*) the secretion of lysozyme, an enzyme specialized for bacterial cell wall degradation, in the stomach (7). This obligatory host–microbiome symbiotic relationship is more lenient than the one described between aphids and their symbionts. Despite the host's complete functional dependency on its plant-degrading rumen microbes, variation in microbial composition is common (8, 11, 13, 14). It is tempting to speculate that the magnitude of variation stems from the stringency level of selection on the host and its microbiome, whereby less variation is observed in higher stringency. In the abovementioned examples of aphids, particular genes coded by the specific symbiont are selected, together with the microbe that carries them, to complement the host metabolic pathways. This jigsaw-like system, created across evolutionary time, makes this specific obligate symbiont essential for host survival, and as a result, no variation is observed in the occurrence of this aphid symbiont across hosts. Alternatively, in ruminants, variation in gut microbial composition is observed across hosts, despite the obligatory dependence on their microbiomes. This could stem from a high degree of functional redundancy across rumen microbial genomes, meaning less importance is given to the microbial species carrier, because the selection is applied on the function itself (15, 16).

Selection for particular traits of specific microbial members is also apparent in host microbial systems in which the host–microbiome relationship could be decoupled and therefore is not essential. This is the case of the bobtail squid, *Euprymna scolopes*, which has evolved to have a specialized organ in which its microbial symbiont, of the species *Vibrio fischeri*, is maintained (17). This microbe is not involved in the squid's metabolic function but contributes greatly to its fitness and survival by providing camouflage ability. The nocturnal host uses the luminescence features of its bacterial symbiont, situated within this specialized light organ, to emit light that imitates the moonlight above and conceals the squid from predators' eyes. Both partners in this symbiotic interaction can be cultivated separately, highlighting their nonessential relationship. Nevertheless, given the host's high dependency on its microbes, individual squid hosts will always house this specific microbe, which provides the desired luminescence function. Interestingly, most (up to 95%) of the bacterial cells used for illumination at night are disposed of at dawn, after which the remaining bacteria in its light organ reproduce rapidly, ensuring illumination during the following night (18). The bobtail squid–bacteria relationship provides an example of a strong nonessential host–symbiont interaction in which the microbial community is not constant in size or composition but changes cyclically depending on the host's needs. More examples of moderate dependency are found in host–microbiome systems in which host-specific microbiome composition exists and

a connection between its physiology and microbiome composition is evident; nevertheless, such interactions are not obligatory. Such relationships are found in mice and pigs and even range into the plant kingdom, where the host can be sustained without its microbes in a germ-free state, despite the fact that microbiome composition affects many of its attributes (19–21).

More extreme examples of host–microbiome system independence exist in the Lepidoptera caterpillars, in which host-specific symbionts are largely absent (22, 23). Although microbes reside within the caterpillars' digestive tract, they show characteristics that point to nonspecific host association. For example, their gut microbes are mainly ingested with food, appear in low density, and naturally exhibit high variation across different animals. This could stem from the environmental conditions in the caterpillar's digestive tract, such as high pH and fast transit of digested food. These conditions are suggested to actively prevent host-specific microbial colonization of the hosts' gut, keeping the microbes entering the system in a highly transient state. Moreover, the use of antibiotic repression on caterpillars' microbiota does not impact host metabolic function or development, highlighting the host's independence from its residing microbes (22, 23). Lepidoptera caterpillars are highly diverse and exhibit high abundance across many ecological niches, potentially suggesting an ecological benefit to a lack of dependence on a gut microbiome.

The different degrees of host–microbiome dependencies beg the question of how the host acquires the relevant microbiome. Mammals acquire their initial microbial symbionts through both vertical transmission from mother to offspring and horizontal transmission, in which symbionts are acquired in each generation from free-living microbial populations in the environment (24). Given the great genetic variation among potential microbial colonizers in the host's environment (25), horizontal transmission may open the opportunity for cosmopolitan microbes that have less fidelity to the host to inhabit its gut environment. The abovementioned bobtail squid also acquires its microbial community within its luminescence organ via horizontal transmission. However, this organ remains uncolonized by nonsymbiont bacteria, even in the absence of its symbiont microbe, *V. fischeri*. Upon initial colonization by the specific microbial symbiont, peptidoglycan and lipopolysaccharide emitted solely by *V. fischeri* strains cause morphological changes in juvenile squids' light organ epithelial walls, allowing for primary successful colonization by the microbial symbionts (18, 26). By reducing organ inhabitability for microbial colonization by non-symbiont bacteria, it may remain available for colonization by *V. fischeri*.

Because maintaining host–microbe symbiotic relationships in future generations and transferring important microbes to the hosts' descendants are crucial to host fitness, one must ask how animals can ensure the transmission of primarily symbiont bacteria to their offspring. Many animals vertically transmit their gut microbial symbionts to their offspring at early stages of life. Vertical transmission ensures the transmission of the symbiotic microbes across generations and therefore allows coevolution through alignment of host–symbiont fitness, which results in phylogenetic congruence and co-speciation (27, 28). One strategy for vertical transmission involves actively feeding fecal microbiota to offspring, as exemplified by koalas and termites (29, 30). Another sophisticated strategy for vertical transmission can be found in the insect world, where the bacteriocytes, which house the microbial symbionts, share an interface with the insect ovarioles, enabling direct transfer of symbionts to the maturing eggs (31, 32). These mechanisms and notions are involved in maintenance and acquisition of the most important microbial members and their functions. However, as mentioned above, in most cases a high level of variability of abundance and presence–absence patterns of microbes exists across hosts.

Interestingly, microbiota variation between hosts arises from differences in the order and timing by which the gut is colonized early in life in a few model animals, including mice, cows, and humans (33–35). These early-colonizing bacteria have a great impact on the microbial habitat through historical contingency effects (36). The rumen provides one clear example for this

process: Close to birth, the rumen is undeveloped and occupies a small portion from the overall digestive tract as the host still suckles milk. At this stage it is an aerobic environment that is inhabited by aerobic and facultative anaerobic bacteria. Once oxygen levels drop, the initial aerobic microbial community is replaced by anaerobic bacteria (37, 38). Hence, the nature of the selected colonizing microbes is determined by the available species pool, as well as the environmental conditions in the gut, which sort specific functions that are encoded in the selected microbial genomes (39). This notion is exemplified in several studies in humans, mice, and ruminants in which the identity of the early colonizers was perturbed by exposure to different species pools at birth as a function of delivery mode (34, 40–43). In these studies, the assembly trajectories and their dynamics were modified as a function of the early colonizers. Nevertheless, in ruminants, despite the different dynamics and compositions exhibited during the ecosystem assembly process, resulting from the different delivery modes, a set of microbes prevalent across all individual animals was shown to colonize the rumen ecosystem at its early stages. This set of consistent microbes was conserved not only in composition but also in the order of arrival into the ecosystem, as well as its persistence across a long time period of three years (34). Although the impacts from the environment used in these studies need to be considered, the findings do suggest that despite individual variability between hosts, a set of early colonizers are shared between hosts in certain host–microbiome ecosystems.

