Trends in Microbiology

CellPress OPEN ACCESS

Opinion

Regulation of microbial gene expression: the key to understanding our gut microbiome

Anurag Kumar Sinha [1,2,*], Martin Frederik Laursen [1,2], and Tine Rask Licht [1,*]

During the past two decades, gut microbiome studies have established the significant impact of the gut microbiota and its metabolites on host health. However, the molecular mechanisms governing the production of microbial metabolites in the gut environment remain insufficiently investigated and thus are poorly understood. Here, we propose that an enhanced understanding of gut microbial gene regulation, which is responsive to dietary components and gut environmental conditions, is needed in the research field and essential for our ability to effectively promote host health and prevent diseases through interventions targeting the gut microbiome.

Highlights

Regulation of gene expression is a complex phenomenon that is essential for bacteria to survive and adapt to changing environmental conditions.

Gut microbiome composition profiling frequently does not correlate with the gut metabolome.

Recent research indicates that regulation of microbial gene expression significantly influences gut microbial metabolite production. However, the factors that affect microbial growth and their gene expression have hitherto not received much attention in gut microbiome studies.

Understanding the influence of dietary and gut environmental factors on gut bacterial pathways for metabolite production requires careful attention in future microbiome research.

Increased integration of bacterial gene regulation in gut microbiome studies is crucial for leveraging the potential of gut microbiota to enhance health and prevent diseases.

Regulation of bacterial gene expression: the way forward for microbiome research

The human gut contains a diverse microbial community, including several hundred species that together produce a multitude of **metabolites** (see Glossary) [1]. These metabolites are intermediates or end products of microbial metabolism generated through the growth and fermentation processes of microbes in the gut. The microbial metabolites act as ligands for receptors on the host cells in multiple ways, eliciting local or systemic host responses [1,2]. These metabolites impact human health as they contribute to mucosal homeostasis and, depending on the context, either protect against or escalate disease conditions such as **inflammatory bowel diseases**, metabolic disorders, and neurological conditions [3,4]. Despite the established role of the **gut microbiome** in human health, we are still far from harnessing the full potential of this knowledge for therapeutic and preventive approaches [1]. Particularly, we lack understanding of the conditions that impact regulation of bacterial gene expression at transcriptional, translational, and post-translational levels in the gut. This knowledge is needed in order to design targeted strategies to manipulate the production of specific gut microbial metabolites *in vivo*. Current microbiome research typically includes sequencing-based **microbiome profiling**, coupled with **metabolomics**, aiming to identify correlations between microbial abundance and metabolites [5–8]. Although this strategy has proved useful [6], correlation analyses have clear limitations and disadvantages. An example is the occurrence of spurious correlations happening at random or due to noncausal covariation and confounding factors in studies aiming to identify microbial contributors for specific metabolites [9]. Furthermore, correlation analyses may miss detection of true microbial producers since the production of metabolites in the gut can be profoundly affected by non-genetic factors such as substrate availability and environmental conditions [9,10]. Indeed, in the gut, the abundance of genes in a given bacterial metabolic pathway does not necessarily correlate with the abundance of the metabolite produced through this pathway [11].

Metatranscriptomics, the analysis of the collective transcriptomes of the microbiome, has been applied in human gut microbiome research [12]. Metatranscriptomic analysis of 372 fecal

[1]National Food Institute, Technical University of Denmark, Kongens Lyngby, Denmark
[2]These authors contributed equally to this work

*Correspondence:
akusi@food.dtu.dk (A.K. Sinha) and
trli@food.dtu.dk (T.R. Licht).

samples collected from 308 healthy individuals identified core versus variably transcribed genes and assigned them to specific microbes [13]. Core genes included glycolysis, nucleotide biosynthesis pathways, and carbohydrate metabolism genes, whereas the variable transcriptome included genes for amino acid biosynthesis, long-chain fatty acids, terpenoids, polyamines, cofactors, and the stringent factor (p)ppGpp alarmone, indicating the dynamic expression profile for these genes [13]. Another fecal metatranscriptomic analysis captured high expression of genes involved in RNA polymerase production, glycolysis, ribosome biogenesis, and energy metabolism in gut microbes [14].

