Understanding the Apothecaries Within: The Necessity of a Systematic Approach for Defining the Chemical Output of the Human Microbiome

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Abstract

The human microbiome harbors a massive diversity of microbes that effectively form an “organ” that strongly influences metabolism and immune function and hence, human health. Although the growing interest in the microbiome has chiefly arisen due to its impact on human physiology, the probable rules of operation are embedded in the roots of microbiology where chemical communication (i.e., with metabolites) is a dominant feature of coexistence. Indeed, recent examples in microbiome research offer the impression that the collective microbiome operates as an “apothecary,” creating chemical concoctions that influence health and alter drug response. Although these principles are not unappreciated, the majority of emphasis is on metagenomics and research efforts often omit systematic efforts to interrogate the chemical component of the complex equation between microbial community and host phenotype. One of the reasons for this omission may be due to the inaccessibility to high-breadth, high-throughput, and scalable technologies. Since these technologies are now available, we propose that a more systematic effort to survey the host–microbiota chemical output be embedded into microbiome research as there is strong likelihood, and growing precedence, that this component may often be integral to developing our understanding of these ultimate apothecaries and how they impact human health. Clin Trans Sci 2014; Volume 7: 74–81

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The plethora of species (500–1,000) comprising our gut microbiota has co-evolved with our physiology such that it represents a formidable influence on health and disease. The list of physiological contributions of the gut microbiota continues to grow but includes the extraction of nutrients from dietary substrate,¹ protection against enteropathogens,^{2,3} immune function,^{4,5} and drug metabolism.⁶ Imbalances or disruptions in the microbiome are associated with diabetes and obesity,⁷ inflammatory bowel disease,^{8,9} neurological disorders including multiple sclerosis,^{10,11} cardiovascular disease (CVD),^{12,13} cancer,¹⁴ bone mass,¹⁵ malnutrition,¹⁶ type 1 diabetes,¹⁷ and infection.¹⁸

The need to reconstruct the key elements in between the microbiome and phenotype is essential.^{16,19–22} There has been significant progress in characterizing the gut microbiota composition in association with disease (chiefly through metagenomic approaches). These studies have confronted us with a staggering level of added complexity to the conventional pursuit of understanding the contribution of genes, environment, and lifestyle to health and disease. And while many of these studies have strengthened the association of host–microbiota physiological linkages, a mechanistic void often resides between the microbial constituents and the associated phenotype (Figure 1A). In essence, the components and sequence of events linking the perpetrators (microbes) to the outcomes (phenotypic changes) are limited or missing.

Although there will likely be a host of experimental approaches and data sources involved in formulating these reconstructions, the chemical constituents (i.e., small molecules/metabolites) are both highly intuitive to deconvoluting this picture and have already demonstrated significant impact.^{12,14,17,23–30} No doubt the contributors between the metagenome and the phenotype will be several and diverse (i.e., proteins, peptides, metabolites, etc.), but the chemical output (i.e., metabolites) will often be a substantial part of this understanding if the paradigms of

microbial communities elsewhere in nature extend to human–microbiome relationships as emerging precedence has suggested (Figure 1B and Table 1).

We believe that the evidence indicates that it is likely that the same chemical currency will be a dominant factor in the inner workings of the human–microbiome ecosystem as illustrated by Figure 2. While it has long been appreciated that metabolic regulation is central to the maintenance of the phenotype through the input of host genetics and diet, the microbiome has contributed an additional layer to maintaining the host phenotype through metabolic regulation. Therefore, in this review, we outline the rationale for a more systematic collection of the chemical output (metabolites) of the human–microbiome system as a central element for bridging metagenome–phenotype associations to advance our understanding of how the microbiome influences human health.