In summary, an interesting picture emerges, in which in most host–microbiome systems where dependency between parties exists, specific microbial groups tend to be prevalent across multiple separate hosts of the same species, especially if they are tightly linked to their host functions. Earlier iterations presented this set of microbial taxa shared among microbial consortia from similar habitats, which further identify the stable, consistent components across complex microbial assemblages as core microbes (44). Within host-associated ecosystems such as animals and plants, the principle of a core microbiota has been proposed to describe the microbial community that is systematically associated with its host (45). In this review, we further discuss how to define such core microbial groups, the mechanisms that ensure their stability, and their impact on their host.

The Core Gut Microbiome Concept

The major incentive for identifying the gut core microbiome is to define the microbiome component that is of major relevance to host metabolism (46). Although the term can appear intuitive, it encompasses different but not mutually exclusive levels of definition, as recently delineated by Risely (47). The core microbiome can be examined at the taxonomic level in which taxon prevalence across hosts, their temporal stabilities and/or influence(s) on the prevailing environment, and their association to the host genetic variations are currently proposed as the key drivers among the different definitions of the core microbiome. Complementing the taxonomy-centric approach, the core microbiome can be examined at the functional level in which shared functions across environments, decoupled from taxonomy, can inform central functions in the ecosystem that are possibly critical for the host. At the functional and/or taxonomic levels, microbial genes or taxa can be classified based on their beneficial or critical contribution to host fitness, or based on their association host genetics. In the following sections, we address the prevailing definitions of the core microbiome.

The common core. The common core, conceptually the most straightforward definition, refers to microbial taxa with the highest occupancies across hosts. Because taxonomic characterization via amplicon sequencing remains the most accessible tool in meta-omics, this definition is the most widely used as a criterion to define the core microbiome in animal and human studies (48, 49). Although the level of occupancy used to determine whether a taxon is part of the common core

varies between studies, high-occupancy microbial taxa would typically suggest that these taxa are well adapted to the studied environment and may carry functions enabling their prevalence. However, this definition of the core microbiome cannot necessarily infer essentiality for host function (50) but may be affected by other factors of the common environment, such as diet (51) and other biotic and abiotic factors.

The temporal and successional core. The temporal core microbiome is a general definition for taxa that are temporally stable across hosts, or with predictable taxa dynamics within an individual [e.g., alpha diversity is stable in rumen microbiomes during the growing period (52)]. The successional core is an additional subdefinition, which determines the abundance of specific taxa within a defined temporal stage, such as season or age [e.g., up to 91 population abundances are stage specific during birth, weaning, and growing periods of the pig microbiome (34, 53–55)]. Of note, the temporal and common cores can overlap when measured at the host population level, because widespread taxa tend to be abundant and more temporally stable (56) and thus are less likely to be extinct than taxa with low occupancies. Compared to the common core, the temporal core provides more information regarding changes in host requirements, which affect microbial sorting in the gut.

The ecological core. The ecological core is composed of microbial taxa that have a key role in shaping the ecological structure of the microbial environment. These taxa might be referred to as keystone taxa, essential for maintaining the structure of the overall community [e.g., initiation of substrate degradation by cellulolytic microbes or dysbiosis caused by microbial pathogens (57–59)]. However, identification of keystone components in a microbial community containing many complex interactions is difficult and must be accompanied by experimental validation (60).

The functional core. The functional core identifies sets of encoded microbial genes that are important to the biological function of the host itself; thus, core functions can be fulfilled by more than one microbial species able to colonize the niche (61). These include biochemical functions, whereby the ability to synthesize a certain metabolite is added by genes encoded by the microbe that provide behavioral, physiological, or ecological functions. Examples of functional core symbionts include microbes that outcompete potential pathogens and prevent their harmful colonization within the host (62), microbes that synthesize key metabolites (51, 63, 64), and microbes that maintain energetic regulation and homeostasis (65, 66). Given the important role of the symbiotes' functions in the interaction between hosts and their functional core, their prevalence is usually measured at the genomic rather than the taxonomic level (65, 67). Because functional genes can be spread across microbial phylogeny, the functional core likely includes more taxonomic diversity than the common, temporal, and ecological core microbiomes, which represent a broader, facultative function association to the host (16, 47). In the microbiome functional catalogs reported by Li et al. (51) in ruminants and Xiao et al. (68) in pigs, all animals shared <0.1% of the core set of microbial genes, while sharing a much higher percentage of KEGG (Kyoto Encyclopedia of Genes and Genomes) functions [63% and 50% of the KEGG functions (69), respectively], indicating that functional redundancy also occurs for microbial genes with similar functions.

The host-adapted core. The last definition of core microbiome based on taxonomy is the host-adapted core, which contains specific microbial taxa possessing functions that increase host fitness, either consistently or under particular ecological contexts, and whose maintenance within the host population is a product of host-driven natural selection. Taxa with highly conserved association with the host along the host phylogenetic tree are likely to be part of the host-adapted core,

although other factors like diet and host habitat can contribute as well. The vertical transmission methods mentioned above are a good example of the host ensuring the enrichment of specific host-adapted symbionts along its lineage and can be referred to as host active selection on the microbial community. Therefore, unlike the functional core, microbial taxa or functions from the host-adapted core are unlikely to have low occupancy, because imperfect vertical transmission may obstruct natural selection, as described previously in the extreme case of the relationship between aphids and their symbiont microbiome.