Importantly, global transcriptomic analysis is challenged by the fact that the majority of the transcripts are associated with fundamental biological processes such as metabolism, translation, ribosomal structure, and biogenesis and ATP production, which are common to all bacterial species and therefore typically not involved in differential production of metabolites with impact on host health. Metatranscriptomic analysis of the fecal microbiota in ten healthy individuals thus concluded fairly uniform and homogeneous microbiota functional activities among individuals in spite of large differences in microbiota composition observed with corresponding metagenomic data [15].

Another major challenge to the use of metatranscriptomics is the significant instability of microbial mRNA, which, in combination with the rapid, environmentally induced changes in microbial transcription profiles, means that the transcription profile of a fecal sample is not representative of bacterial gene transcription inside the intestinal tract [12].

Additional challenges associated with metatranscriptomics include the need for large amounts of microbial mRNA and filtering out the highly expressed and much more stable microbial rRNAs as well as RNA originating from the host [12]. Moreover, the production of gut bacterial metabolites depends not only on the transcription of relevant genes but also on various other aspects of gene expression, such as translation and post-translation.

Here, we argue that the impact of regulation of gene expression on gut microbial metabolic output has been largely neglected in the gut microbiome research field. We propose that the regulation of transcription, translation, post-translational modifications, and enzymatic activities plays a significant role in the metabolite pool generated by the microbiome. Multiple studies of defined microbial cultures have established the pivotal role of regulation of gene expression for bacterial survival and adaptation to changing environmental conditions and ultimately the production of specific metabolites [16–22]. In the gut, as in any other environment, bacterial genetic pathways must be efficiently regulated, as evidenced by recent studies [23,24]. Later, we outline how an improved understanding of bacterial physiology, characterized by the fundamental processes of bacteria, including their response to environmental factors that affect their survival, growth, and metabolism under changing conditions, is needed to interpret the multiple associations and intervention responses reported from gut microbiome studies.

Microbiome metabolites: beyond the genomic abundance

Through targeted modeling of a fermentation process involving a bacterial community consisting of ten representative gut species and using their genome-scale metabolic models, it has been concluded that correlation-based analysis lacked predictive power in identifying the primary contributors to specific metabolites [9]. Similarly, a comparison of the microbiome and the microbial metabolome present in stool samples from seven exclusively breastfed neonates, collected at first transitional stool (0–24 h), on the day of discharge from the hospital (30–48 h), and 3–5 days after birth, has shown that multivariate composition of the metabolome did not correlate with

Glossary

Gut microbiome: refers to the diverse and complex community of microorganisms such as bacteria, viruses, and fungi that inhabit the gastrointestinal tract. The gut microbiome plays a crucial role in maintaining the health of the digestive system as well as overall health of the host.
Inflammatory bowel diseases: inflammatory diseases of the gastrointestinal tract. Crohn's disease and ulcerative colitis are two common types of inflammatory bowel disease.
Metabolites: the intermediates or the end products of metabolic pathways that occur in living organisms for their survival and growth.
Metabolomics: refers to the analysis of all small molecules, or metabolites, present in a biological sample. The common methods of metabolomics include liquid chromatography–mass spectrometry (LC-MS) or gas chromatography–mass spectrometry (GC-MS).
Metatranscriptomics: the analysis of mRNAs of a complex microbial community to quantify the expression levels of their genes.
Microbiome profiling: refers to the characterization of the microorganisms in a particular environment, such as the human gut. The method involves sample collection, DNA extraction, next-generation sequencing, and analysis.
Proteolytic fermentation: a metabolic process in which microorganisms break down proteins and peptides into amino acids and amino acid–derived molecules. This type of fermentation involves the use of proteins or peptides as a carbon and energy source by microorganisms, leading to the production of various metabolites.
Saccharolytic fermentation: a metabolic process in which microorganisms, such as bacteria and yeast, break down complex carbohydrates (fibers) into simpler compounds such as acetate, propionate, and butyrate, collectively referred to as short-chain fatty acids, and gases.
Short-chain fatty acids (SCFAs): produced through the fermentation of dietary fibers by gut microbes. The common SCFAs include acetate (C2), propionate (C3), and butyrate (C4), each with distinct metabolic and physiological effects.