The Rationale for Surveying the Chemical Landscape in Microbiome Research

Chemical communication

Microbial communities rely on small molecules for communication throughout nature⁴⁴ (Figure 1B). In microbiology, the most familiar form of microbial communication via small molecules (metabolites) is quorum sensing (QS).³⁷ QS, via a growing list of molecules such as acyl-homoserine or furanosyl diesters, is a well-established mechanism for cell to cell communication. In QS, cells produce small molecules that traverse the extracellular environment where they are received by neighboring cells, altering metabolism, and gene expression.⁴⁵ Ultimately, these chemical signals potentiate molecular changes in the cells that drive functions like virulence, swarming, biofilm formation, etc.^{35,37,43,45,46} Bacteria also use small molecules to modulate riboswitches and allosterically regulate transcription and enzyme activity.⁴⁷

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Figure 1. Adding mechanistic detail to microbiota–host associations. **(A)** The interest in understanding the complex host–microbiome relationship has generated a massive effort toward the identification of the precise microbiota linked to specific phenotypes or outcomes, particularly with metagenomic approaches. In between the outcome and the identified microbiota are the means (mechanism) by which the microbiota and the host system conspire to affect outcome. **(B)** Borrowing from the lessons in microbial communities elsewhere in nature (plant–microbial, microbial communities resulting in biofilms or mats, and complex multispecies relationships involving yeast, bacteria, insects, and actinomycetes), may offer insight into understanding the human microbiome since all of these communities rely on chemical communication and chemicals initiate an array of activities that support these relationships.

Producer	Compound	Effect/interaction	Citation
<i>Pseudomonas aeruginosa</i>	cis-2 decenoic acid (fatty alcohols)	Destroys biofilms of bacteria and fungi	Ref. ³¹
Actinomycete bacterium	Mycangimycin	Kills a fungus invasive to pine beetle larva	Ref. ³²
Insect gut microbiota + <i>Bacillus thuringiensis</i>	<i>Bacillus thuringiensis</i> toxin + activator compound from gut microbiota	Anti-insecticidal activity	Ref. ³³
<i>Actinomyces naeslundii</i> and <i>Streptococcus oralis</i>	AI-2 (furanosyl borate diester)	Dual species biofilm formation	Ref. ³⁴
<i>Xanthomonas campestris</i>	cis-11-methyl-2-dodecenoic acid	Inhibition of filamentation by <i>C. albicans</i>	Ref. ³⁵
<i>Candida albicans</i>	Farnesol	Decreased swarming of <i>P. aeruginosa</i> by reducing another chemical, pyocyanin	Ref. ³⁶
<i>Bacillus subtilis</i>	Surfactin	Inhibition of <i>S. coelicolors</i> sporulation	Ref. ³⁷
<i>Vibrio cholera</i>	Norspermidine	Biofilm formation	Ref. ³⁸
<i>Rhodopseudomonas palustris</i>	p-coumaryl-homoserine lactone	p-coumaric acid likely comes from plant	Ref. ³⁹
<i>Streptococcus</i> spp	Lactic acid	Supports growth of <i>V. atypical</i> and <i>A. actinomycetemcomitans</i> in biofilm formation during plaque formation on the tooth	Ref. ³⁷
<i>Pseudomonas aeruginosa</i>	4-hydroxy-2-heptylquinoline-N-oxide (HQNO)	<i>S. aureus</i> cells shift growth morphology to become small colony variants (SCVs)	Ref. ^{37,40}
<i>Pseudomonas aeruginosa</i>	3-oxo-C12-homoserine lactone	Potent inflammatory response	Ref. ⁴¹
<i>Pseudomonas aeruginosa</i>	Dynorphin a (13 aa peptide)	Increased virulence by increasing production of the 3 QS signals and QS operon upregulation	Ref. ⁴²
Gut microbiota and human metabolism	AI-3, epinephrine/norepinephrine	Virulence genes activation in <i>E. coli</i> (EHEC)	Ref. ⁴³
<i>Salmonella enteric</i>	Norepinephrine	Pathogenesis of <i>Salmonella</i> -increased motility	Ref. ⁴³
Plants	Flavonoids, QS molecule, nod factor	Bacterial attraction, subsequent QS, production of nodulation factors (lipochitoooligosaccharide) and root module formation	Ref. ³⁷

Table 1. Sampling of reported inter- and intraspecies chemical communication.