The host-adapted core is composed mainly of obligate symbionts, as dependence tends to be reciprocal between the symbiont and the host (70), whereas the functional core is extended from facultative to obligate symbionts. Not all species have host-adapted microbes; for example, animal symbiosis dependence is very strong in cows (50) but lower in certain frugivores and nectivorous birds (71), which also feature simpler gut anatomies and shorter retention times. One example of host-adapted core functions are those that facilitate growth during development by interacting with the somatotropic hormone axis (72) or diverse housekeeping functions (3). Finally, based on recent evidence that host genetics influence gut microbiome composition (73–75), a host-specific core can be defined as the microbial communities whose colonization is favored by specific genetically determined features of the host (e.g., gut chamber size and pH), which leads members from the same host family to share a common functional and taxonomic microbiome, owing not only to maternal vertical transmission but also to their genetic content. This was first exemplified in studies with cows, in which Weimer et al. (73) demonstrated, using rumen content exchange between cows, how the rumen bacterial community is host specific. Roehe et al. (74) emphasized this notion, showing a genetic variation among the archaea:bacteria ratio of steers with different sires, as did Wallace et al. (75), who revealed that a large proportion of the rumen common core microbiome of dairy cattle was heritable (e.g., its variance was determined by the host's genomic content).

HOW CAN WE FURTHER DEVELOP AND QUANTIFY THE CORE GUT MICROBIOME?

Considering the fluidity of the core microbiome definitions, it can be hard to standardize them across studies (76). Furthermore, it can be calculated via various means, under different host conditions and with different connotations (i.e., structure versus function), which complicates efforts to standardize such a definition and use it as a tool in the animal sciences. The molecular and computational tools used to perform these analyses are also evolving rapidly, as are the scale of the available data sets and the boundaries that define where a microbiome's influence begins and ends. All these culminate in a dynamic interplay that must be considered when attempting to connect a core microbiome to animal development and function.

Controlled experiments are required. One of the challenges with defining and quantifying core populations is designing controlled experiments that enable data to be reusable and comparable in other studies, while creating standardized conditions that extend from sample collection and storage all the way down to molecular experimentation. For example, although a plethora of rumen core microbiome studies exist, quite often they each contain their own unique circumstances, such as animal breed (77, 78), animal age (79), sample location (80, 81), and molecular method used to calculate microbiome composition across the study (80). Global surveys defining core microbiota via standardized methodologies, while encompassing multiple breed types and geographical locations (48), have become the gold standard for any future attempts to upgrade our definition of what constitutes a core microbiome.

Meta-omics and computational analysis as powerful tools. Currently, the core microbiota is defined mostly based on DNA sequences with taxonomic information. To date, this analysis has used amplicon sequencing of marker genes, such as segments of the 16S ribosomal RNA (rRNA) gene. However, such a strategy obviously has resolution limitations and cannot be readily extended to the metabolic functions that drive the phenotypes we aim to control. In the context of host-associated ecosystems, core microbiota could be defined via essential functions that are directly connected to holobiont fitness, i.e., the sum of the host and all its inherent microbiota (45). Nevertheless, the most accurate way of quantifying microbiome function is via study and assessment of the representative microbial communities' metagenomic content.

In that context, metagenomic approaches used to reconstruct population genomes and/or metagenome-assembled genomes (MAGs) from animal-associated microbiota have gone from hundreds (78, 82, 83) of recovered genomes to hundreds of thousands in just several years (84, 85). The power of such MAG-centric methodology is that it grants access to an immense amount of genomic data that can be connected specifically to taxonomic information. Concurrent with this MAG revolution, functional RNA- and peptide-based expression studies have also experienced transformation, albeit at a lesser level of application by the animal microbiome community, with the exception of human gut studies (86, 87). Metatranscriptomic methods have been used to analyze gene expression patterns across entire animal microbiomes and in human studies have shown that a functional core is universally transcribed over time and across individuals, often by different microbes (86). Within ruminants, similar taxa (*Prevotellaceae*, *Succinivibrionaceae*, and *Fibrobacteraceae*) and CAZymes are prevalent in multiple studies, indicative of core expression patterns (15, 88, 89). Multi-omic approaches that combine large metagenomic and metatranscriptomic or metaproteomic data sets can further improve resolution to a specific individual population genome and the mechanisms they use to actively degrade the animal's feed (78, 90). Such multi-omics methods have shown that specific populations are affiliated to fiber digestion in pigs (91), that *Sharpea azabuensis* and *Megasphaera* spp. are prevalent in low-methane-producing sheep (92), and that core *Bacteroidetes* saccharolytic machineries (polysaccharide utilization loci) are critical for rumen fiber digestion in moose (78). Although these genome-centric studies are still in their infancy, they illustrate that such methods can be used to create deeper understanding of microbial plant fiber degradation. Furthermore, the broader application of multi-omics could significantly enhance microbiome visualization of larger community dynamics in play, which are constantly varying in response to diet, time (i.e., such as before and after eating), and age, as well as individually varying features of the animal host.

As data sets increase in both size and scope, computational methods are needed to analyze and interpret their biological meaning. Currently, high-throughput meta-omic annotation pipelines exist both as stand-alone packages [e.g., DRAM (93)] and as part of larger community platforms [e.g., KBase (94)]. An additional hurdle is also emerging with regard to how animal microbiome omic data can be computationally integrated with host omic and/or metadata. Such systems-wide analysis should better portray core functions at a holobiont level, such as the interplay between the host animal, its microbiome, and the greater environment, as well as how it affects animal health and productivity (95). Computational approaches for analyzing holo-omic data will be the next step to enable researchers to follow the flow of feed components through multiple core microbial populations and into the host animal.

Microbial knowledge gaps: the eukaryotes and viruses. Although meta-omic resources are transforming the way we study animal microbiomes, they critically do not always address elemental biases that impede obtaining a complete understanding. Namely, a huge discrepancy often exists between our genomically sampled bacterial and archaeal populations in comparison to

eukaryotic and viral populations. Recent 16S rRNA gene studies in healthy mice have identified core taxa within their gut mycobiome and suggested it is influenced by the environment, including diet, and correlates significantly with metabolic outcomes (96). In pigs, environmental factors such as diet appear to be influential in the structure of eukaryotic communities; however, data also suggest that the pig gut microbiome lacks a stable eukaryotic population that may play an important role in host health (97). Similarly, in the human gut microbiome, the role of eukaryotic populations in human health is acknowledged but remains understudied (98–100). Using the ruminant microbiome as an example, microbes are still widely believed to be core in terms of being taxonomically observed across a wide range of geographic and genetic backgrounds (48) and are acknowledged to be key contributors to fiber digestion and enteric gas formation. For example, rumen protozoa make up approximately 20–50% of the microbial biomass within the rumen (101, 102) and are recognized as hydrogen producers via hydrogenosomes (103). In addition, they have a large impact on rumen ecosystem structure and productivity, where their predation strategy modulates ecological niche organization (104) and intraruminal nitrogen recycling. The first obligately anaerobic fungi in nature were confirmed to exist in ruminant microbiomes in the 1970s (105, 106) and have since been shown to harness CAZymes that deconstruct the plant cell walls and structural integrity of ingested grasses (107) and to produce hydrogen (108), both of which support microbial symbioses benefitting the host. Culture-independent omic studies have additionally highlighted that anaerobic fungi and ciliates contribute an unexpectedly large share of transcripts and/or proteins for cellulose- and hemicellulose-degrading enzymes (15, 88, 109, 110).