microbiome composition [24]. Moreover, analysis of stool samples for microbiome–metabolome relatedness from infants in a New Hampshire Birth Cohort Study aged approximately 6 weeks (n = 158) and 12 months (n = 282) revealed that the microbial community structure was only weakly predictive of stool metabolite relative concentrations [25]. Considering the discrepancies between the results obtained from different studies and cohorts in finding microbially associated metabolites, a machine learning (ML) pipeline was developed with the aim of identifying universal microbiome–metabolite links [10]. Data from 1733 samples from ten independent human gut microbiome–metabolome studies were analyzed, and the ML pipeline was used to compare the predictability of each metabolite across datasets. A few metabolites were robustly well predicted on the basis of microbiome data, but for several other metabolites, the model was not applicable. Furthermore, many metabolites exhibited significant variation in predictability across different datasets, highlighting the complexity and challenges in microbiome–metabolome relationships [10].

In line with this, it was recently shown that microbiome-derived metabolites do not correlate with the metagenomic abundance of the microbial genes responsible for their production [11]. For example, the metagenomic abundance of the genes encoding the succinate, acrylate, and propanediol pathways, the three known pathways responsible for gastrointestinal production of propionate, show no correlation to the concentrations of propionate measured in feces or plasma, suggesting that the pathway-specific gene regulation, rather than just the abundance of the producer species, plays a significant role in determining metabolic flux [11]. These examples illustrate that we cannot rely only on mere abundance of producer species/genes when trying to understand production of microbial metabolites in the gut.

Microbial fermentation in the gut: metabolomic results from dietary interventions unexplained

In a broader context, a fiber-rich diet promotes **saccharolytic fermentation** by the gut microbiota, resulting in the production of **short-chain fatty acids (SCFAs)**, primarily acetate, propionate, and butyrate, as fermentation by-products. Conversely, a protein-rich diet promotes **proteolytic fermentation**, leading to the generation of metabolites derived from amino acids and peptides (Figure 1) [1,2]. However, significant interindividual differences in the gut microbiota's production of specific metabolites in response to a given diet have been observed [26]. These variations can lead to inconsistencies in the results obtained, often leaving researchers with unexplained observations. For example, a homogeneous dietary intervention in healthy adults was proposed in one study to be insufficient in reducing interpersonal and intrapersonal variation in microbiome composition, function, and metabolic output [27]. Another randomized crossover study involving 46 healthy adults found that fecal butyrate levels exhibited significant individual variation following a 4-week dietary intervention with resistant starch [28]. Similarly, the microbiota response to fully controlled diets supplemented with resistant starch varied substantially in 14 obese males [29]. A common limitation of many dietary interventions aimed at manipulating gut microbial metabolism is that the inherent environmental conditions, such as pH, substrate availability, and bacterial interactions in the gut, as well as their effects on microbial gene regulation, are not sufficiently investigated.

Bacterial genetic regulatory networks: a driving force behind gut bacterial adaptation, survival, and metabolic output

Bacterial gene regulation predominantly controls nutrient utilization, fermentation processes, niche adaptation, and interspecies competition [30,31]. In the following, we discuss instances where bacterial gene regulation plays a central role in the functioning of the gut microbiota and production of its metabolites.