Figure 2. Small molecules (metabolites) dictate metabolic homeostasis and the maintenance of the phenotype. The microbiome is expanding the network of influences on metabolic regulation beyond host genetics and diet. The microbiome forms a new “edge” in this network by contributing to the maintenance of the phenotype with its ability to: (1) be directly modified by small molecule inputs from drugs, xenobiotics, and nutrition; (2) modify small molecules from drugs, xenobiotics, and nutrition; (3) directly produce small molecules from their metabolism or modify the structures or levels of metabolites produced by host metabolism and; (4) respond to the changes in excretion of host molecules. Thus, small molecules (metabolites) can be viewed as the “currency” of the complex host–microbiome interaction. Hence, by surveying this currency from a cohort of interest, in addition to metagenomic characterization, the key elements of microbiome-influenced metabolic alterations can be elucidated to reveal mechanistic detail of the host–microbiome interaction.

In addition to intraspecies QS, there is a growing appreciation that these communication systems are operative across species and kingdoms,³⁵ forming even more complex niches, including the human microbiome (Table 1).

Communication is operative to the function of any community as there is a need to constructively coordinate behavior (Figure 1B). There are many ecological subsystems in nature where microbes form the basis of organ-like environments or niches that exchange chemical information to constitute a language that members use to define action including fuel for cell growth, waste products, and defense. These relationships are exemplified in biofilms and microbial mats where individual cells in the population execute distinct functions to ensure coordinated actions that maintain the entire population and in plant root systems where chemicals draw microbes in to take up residence for the purpose of nitrogen fixation. In considering the human gut microbiota, it is important to take these lessons into account and recognize that many of these niches run across species and even across kingdoms. In fact, there have been many recent reports describing communication whereby metabolites produced from microbes signal to the host and molecules from the host communicate to the microbes (Table 1).⁴³

Although the evidence for the existence of chemical communication is clear, especially in the case of single-species developmental systems,⁴⁶ the understanding of how multispecies

signals precisely operate within their ecological context is in its infancy, particularly within complex mammalian systems. And, while chemical communication with secondary metabolism is well appreciated in systems such as biofilms and plant roots, the potential for small molecules to potentiate or drive a significant component of the microbiota-induced host phenotype appears to be secondary to metagenomic analysis. However, many research groups do recognize that the microbiota–host interaction is likely comprised of a complex web of host–microbe metabolic interactions—essentially, a chemical communication highway between the host and the subecology created by the resident microbial community.¹

Homeostasis and metabolites

Inherently, human metabolism is under tight homeostatic regulation. Metabolism is the simplified unit that all life evolved around and it is tightly regulated with normal homeostasis⁴⁸ whether the initiator of this change is induced by the environment (i.e., lifestyle, diet), microbiome, or the genetics. Thus, in the case where the vast complexity of microbiome, environmental, and host genetics conspires to generate a particular disease state, metabolites are a functional barometer for signifying and understanding these states irrespective of the source.^{12,49,50}

As noted above, there are a large number of publications that offer intriguing associations between the microbiota and disease but, mechanistic links are

frequently absent. A recent report that exemplifies this point is work describing metagenomic signatures of type 2 diabetes in European females⁵¹ and in separate work deriving a metagenomic signature for type 2 diabetes in Chinese individuals.⁵² Despite both studies successfully producing metagenomic predictive signatures, the metagenomic models were incongruent. The authors reconcile this by suggesting that the microbially encoded functions that contribute to disease progression are likely to be common across these distinct populations. Although this is speculative, the readout of a chemical signature between the two groups may be a definitive way to determine this possibility.

Emerging evidence for metabolite involvement in defining human microbiota-induced changes

There have already been substantial contributions where metabolites have offered mechanistic details about microbiota-induced changes (Table 2).²³ These include melamine toxicity,²⁴ short chain fatty acids (SCFAs) in adiposity and inflammation (via antibiotic use or gastric bypass,^{25–27} the conversion of components in certain eastern medicines,²⁸ the production of pheromones in the locust gut leading to swarming behavior,⁵³ the generation of trimethylamine N-oxide (TMAO) as a cardiovascular risk factor,¹² the generation of variants of vitamin B leading to binding and signaling through major histocompatibility complex (MHC1),²⁹