Genomic information remains scarce for both protozoa and fungi, irrespective of the animal host, which is due largely to the difficulty of growing them axenically and the ability to purify, sequence, and annotate the genomes of both cultured and uncultured representatives. To date, there exists only one draft macronuclear genome sequence of a ruminal protozoa (*Entodinium caudatum* MZG-1) (111), whereas representative fungal genomes from cultures of the genera *Anaeromyces*, *Neocallimastix*, *Orpinomyces*, and *Piromyces* are available (112). Developments in metagenomics and the bioinformatic processing of these data have recently created the first iterations of representative genomes from uncultured protozoal and fungal populations in both humans (113) and ruminants (114). Single-cell genomic methodologies have also been applied recently to sequence uncultured fungal taxa (115). It is additionally hoped that long-read sequencing technologies (Oxford Nanopore, PacBio) will rapidly improve the assembly of eukaryotic genomes (116), perhaps in combination with genome binning software (EukRep), which was designed recently to enable reconstruction of eukaryotic genomes from complex microbial communities (117).

Viruses are another component of the gut microbiome, although their research has been conditioned by only recent adaptation of omics technologies into this field (118). The virome is the compendium of pathogen populations of the microbiome that infect prokaryotic and eukaryotic organisms, the former (bacteriophages and archaeaphages) being the most widely investigated. In terms of viral taxonomic families, Podoviridae, Myoviridae, and Siphoviridae have been observed widely across different livestock species, e.g., rumen of cattle, goat, sheep, and reindeer, and also in poultry and pigs (118, 119), and these families are likely to predate core bacterial species (118). Investigation of the functional rumen virome has revealed that 50–70% of total reads were related to viral replication (KEGG pathway of nucleotide metabolism, replication, and repair) (118). The remaining genes were diverse and included viral structure genes or were auxiliary metabolic genes with the ability to encourage their replication by controlling host (bacteria, archaea) metabolism, altering the metabolic potential of the rumen microbiome (120). By lysing bacterial and archaeal cells, phages modulate the microbial populations and make microbial proteins and nucleotides available for other microbes (intraruminal recycling), but also microbial enzymes for carbohydrate breakdown (78). Moreover, bacteriophages are also responsible for phage virus-mediated

horizontal genetic exchange, helping to maintain bacterial population diversity and adaptation (e.g., genes for substrate utilization ability) but also with potentially harmful consequences when promoting the transfer of genes for antibiotic resistance or toxin production (118, 121).

Pure and cocultures are still needed. In a short time, advancements in culture-independent techniques have rapidly heightened our understanding and appreciation of the genetic and metabolic diversity that exists within the rumen microbiome. For example, a decade ago, our knowledge of the different strategies that gut microbiota employ to degrade fiber was limited to free enzymes and cellulosomes (122). Today, we recognize the fibrolytic contributions of polysaccharide utilization loci in both Gram-negative and Gram-positive populations (123, 124), outer-membrane vesicles (125), and type 9 secretion system–secreted multi-modular enzymes (90). However, all of these functional inferences were enabled by comparing omic data against databases that house detailed biochemical descriptions of each respective mechanism, arising from a culture-based study. Thus, to obtain accurate and reliable functional annotations, we must continue to build and update biochemical evidence and reference databases. Since the first human microbiota isolation project coordinated via Virginia Polytechnic University in the 1970s (126), efforts have been renewed in recent years, most notably the Human Gastrointestinal Bacteria Culture Collection and the Rumen Microbial Genomics network. The former currently consists of 737 whole-genome-sequenced bacterial isolates (127), whereas the latter established the launchpad for the Hungate1000 Project to isolate, sequence, and annotate 410 rumen isolate genomes (128). In addition, culturomic approaches have sought to identify factors to boost microbial richness that exists in a cultivable state in samples originating from both humans (129) and ruminants (130). It must also be noted that in partnership with isolation efforts, enzymological and biochemical evidence is needed to elucidate the actual metabolic or physiological functions attributable to microbial gene products.

In addition to pure culture studies, coculturing is an essential tool to study microbe–microbe interactions, which form the basis of many key core metabolic functions. Importantly, coculture studies create a bridge into potential in vivo relationships without the noise that comes with complex omic studies, and in doing so, can create a deeper mechanistic understanding. This is particularly relevant for critical symbiotic partnerships, such as those that exist between fibrolytic populations that produce hydrogen and hydrogenotrophic microbiota such as succinate producers (131) and methanogens (132). Again, these interrelationships, such as the role of proton-reducing acetogens in the three-step process of anaerobic digestion (133), were revealed decades ago by culture-dependent studies but largely escape detection (and validation) by the culture-independent approaches favored today in "microbiome" research. More recently, approaches have alternated between omic and coculture studies to first identify core microorganisms that mediate hydrogen cycling, while using model organisms to understand how and why ruminant bacteria regulate hydrogen metabolism, before finally comparing their acquired knowledge in real-world scenarios of animals producing varying levels of methane (134) (**Figure 2**). In that context, this conceptual approach was first used in the study of the foregut microbiota of the Tammar wallaby (*Macropus eugenii*) to support the omics-based identification and subsequent isolation of a novel member of the *Succinivibrionaceae* (135, 136), which developed a rational basis for the role of these microbes in low-methane-emitting phenotypes, which are now confirmed to exist in cattle (74).