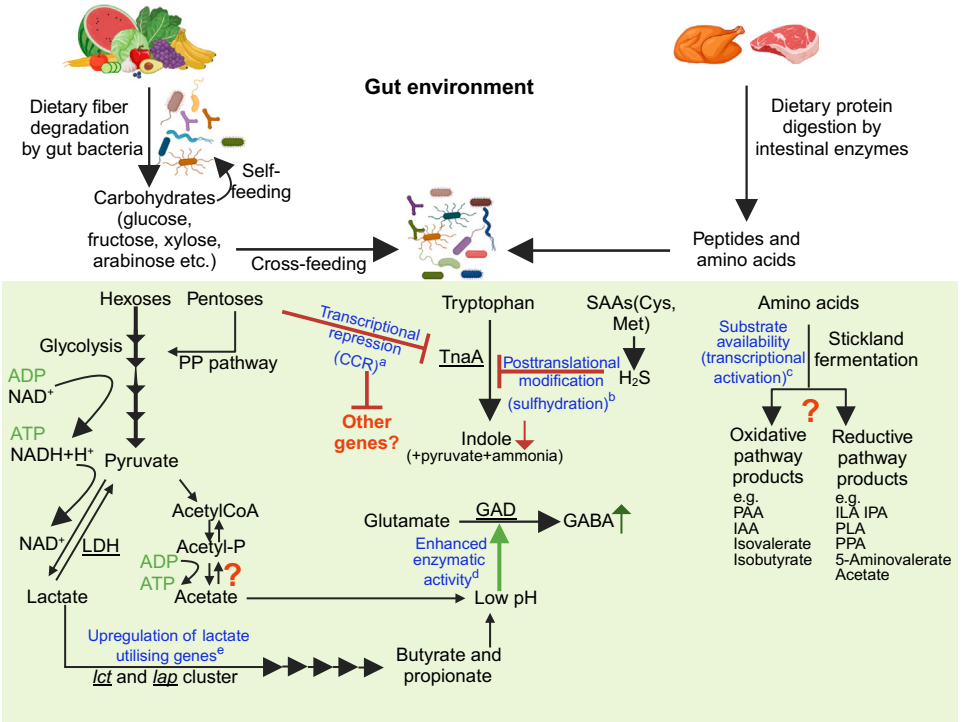

Figure 1. Examples of microbial gene regulation resulting in alteration of metabolite production. Top panel, white: specific fiber-degrading bacteria selectively break down dietary fibers in the human colon, providing monosaccharides for their own use and/or cross-feed to other bacteria. Unlike fibers, dietary proteins are enzymatically digested into peptides and amino acids and absorbed in the small intestine. However, a notable portion of the proteins reaches the colon and becomes accessible to the colonic microbiota. Bottom panel, green: in the predominantly anaerobic environment of the colon, bacteria ferment carbohydrates and amino acids to generate energy (ATP), use them as sources of carbon and nitrogen, and employ their products as both electron donors and acceptors to support bacterial proliferation. Details of the diverse fermentation pathways used by gut bacteria are reviewed and discussed elsewhere [1,2,42,43,72]. The shown examples (discussed in the main text) illustrate how gene regulation at the transcriptional, post-transcriptional, and enzymatic activity levels impacts and modifies gut microbial metabolic output. Specific references related to the examples: [a][36], [b][23], [c][45], [d][52], and [e][22]. Created with BioRender.com. Abbreviations: CCR, carbon catabolite repression; CoA, coenzyme A; GABA, γ-aminobutyric acid; GAD, glutamate decarboxylase; IAA, indoleacetic acid; ILA, indolelactic acid; IPA, indolepropionic acid; LDH, lactate dehydrogenase; PAA, phenylacetic acid; PLA, phenyllactic acid; PP, pentose phosphate; PPA, phenylpropionic acid; SAA, sulfur-containing amino acid; TnaA, tryptophanase enzyme.

Transcriptional regulation

Carbon catabolite repression (CCR) is the best-studied gene regulation system in a diverse group of bacteria and fungi [32]. This system entails the capacity of the organism to prefer the use of specifically selected substrates over others from a diverse mixture of carbon sources. The presence of preferred carbon sources hinders the expression of catabolic systems for the use of other secondary substrates through the CCR [17,32,33]. A textbook example of CCR is the repression of lactose-utilizing genes in the presence of glucose in *Escherichia coli* [33]. In the anaerobic environment of the gut, colonic bacteria ferment carbohydrates and amino acids to generate energy (ATP), often through substrate-level phosphorylation, as well as use them as sources of carbon and nitrogen to sustain their growth (Figure 1) [34]. In the presence of complex mixtures of carbon and nitrogen sources available in the gut environment, it is anticipated that colonic bacteria would often employ CCR to prioritize energy sources, thereby influencing the generation of their fermentation products. The presence of dietary fibers stimulates the activation of polysaccharide utilization loci in *Bacteroides* species, thereby