Metabolite	Physiology induced or associated with	Citation
Colibactin	Colitis-associated colorectal cancer (CRC)	Ref. ¹⁴
Various drugs (>40 known)	Various, negative and positive	Ref. ⁶
Metformin	Metformin may be modulating the gut microbial community to result in an improved metabolic profile	Ref. ⁵⁵
Herbal medicines	Transformed by microbiota to deliver efficacy	Ref. ²⁸
Deoxycholic acid	Liver cancer	Ref. ⁵⁶
Cyanuric acid	Melamine toxicity	Ref. ²⁴
Short chain fatty acids (SCFAs)	Adiposity, gastric bypass efficacy	Ref. ²⁵
Guaiacol	Locust swarming	Ref. ⁵³
Trimethylamine N-oxide (TMAO)	Heart disease	Ref. ^{12,13}
Vitamin B metabolites	T-cell activation	Ref. ²⁹
Medium chain fatty acids	Immune and inflammatory response	Ref. ²⁶
Portal circulation TLR agonists	NAFLD	Ref. ⁵⁷
Polysaccharide A	Immunologic tolerance	Ref. ⁵⁸
Testosterone	Autoimmunity and type 1 diabetes	Ref. ¹⁷
Secondary bile acids	TGR5 agonism (energy expenditure, GLP-1 secretion)	Ref. ²¹
Taurocholate	Bloom in proinflammatory <i>B. wadsworthia</i>	Ref. ³⁰
Sialic acid	Expansion of enteric pathogens	Ref. ⁵⁹
Bile acids	Obesity—possibly via farnesoid X receptor (FXR)	Ref. ⁶⁰

Table 2. Sampling of reported gut microbiome–host chemical interactions.

susceptibility to autoimmunity via microbiota-driven sex hormone alterations,¹⁷ in starvation via kwashiorkor with altered gut microbiota and associated sulfur metabolism,⁵⁴ cancer inducing activity,¹⁴ and the growth of a proinflammatory strain via bile acids stimulation.³⁰ As detailed below, these examples generally fall into three categories of interaction where the microbiota: (1) influence the levels of an endogenous host metabolite; (2) directly transform xenobiotics or host metabolites to potentiate a biological effect; or (3) directly produce a metabolite that effects the host biology.

Microbiota and endogenous metabolites

Although microbiota have been described to influence the levels of many endogenous metabolites and peptides,¹ a recent investigation into the genesis of the autoimmune link of type 1 diabetes (T1D) revealed a striking influence of the microbiota on the abundance of an endogenous steroid.¹⁷ Early-life microbial exposure in the mouse model of T1D showed that when females were colonized with gut microbiota from mature male mice, their serum testosterone levels were raised, resulting in strong protection from progression to T1D and insulinitis. Several lines of evidence showed that the protection was isolated to testosterone activity. Collectively, the results showed that a shift in the microbiome composition drove testosterone-dependent attenuation of autoimmune-induced T1D development. In addition to this recent example, microbiota have been described or suggested to effect levels of the intestinal incretin glucagon like peptide-1 (GLP-1).⁶¹ It has been observed that GLP-1

secretion may be reduced and delayed in obese patients as compared to lean control individuals and it is appreciated that obese patients have a different microbiota.⁶² Thus, microbial communication can affect host hormonal signaling and potentially have a profound effect on physiology. Conversely, endogenous host metabolites such as cholesterol, or those derived from the processing of specific diets, have been reported to influence the composition of the microbiota.^{63,64} One striking example of this is recent work showing how pathogenic strains (*S. typhimurium* and *C. difficile*) expand by consuming host mucosal carbohydrates that are normally liberated and catabolized by resident species when resident microbiota are cleared by antibiotics.⁵⁹ Hence, in this case, an endogenous host metabolite (e.g., sialic acid) is converted to enteric pathogen biomass, resulting in host infection. This same type of relationship may also extend to microbiomes outside of the gut, as shown in work examining the metabolome of patients with progressive forms of dental disease. In this work, a combination of host metabolites and bacterially derived metabolites were influenced with disease progression and provided insight into the host–resident microbiota–inflammatory network.⁶⁵

Direct transformation of xenobiotics or host metabolites

Arguably, this influence is the most well appreciated with the impact of the microbiome on the conversion of dietary components into host fuel sources like SCFAs,¹ the conversion of nonbioavailable herbal medicines into active forms,²⁸ the conversion of toxins into benign and readily excreted molecules, or the conversion of essential host metabolites such as choline, carnitine, tryptophan, or bile acids into bioactive compounds.¹ These activities are also why the microbiome is sometimes described as an “organ”—because of the propensity to convert molecules into energy sources, and bioactive species (i.e., akin to a liver). Notable recent examples include the melamine-induced renal toxicity invoked by gut microbiota metabolism.²⁴ Melamine gained notoriety as a cause of renal failure in children after ingestion of infant formula supplied with melamine in an effort to disguise the true protein content. Zheng and colleagues showed that clearing the gut microbiota by antibiotic treatment attenuated toxicity in rats. Further interrogation showed that *Klebsiella terrigena* converted melamine to cyanuric acid resulting in the renal toxicity.