LINKING THE CORE MICROBIOTA TO ANIMAL PHYSIOLOGY, GENETICS, GROWTH, AND DEVELOPMENT

Linking the impact of the core microbiome on the host can be done according to the different definitions of the core microbiome, from the more classical definition of the common core,

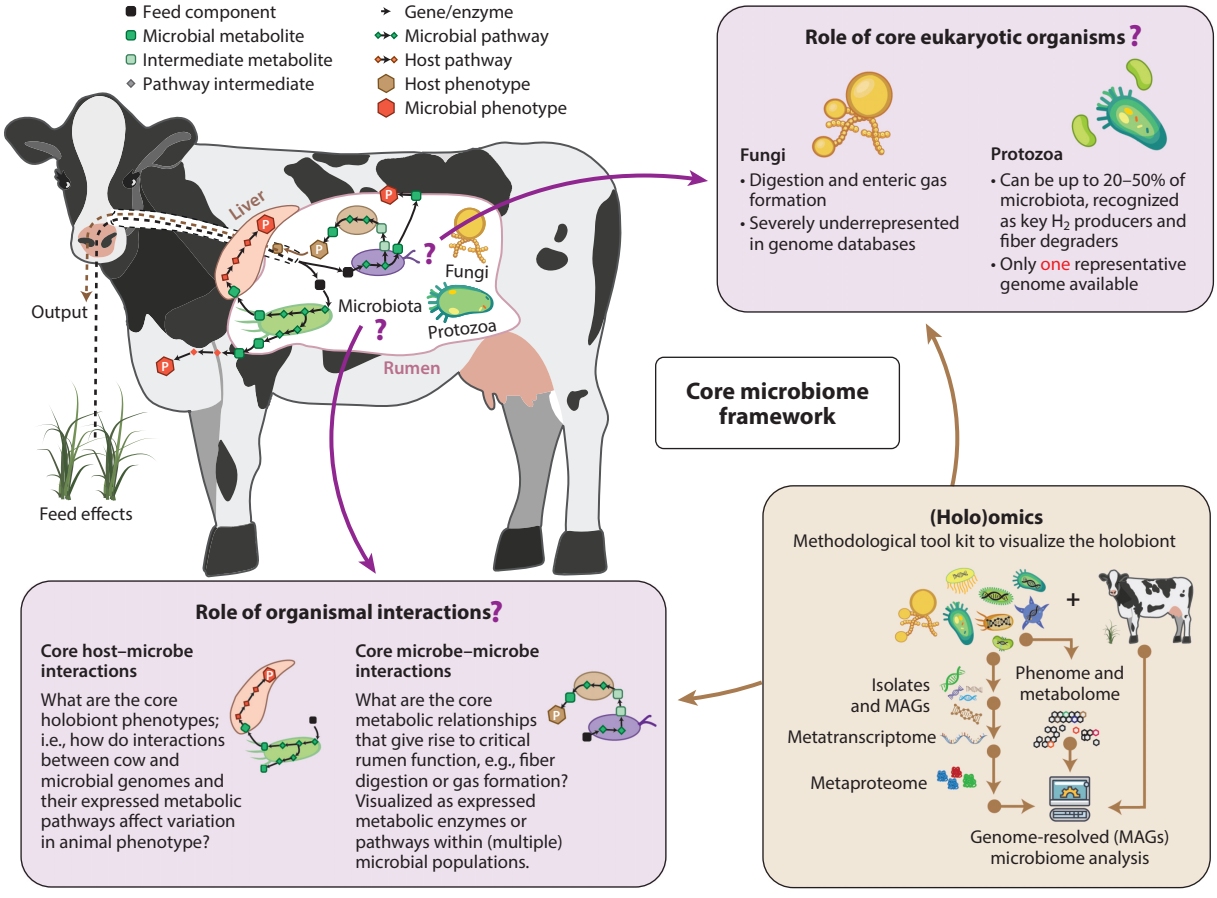

Figure 2

Current approaches used to decipher the regulation of host phenotype by the ruminant microbiome. Abbreviation: MAG, metagenome-assembled genome.

referring simply to recurring taxa, to the functional core concept. As mentioned above, the various definitions are not contradictory but rather complementary, as, for instance, common core taxa would likely carry several core functions, elucidating the ecosystem fundamental requirements.

As discussed above, the rumen microbiome provides a hallmark example of an obligatory relationship with high fidelity of the ruminant host to the functions contributed by its symbiont microbiome, yet also exhibits microbial variability. Thus, this system serves as an excellent model for understanding host–symbiont interactions and for defining the role of core taxa and core functions (see the sidebar titled The Rumen Core as a Case Study). A core taxonomic community in cattle has been identified recurrently in several largely agreeing studies and was recently compiled in a review (8). One such study, which surveyed 1,000 dairy cows from two different species across different European farms, examined the structure and role of the gut microbiome groups in the rumen. This study identified a core microbiome, consisting of 454 species-level prokaryotic operational taxonomic units (OTUs) that were present in at least 50% of animals across all farms (75). Although these microbes represented only 0.25% of the whole microbial species

THE RUMEN CORE AS A CASE STUDY

Given the strict obligatory connection between ruminants and their rumen microbiome, the host-adapted core was first exemplified in cows, and several studies have demonstrated the tight specificity of the rumen microbiome to its host (73–75). In parallel, efforts to define a core microbiome across gut microbial ecosystems of several livestock species based on a high-occupancy criteria highlighted the dominance of *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* phyla (48, 75, 83, 157, 158), although their abundances vary with host phylogeny (158). The global rumen census (48) attempted to determine the core rumen microbiome of rumen samples from 742 individuals from different ruminant species around the world, including cattle, buffalo, goat, sheep, and alpacas. The common core rumen microbiome comprised 67% of all bacterial sequences and consisted of *Prevotella*, *Butyrivibrio*, and *Ruminococcus* genera and unclassified *Lachnospiraceae*, *Ruminococcaceae*, *Bacteroidales*, and *Clostridiales*, most of them (~75%) assembled later on in the cultured Hungate1000 collection (128). Recently, Stewart et al. (83) updated the rumen census by adding 4,941 metagenome-assembled genomes found in cattle rumen and observed similar common core members but also highlighted that members from *Proteobacteria* phyla (e.g., *Succinivibrio* genera) were highly abundant across cows.

Archaea compose the other ubiquitous prokaryote lineage in rumen and are subjected to much lower diversity than bacteria are. They are dominated by methanogen archaea from Euryarchaeota phyla, which carry specific coenzymes (e.g., methyl-coenzyme M reductase complex) for hydrogen utilization, and formate or methyl compounds to a lesser extent derived from fermentative microbes to synthesize methane and obtain energy for their growth (159). Methanogen uptake of hydrogen is symbiotically coordinated with some of the degrader microbes, in particular the cellulolytic bacteria and fungi (7), as methanogens release intraluminal pressure that otherwise will inhibit microbial fermentation rate (160). Hydrogen-consuming methanogenesis is the most widely spread pathway for methane synthesis, and members of hydrogenotrophic *Methanobrevibacter* genera are part of the common microbial core (48). In the global rumen census, only two species, *Methanobrevibacter gottschalkii* and *Methanobrevibacter ruminantium*, accounted for 74% of all archaea (48). Whether viruses, fungi, and protists are part of the common core rumen microbiome remains to be determined.