A final example of the potential for gut microbiota to convert diet-derived metabolites to bioactive compounds that have a role in disease is the recent discovery of the levels of TMAO and its association with CVD.^{12,13} TMAO was first revealed to be associated with CVD using a metabolomics screen revealing that choline, TMAO, and betaine serve as predictive biomarkers for the development of CVD.¹² It was subsequently demonstrated that gut microbiota were responsible for the conversion of choline to TMAO and that by manipulating the gut microbiota TMAO levels

could be modulated along with atherosclerosis. In separate work, it was revealed that dietary L-carnitine could also be converted to TMAO by microbiota.¹³ In this study, clinical outcome data showed that concurrently high plasma TMAO and L-carnitine predicted increased risks for CVD and adverse cardiac events (myocardial infarction, stroke, or death). The above examples illustrate how the gut microbiota can extend our own metabolism to affect disease. Hence, recent developments toward understanding the effects of microbiome-related metabolic influences on human health have provided the impetus to consider the microbiome as a malleable factor in human health⁶⁶ and view microbe-specific enzymes as “druggable targets.”⁶⁷

Alteration of drug efficacy and toxicity

This same dynamic of microbiota acting on xenobiotics is well established for over 40 drugs⁶ to either the benefit or detriment of patients taking them. Relationships with these drugs are often complex, with drugs being acted on by microbiota or in some cases influencing the composition of the host microbiota. In fact, one recent study revealed an intriguing possibility with the longstanding mystery of the mechanism of action of metformin. In this work, it was shown that metformin increased the population of *Akkermansia* in the gut and modulation of the levels of this microbe improved glycemic control of diabetic mice.⁵⁵ This is even more intriguing given the recent report of a study on human microbiota that showed an association of *Akkermansia muciniphila* with type 2 diabetes.⁵² In addition, the pharmaceutical company Elcelyx Therapeutics reported results with their delayed-release formulation of metformin that, despite being 50% less bioavailable than metformin, was equally efficacious since the drug was more targeted to the gut (American Diabetes Association [ADA] Scientific Sessions in Chicago). Other examples have shown notable impacts of diverse host microbiota on alterations in both drug efficacy and toxicity, where gut microbes influence absorption, bioavailability, and preabsorption drug metabolism to impotent or even toxic metabolites.^{66,68,69} Particularly compelling examples are with a nicotinic acid receptor (NAR) agonist that unexpectedly induced acute renal toxicity in mice due to the marked elevation in 3-indoxyl sulfate (3-IS), a metabolite produced by microbiota catabolism of tryptophan.⁷⁰ In this case, this host–microbiota interaction was circumvented by using 3-IS as a biomarker to show that a second NAR agonist did not induce the production of 3-IS or renal toxicity. Also, recently, it was described that select strains of the gut microbiota *Eggerthella lenta* possessed digoxin-inactivating cytochromes and were the source of the well-established inactivation of this important cardiac drug. These strains were predictive of digoxin inactivation in humans and digoxin levels followed the manipulation of these strains in mice.⁷¹ Gut microbiota may also, in part, determine response to cholesterol-lowering statins as described in recent human serum metabolomic work that showed that bile acids produced by the microbiota delineated response to simvastatin.⁷² Collectively, this evidence exemplifies the need to evaluate the metabolic impact of the gut microbiome during drug development, and consider its implication with respect to seemingly inexplicable adverse events, potentially revealing a mitigation strategy for “troubled” late stage drugs.

Microbiota produced metabolites

What may eventually turn out to be the most important in terms of understanding symbiotic function are metabolites that are the

direct output of the diverse biosynthetic capability of microbes. This may not currently be the widest class of microbiota-related known small molecule influencers, but we hypothesize that this may be due to a lack of deep systematic surveys for these metabolites (discussed below). However, there is a growing body of examples where metabolites produced by microbiota directly influence host response.