In addition, as discussed above, studies of the temporal, ecological, and functional core of the rumen microbiome have been initiated in cows only recently (34, 51, 58). Concerning the functional core, only a minimal fraction of the genes (approximately 1%) appear to be shared by 90% of animal hosts (87 animals), though approximately 75% of the KEGG orthologs and even close to 80% of CAZy families could be identified across 90% of the animal cohort (51), attesting to the significance of the functional core within the rumen ecosystem.

pool, they encompass a high proportion of the overall microbiome observed in the sampled cows. Furthermore, these core microbes maintain a highly conserved rank abundance structure across geography, breed, and diet, and the abundance pattern of each species remains almost constant across different animals. This conserved structure portrays the crucial internal connections between these keystone symbionts and the functions they provide to the entire system, directly linked to the survival of both interacting partners. The rumen core community was shown to represent a key element of the microbial community and was more likely than noncore species to be linked to phenotypic features of the host, such as end-product production and efficiency (11, 75, 137, 138). Additionally, despite their small proportion from the total number of species, core microbes may disproportionately affect the overall composition of the community. This is evident from co-occurrence networks in which core microbes were significantly more connected than other members of the community, suggesting that these core microbes likely represent keystone species, i.e., species that have critical importance and effect on the entire ecosystem. To what degree these core species carry core functions when compared to other community members has yet to be determined.

Host Control over the Core Microbiome

A general notion in biology is that living organisms maintain and control their metabolism by carrying and expressing specific genes to cope with their environment and maintain homeostasis. Taking the holobiont and hologenome concepts into account, this raises the question of the connection and control of the host genome to microbiome composition. Indeed, in extreme examples, such as the ones mentioned for the aphid host and its *Buchnera* symbiont, this connection is clear. Evidence of a link between the host genome and microbiome members can also be observed in other systems, although in most cases a mechanistic understanding is still to be studied.

Kokou et al. (139) exemplified this in a study that examined the connection between the gut microbiome composition and host genome selection by looking at genetic lines of poikilothermic organisms such as blue tilapia fish. These lines consisted of both fish families that were selected for the ability to withstand cold temperature and families from the ancestral population that were not exposed to such selection. Differences in the core gut microbiome were found between these two populations, further connecting genetic selection and core microbiome composition. Interestingly, the microbiome of the cold-resistant fish exhibited higher resilience to colder temperatures in comparison to the microbiome of the sensitive ancestral fish population, suggesting that the genetic selection also acted at the functional level of the microbiome. The more dramatic microbiome response of the sensitive-fish microbiome was manifested by clearance of noncore microbes and invasion and dominance of core microbes to the system. This might suggest that the fish core microbes are connected to the ability to withstand colder temperatures.

In the rumen, the subset of the core microbes identified as linked to host phenotype and microbiome structure also exhibited strong heritability values (75), i.e., the degree to which variations in their abundance between cows can be explained by overall genetic variation between the animals across the cohort. Similarly, an additional study demonstrated potential levels of control of the host genome on the microbiome, showing how genome content had an effect on the microbial diversity indices, relative abundance of $\sim 34\%$ of microbial taxa, and the copy number of total bacteria had a high heritability estimate (h^2) ≥ 0.15, suggesting that these microbiome features are heritable elements affected by the ruminant host additive genetics (138). In the mentioned studies, these core heritable microbes represented central hubs within the microbial interaction networks, highlighting their impact on the entire microbial system and stressing the primary role of the core microbiome in host function and microbiome metabolism. These findings of high heritability of core species established in both studies of the rumen microbiome support the possibility of some level of host control on this subset of core microbes and strongly suggest their central role in the rumen ecosystem, and perhaps even in the coevolution of both interacting partners.

A connection between the host genome and core microbiome was also found in mice and rabbits. In a study that analyzed the connection between prevalent microbes that appeared in at least 50% of the mice and were termed "measurable microbiome" but also fit the definition of the common core microbiome, a significant genome-wide linkage with the relative abundance of some microbial members of the measurable microbiome was found (140). In rabbits, a functional core microbiome comparison between an obese and a lean genetic line, both reared under the same controlled environment, revealed differential abundances in specific microbial genes (e.g. encoding lipopolysaccharides and peptidoglycans biosynthesis), suggesting a link between the host gene content for obesity and these core microbial functions (141). In humans, heritability of core microbes remains disputable, but even the most stringent studies acknowledge the presence of a link between the host genome and a selected number of microbes (142, 143). Importantly, heritability measures are more likely to be found for high-prevalence taxa, a feature that will inevitably define them as common core taxa. This might suggest that less prevalent members of the

host-associated microbial community may also be connected and controlled by their host genome. Nonetheless, a study characterizing the microbial communities of twin pairs and their mothers ($n = 154$) revealed a wide array of shared microbial genes among sampled individuals, comprising an identifiable functional core microbiome, without taking into account the taxonomic level of the gene-containing bacteria (65). These genes are important for many metabolic functions, and the fact that they were identified across individuals in taxonomically distinct gut bacteria indicates their great redundancy in the human gut microbiome. This study also demonstrated that deviation from this functional core set was associated with host obesity, showing the effect of the core microbiome composition on human physiology. An additional study examined a larger set of twin pairs ($n = 416$) and identified many microbial taxa whose abundances were influenced by host genetics, where the most heritable taxon, the family *Christensenellaceae*, was shown to be enriched in individuals with low body mass index (144). Yet, the obstacles for detecting heritable taxa within the human microbiome may have a technical component, because a large array of parameters cannot be accounted for in human surveys. This possibility is reinforced by a recent study in mice, which showed that wild mice of different backgrounds maintain the original microbial composition across multiple generations after being transferred to a laboratory setting, suggesting a strong connection between host genome and its microbiome in this ecosystem (24).

However, another possibility may be connected to the lower host-symbiont fidelity within the human microbiome. Higher dependence of the host on its microbes may play a role in establishing an evolutionary link between key components of the microbiome and the host genomic background and result in greater heritability between hosts and their symbionts: Heritability may be better established within more stringent interactions, like those found in ruminants, in which host–symbiont fidelity and dependence are greater than those found in lower-fidelity interactions within the human microbiome.