For example, polysaccharide A (PSA) signals through Toll-like receptor (TLR) 2 to promote immunologic tolerance.⁵⁸ Signaling typically occurs with microbial ligands through TLRs to activate the immune response. Thus, this work illustrated the capacity of symbiotic signaling through a receptor that typically engages a vigorous immune response from ligands of foreign invaders. In another example, colibactin, the genotoxic polyketide produced by commensal *E. coli*, together with inflammation provides a “two-hit” formula to induce colitis-associated colorectal cancer in the mouse.¹⁴ A similar “two-hit” mechanism is proposed to drive nonalcoholic fatty liver disease/nonalcoholic steatohepatitis (NAFLD/NASH) pathogenesis; recent work suggests that the inflammation associated with progression may be linked to inflammasome-mediated gut microbiota.⁵⁷ When inflammasome proteins NLRP6 and NLRP3 are absent, an altered population of gut microbes results, leading to the production of TLR4 and TLR9 agonists that potentiate NASH and perturb glucose homeostasis. Importantly, this mechanism is proposed to be initiated by agonists that efflux from the intestines of NLRP6- and NLRP3-deficient mice through the portal circulation to the liver where they activate TLR4 and TLR9, enhancing the progression to NASH. These agonists may consist of proteins, small peptides, or chemicals; the discovery of their exact identity could provide the foundation for understanding how to target this particular route to NASH.

Recent work has described microbiota-derived vitamin B ligands as activators for the MHC class I-like molecule, MR1, that activate mucosal-associated invariant t-cells (MAIT cells). MR1 possesses an antigen-binding site distinct from MHC and cluster of differentiation 1 (CD1) gene families that is tailored for binding B-vitamin metabolites of bacterial vitamin B2 biosynthesis. Thus MAIT cells are adapted to conduct immune surveillance for nonsymbiotic microbes possessing specific vitamin B biosynthetic capacity. Finally, SCFAs are prominently produced by microbiota through the fermentation of dietary fiber and have also been shown to signal through G-protein coupled receptors (GPR43) to regulate immune and inflammatory responses.²⁶

Pharmaceutical and functional foods based on bioactive metabolites of the microbiome

Conducting an extensive survey of microbiome metabolites may extend beyond building knowledge. It may also provide highly practical considerations for functional foods, nutraceuticals, and pharmaceuticals. Just as secondary metabolites with important biological properties have been identified in plants and exotic organism from around the planet for decades, the gut microbiota possess diverse secondary (and primary) metabolic machinery.^{73,74} These microbially produced secondary metabolites with drug-like activity can potentially be harnessed for therapeutic purposes. In contrast to the search for potent bioactive secondary metabolites from exotic species not resident to human (e.g., marine organisms, plants, etc.), a metabolite discovered from the human microbiome will have a long track record of years of tolerability. Further, although the future of therapeutically harnessing the microbiome may be by invoking or transplanting

Figure 3. Overview of a thorough systems biology effort to understand the influence of the microbiome and host genetics on the phenotype. With this approach, it is expected that, in addition to associations derived from metagenomics, the comprehensive survey of metabolites by metabolomics will serve to enrich findings from a mechanistic perspective. Ideally, host genetics would also be incorporated to complete the understanding of this on the microbiome. One simultaneous complication and strength of studying the human metabolic footprint is that the phenotypic expression will produce integrated signal from host metabolism, microbiome metabolism, and the diet/xenobiotic contribution. This is a strength as it allows for the summation of the system's metabolism to be surveyed to reveal the important perturbations. In addition, metabolism inherently mitigates this complexity because, with the exception of novel microbiome-produced metabolites, human metabolism is well annotated such that the nature and source of these signals can frequently be distinguished. The same paradigm could also be applied to germ-free mice colonized with different strains or fecal lineages associated with particular phenotypes. In this case, cecal samples can also be added to the analysis.

beneficial species, the manufacturing and regulatory avenues have very little precedent. In contrast, channels for moving a small molecule agent or modified variant forward is well defined from a development and regulatory perspective. As a final point, the identity of metabolites produced by the microbiota that impart negative health benefit, or increase susceptibility to disease, can assist in elucidating the precise pathway that should be targeted in conjunction with metagenomic data identifying the relevant strain(s).

Approach for Studying the Chemical Output of the Host–Microbiome Interaction

When microbiome researchers embark on adding the chemical constituent of the host–microbiome relationship to their research, there are a number of important considerations to maximize the probability of success. First, it will be essential to employ approaches that survey the widest breadth of canonical and noncanonical metabolites. This is because, whether urine, stool, or blood are analyzed, there are multiple sources constructing the chemical composition of these host-derived sample types—endogenous host metabolism, diet/xenobiotics and, as discussed above, the influence of the microbiome (Figure 2). Also, in addition to classes of host metabolites that are influenced by the microbiota, it is likely that the microbiota will produce many diverse and uncharted (i.e., novel) metabolites.⁷⁵ In an effort to identify novel metabolites, some have advocated the use of a natural products platform that is able to elucidate structures of novel metabolites.⁷⁶ However, there are some metabolomic technologies that simultaneously provide both broad coverage of known metabolites and facilitate the discovery of novel metabolites.

Metabolomics is often described as a systematic study of the low molecular weight (approximately 50–1,500 Da) metabolites (chemicals) within a given sample.⁷⁷ This survey encompasses chemicals consisting of the substrates, intermediates, and products

of enzymatic activity in the support of cellular processes and ultimately provides information about the physiology of a cell, organ, or organism.

Relative to the volume of studies employing metagenomics to understand host–microbiota interactions, few metabolomic studies have been conducted that have coupled a high breadth survey of metabolism with a robust process to identify novel metabolites.^{1,78} Increased use of robust metabolomics approaches holds promise for future metabolite discoveries in the microbiome field. The metabolomic approaches taken to date have described involvement of canonical metabolites such as p-cresol, indoxyl-sulfate, bile acids, or SCFAs, in host–microbiota interactions. In these cases, it is often noted that a more thorough interrogation is warranted and that the metabolites denoted to be important to the microbiota–host phenotype association may in fact be just one part of the story. These results suggest that researchers supplement other lines of inquiry with metabolomics studies to generate metabolomic data to help resolve the penetrance of key elements.

The concept of combining multiple “omic” datasets is not unique; there are several robust examples in the literature that illustrate how combining multiple data sets refines rather than confuses inquiry.⁷⁹ Recent examples of combining GWAS with metabolomic approaches has helped to confirm and extend known genetic–metabolite associations as well as identify and ascribe function to other genes and their associated single nucleotide polymorphisms.⁸⁰ Combining metagenomics, and more recently metatranscriptomics, with metabolomics may help to distill key features relevant to communication and host–microbiota interactions that impact human health (Figure 3). Although there are small molecules common to both the host and the microbiome (Figure 2), there is a diversity of molecules unique to the microbiota. Thus, the small molecule information obtained from metabolomics analyses may allow for a focus on relevant impactful communication schemes when all of the data

are compiled. Furthermore, using approaches that combine data from clinical samples, preclinical models and even monocultures of selected microbial strains, will facilitate distillation of the data into valuable, actionable knowledge. Notably, a complete translational approach could be adopted whereby germ-free mice colonized with different strains or fecal lineages associated with particular phenotypes are profiled as well. This may in fact be an ideal way to more tractably begin to build out a more extensive host–microbiota metabolic map.

The Human Microbiome Project has already provided valuable information, mostly genetic sequences, of all the microbes on and within humans. However, data-gathering and analysis are thought to become stifling efforts in the extraction of meaning from the information.⁸¹ Yet, given the obvious integral role the microbiome is playing in human health, scientists should be intrigued and excited about this new frontier and have the desire to meet the challenge head-on. One approach that will undoubtedly facilitate understanding of the human–microbiome system is the capture of the information contained within small molecules from host plasma or tissue, feces and/or urine. Combining these chemical data with other data streams will undoubtedly enrich our understanding of the gut microbiome and its impact on human health. Since diet has been shown to produce geographic/ethnic-dependent diversity in the human microbiome, it will be important to incorporate such diversity into this broad systems analysis to ensure precise annotation of metagenome–metabolism–phenotype relationships. A systematic approach could maximize the probability of the identification of novel targets for drug development and treatment strategies.

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