Core Microbiome Across Time and Environmental Changes

Although most studies typically define a core community across a short temporal scale or even a single time point across large cohorts of hosts, the core community can also not be limited to a single point in time and can be appreciated across a larger temporal scale in the life of the host. Thus, a core composed of microbes that, once appearing, can remain stable throughout the life of the host, or alternatively a temporary core restricted to specific stages of host development, would prevail as host requirements and environmental conditions change (owing in part to the microbiome modifying the niche).

A recent study established this concept in ruminants by showing that temporally dependent core microbes arriving at various stages of development within the first 140 days of life have a key role in microbial assembly dynamics, governing later development of the rumen microbiome and affecting consequential community processes (34). In a period of 800 days, the core successional microbes were three times more persistent than noncore microbes introduced to the microbial community at the same time. This indicates that the day of arrival is not the main contributor to temporal persistence and potentially that forces linked to adaptation and higher fitness of these microbes have a greater contribution to their high level of persistence. Another example for temporal stability of the core microbiome is a study that followed the seasonal changes in the gut microbiome of the North American red squirrel (*Tamiasciurus hudsonicus*) over a three-year period (145). They demonstrated a notable seasonal rhythm in the microbial gut composition, associated with recurring dietary shifts and including fluctuations in the relative abundance of two core gut microbial genera: *Oscillospira* and *Coprococcus*. The first microbial genus was positively correlated with spring diet and showed high relative abundance during this period, whereas the second was

highly correlated to summer diet, and its relative abundance rose at the expense of the former. The opposite process occurred in the beginning of spring, where *Oscillospira* abundance rose at the expense of that of *Coprococcus*. Importantly, both genera were present in all samples in the study, indicating that these are successional core microbes and not foodborne ones that were reintroduced to the system. This study showed that the gut microbial community is adapted to recurring changes over time and can rapidly shift its metabolic function via composition fluctuations made possible owing to the continuous presence of microbial core species *Oscillospira* and *Coprococcus*. Similar seasonal fluctuations were recorded in other model animals, including honeybees (55) and wild mice (146).

As detailed above, further extension to the core definition relates to its existence in specific time periods and is often termed the temporary core. This definition relates to an age-specific microbial core group that is limited to a specific age or general developmental stage of the individual but still exhibits a core-level occupancy, as defined previously. In cattle, sampling of ruminal microbial communities of calves from birth up to adulthood identified three separate clusters across the different animals sampled, each representing a different age period: in the first month of life, in the second and third months of life, and from four months to the third year (34). Hence, throughout the life span of the animal, its microbiome would include both core species that are prevalent across different animals regardless of life stage and are persistent from birth and other core species that depend on the animal's specific life stage. Both types of core clusters can be used to better understand the temporal dynamics of the core microbiome, taking into account its stability and temporary elements and its connection to specific host features in various developmental stages.

Resilience of the Core Microbiome and Potential Mechanisms

Overall, the core community may represent a stable backbone for the microbial ecosystem, and several studies have showcased the resilience of host-associated microbial communities and of its core microbes across time and growth conditions. As mentioned above regarding microbial composition during different life stages of cattle, Furman et al. (34) also demonstrated how changes in the diet and life stages can cause changes in the composition of the core microbes in the rumen. However, the authors also reported that although core microbes may exhibit changes in abundance, they persist in the ecosystem across a large temporal scale (34). Similarly, Petri et al. (147) demonstrated how acidotic episodes (repeatedly occurring periods of ruminal pH decrease) affect the core microbial taxa. They observed that even though core taxa abundance was affected during and directly following an acidotic event, full recovery of the rumen microbiome in all animals tested was achieved within a week, and no alterations to the core taxa were reported. This might suggest that microbiome structure was recovered owing to the flexibility of the core microbial taxa and demonstrated the core microbiome's ability to adapt to a large breadth of conditions. Ren et al.'s (145) abovementioned paper discussing the seasonal rhythm of the North American red squirrel microbiota demonstrates the flexible nature of the microbial system and the ability to shift between steady states. Additionally, Lima et al. (148) showed the resilience of the predominant core bacterial genera of ruminal animals before and after parturition. The prepartum and postpartum diets of dairy cows are naturally extremely different. Therefore, studying the microbial core structure before and after this diet shift can shed additional light on its stability and flexibility of the core and the system as a whole. This study found strong resilience of the predominant core bacterial genera with regard to parturition and dietary shift. This notion is reminiscent of the fish temperature selection study discussed above (139), in which stable, continuous association with the core gut microbiome after temperature perturbation highlighted the resilience of the core gut microbiome. These examples show the robustness of the core microbiome to resist perturbations, potentially serving as the key for supporting the overall microbial community network (13, 149–151).

What mechanisms contribute to the stability of the core microbiome within the host environment, even under changing conditions? The drivers of robustness and stability of the core gut microbiome across time are not entirely clear. Nevertheless, we must understand what might define these core groups, why they are prevalent across animals, and why they have such important impact on the overall community. These questions were evaluated in a study assessing the persistence of microbes in European seabass across time, diets, and gut parts (152). Using different diets, the authors showed that a key characteristic of core microbiome species is their tendency to take part in more nonobligatory positive interactions between them and other microbes compared to noncore microbes. A combination of pairwise coculture experiments of the core microbes and analysis of overlapping metabolic pathways inferred that these core microbes exhibited more positive interactions and co-occurrence patterns (in which both species in the pairwise coculture survive), whereas the noncore gut microbes in their analysis showed mainly co-exclusion patterns (prosperity of the more fit species and eradication of the other) through competition. Furthermore, the core microbes were characterized by a significantly higher strain variability, suggesting interspecies diversification that could explain their resilience across different conditions. Considering the wide range of habitats in which the core taxa were found, they were defined as ecological generalists, a feature that allows them to be found across multiple changing conditions, including different hosts, diets, ages, and even different gut locations. Their generalist nature might confer these microbes with the ability to indicate high niche partitioning, a process in which natural selection drives competing species into different patterns of resource utilization. This ecological process enables generalists, owing to their intrinsic nature, to share their habitat with other microbes that are potential competitors and thus reduce negative interactions they endure. When discussing an already-assembled, diverse community, strong beneficial interspecies interactions contribute to its ability to bounce back quickly from perturbations, contributing to the stability of the core microbes and therefore of the entire system (153). Despite the co-occurrence pattern, core species did not show obligatory interdependence between other microbial individuals and could grow as isolates as well. This is important, as obligatory interactions and interdependence can lead to instability because microbes in a smaller community might be at risk of extinction if their cooperative partners and the functions they encode for are missing. In contrast, when there is low interdependence, the system is less likely to collapse and more likely to reassemble in the absence of one of the species or functions (153, 154). Another feature of the core microbial species that contributes to their apparent stability in the ecosystem is high strain variability compared to other, noncore microbes. This variability potentially allows different strains to be clustered in different parts of the gut based on their specific fitness. This reduces intraspecies competition owing to niche partitioning at the species level, allowing a single species to persist across different environments and conditions. However, this study of fish core gut microbes (152) aimed to investigate core microbes at the species level, and further investigation regarding core strains should be conducted.

What Are the Gaps, What Needs to Be Done, and How Do We Get There?

Manipulating or controlling animal microbiomes has long been viewed as a key strategy toward improving feed digestion, animal productivity, and well-being, as well as reducing detrimental byproducts such as methane. To be able to predict, control, and adapt microbiome function to a desired outcome in terms of animal phenotype, we must first establish stable and constant oversight that reproducibly quantifies all variables at high resolution. Such a level of understanding requires knowledge of not only core microbiome function but also its intimate connection to variables that influence its composition and structure, such as host species and diet. Diet has long been

viewed as the dominant variable shaping the structure and function of gut microbiota inhabiting animal hosts (155), especially ruminants (48). In addition, the dietary fiber of many plant-eating animals consists of an extraordinary diversity of glycan structures, which has been estimated to require thousands of microbial enzyme combinations to facilitate their degradation (156). Therefore, variation in carbohydrate structures could also help explain some of the observed diversity of the core microbiome. Finally, as outlined above, recent studies are building a consensus that host genetics is also important. Structural variation of rumen microbiota within individual animals has been demonstrated for both beef cattle (88) and dairy cattle (157) that were fed the same diet and managed in the same environment. In this broader context, a core microbiota must undoubtably encompass the high-dimensional multispecies molecular phenotype of animals and their residential microbiomes (i.e., the holobiont), as well as any biological link to its greater environment (i.e., feed structural composition).

This exciting research direction points toward a greater conceptualized definition of what the core microbiota represents: not just an assemblage of stable and predictable populations with conserved metabolic functions but an axis that spans across the holobiont from host genetics to microbiome function, to specific glycan profiles in their diets, and beyond to host phenotype. Although results from host-associated genome-wide association studies are already stimulating new lines of research, they have yet to elucidate how the different theaters of diet, microbiome, and host interact at a profound functional level, i.e., how specific dietary components are connected to expressed metabolic enzymes or pathways within (multiple) microbial populations, which are in turn linked to host genotypes (**Figure 2**). Compounding these difficulties is the circumstance that many heritable populations are assigned to taxa for which no cultured isolate, genome, or metabolic information is available. Such culture-derived knowledge gaps create a disconnect between functional prediction and biochemical evidence. Collectively, these knowledge gaps mean we lack a deeper understanding of core holobiont phenotypes, such as how important interactions among cow and microbial genomes, and their expressed enzymes/metabolic pathways, affect variation in digestion and animal phenotype.

The complexity of many animal microbiomes is well founded and recognized at both a technical and financial level as the predominant roadblock restricting the advancement of holobiont theory into knowledge that could enable axis-wide core definitions to lead to scientific and industrial practices. In recent years, molecular tool kits, such as metagenomic sequencing and MAG curation, have developed rapidly to a stage where it is now becoming routine to create thousands of host-associated microbial genomes (85). Although such metagenomic advancements are encouraging, our eagerness to create genomes must also be supported by real-time functional data with equal enthusiasm. This means we must match genome inventories, from both host and microbiome, with gene expression information (RNA and protein) and metadata in the form of metabolites, feed compositional information (such as glycan structures consumed in animal feed), and phenotypic data from the host. In parallel, open and accessible computational methods are needed to store, maneuver, and process the massive amounts of contrasting data types to disentangle extremely complex interactions between feed, the gut microbiome, and host genetics.

Of course, a feed–microbiome–host axis core definition is still very much at a theoretical stage, and what we propose above is an immense task. However, science-driven community efforts are already mobilizing around these topics with an aptitude that empowers confidence in our hypothesis that it will soon be possible to study the holobiont at an axis level, which will redefine what we consider core functions. This new baseline of knowledge is envisaged to identify the core genomes, which core microbiome and host genes are expressed, and what these genes produce in terms of enzymes and interacting core biochemical reactions. The outcome of such high-dimensional comprehension would be its incorporation into commercial feed design and breeding programs used

to optimize fiber digestion and animal production confidently and reproducibly, with the goal of being able to theoretically customize diets for specific animal breeds with specific dietary components, which would match the enzymatic/mechanistic capabilities of their host-linked microbiota.

CONCLUDING REMARKS

In this review, we summarize current knowledge of the host-associated microbiome component, termed the core. The need to define such a group of microbes stems from our inability to measure the importance of each and every member of the microbiome for its contribution to host–microbiome symbiosis owing to the extensive, complex web of microbe–microbe and host–microbe interactions. Instead, we identify reoccurring microbiome members and functions in multiple individual hosts, under the assumption that they are important to the holobiont phenotype. We note that fidelity of the microbiome to its host is somewhat connected to the exhibited variance in core members and functions, where lower fidelity is linked to higher variance in core members. Indeed, we mention some cases in which this connection is self-evident. Only in very few cases have the importance of core microbes and function to the holobiont phenotype, as well as the factors that contribute to their stability or variance, been fully studied or elucidated. Identification of real imperative components (microbes and functions) and their separation from cosmopolitan and nonessential members is a standing challenge. Such knowledge is crucial to our understanding of host microbiome systems and the identification of the most important components within such ecosystems.

To fully understand the functionality of the core and its importance and interaction with its host, more studies investigating this connection should be implemented. Specifically, germ-free host systems complemented with current omics technologies and increased microbial isolate collections are essential for such endeavors. These tools would enable us to leap beyond association studies to causal ones in which the role of individual microbiome members in affecting the ecosystem itself, including other microbiome members as well as the holobiont phenotype, could be elucidated. Achieving such knowledge will help us understand why and how core microbes and functions are consistent across hosts, as well as the potential targets for holobiont phenotype modulation. Moreover, this kind of knowledge will essentially be used as a tool for modulation and intervention in the holobiont phenotype.

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