facilitating the breakdown of dietary fibers. This enables *Bacteroides* to cross-feed various oligo-, di-, and monosaccharides to other gut bacteria [35]. Consequently, the availability of monosaccharides may instigate CCR in gut bacteria toward less preferred carbon sources. For example, we recently revealed that pectin degrading *Bacteroides thetaiotaomicron* mediates cross-feeding of arabinose and xylose to indole producers such as *E. coli*, which represses the expression of tryptophanase gene, responsible for breakdown of tryptophan into indole and pyruvate, through CCR and thereby inhibits the intestinal production of indole, a precursor for harmful uremic toxins (Figure 1) [36]. In addition to *Bacteroides*, many species of *Bifidobacterium* have the capacity to use and ferment a variety of fibers, thereby generating acetate and lactate through the 'bifid' shunt [37]. These metabolites thereby become available to other gut bacteria. For example, acetate and lactate, produced by *Bifidobacterium adolescentis*, are fermented by *Anaerostipes caccae* and *Anaerobutyricum halli*, leading to butyrate production [37]. Interestingly, lactate utilization in *A. caccae* and *A. halli* is abolished when the growth medium is supplemented with glucose, suggesting CCR of the lactate utilization pathway [35]. Given the widespread occurrence of CCR across various bacterial genera, we expect that this mechanism has a broad impact on the regulation of gut microbial metabolite production.

Another form of transcriptional regulation entails global regulatory networks, such as in the infant gut, where the global transcriptional regulator NagR facilitates the expression of transcriptional regulatory networks that encode enzymes enabling bifidobacteria to metabolize human milk oligosaccharides (HMOs) [38]. Degradation of 2′-fucosyllactose (2′-FL), the most prevalent HMO present in human milk, performed by *Bifidobacterium bifidum*, cross-feeds fucose and lactose to fucose-using *Bifidobacterium breve* and thereby expands the resulting catabolic end products. Considerable production of formate and 1,2-propanediol is thus produced in addition to lactate and acetate [39,40]. A better understanding of the regulatory circuits driving the expression of NagR will allow control of the production of the downstream metabolites.

Stickland fermentation is the primary source of ATP generation for members of the *Clostridium* genus [41]. This reaction constitutes the coupled metabolism of pairs of amino acids, where one amino acid donates an electron (and gets oxidized) to generate ATP, whereas another amino acid acts as an electron acceptor (and gets reduced) [41,42]. Stickland fermentation products play important roles in human health and diseases [42–44]. We recently showed that supplementation with tryptophan proportionately increases the abundance of Stickland fermentation products of tryptophan, independently of the abundance of the producer species, suggesting that substrate availability is the key regulator of Stickland fermentation (Figure 1) [36]. In another study, supplementation of proline in the growth medium activated transcription of proline reductase gene and inhibited transcription of glycine reductase gene to generate ATP via the Stickland fermentation of proline in *Clostridioides difficile* [45]. This suggests that the availability of substrates governs the production of Stickland fermentation products through transcriptional regulation of genes involved in Stickland fermentation.

Furthermore, the bacterial stress response is likely to govern transcription of bacterial genes in the intestinal environment. During nutrient starvation, bacterial physiology is controlled by the so-called stringent response (SR), characterized by the synthesis of guanosine pentaphosphate and tetraphosphate [collectively referred to as (p)ppGpp]. SR is a coordinated alteration of gene expression that allows bacteria to restructure their transcriptional network to be able to respond quickly and efficiently in order to survive under stress conditions [46]. Under conditions of starvation, SR thus allows gut bacteria to adapt to the use of host glycans, resulting in a change in their metabolic output [47,48].

Assaults from bacteriophages is another example of an environmental factor with the potential to trigger transcriptional responses within the target bacterium. Interestingly, a specific lytic bacteriophage targeting *Bacteroides fragilis* induces inversion of the DNA in the promoter region of the polysaccharide A (PSA) gene, thereby turning this gene off and reducing the production of PSA, an anti-inflammatory polysaccharide [49].

Translational, post-translational, and enzymatic activity regulation

In addition to the multiple examples of gene regulation at the transcription level as discussed earlier, also translational and post-translational regulatory mechanisms play a role in the gut. For example, *B. thetaiotaomicron* regulates the use of oligosaccharides such as raffinose and stachyose by regulating the protein translation of polysaccharide use loci mRNAs by RNA-binding proteins. These proteins interfere with the binding of mRNA to the ribosomes, presumably by inducing structural changes in the target mRNA or by facilitating small RNA binding [50].

A recent study showcased the post-translational control of gut bacterial metabolic activity. It demonstrated that the fermentation of sulfur-containing amino acids from the diet, such as cysteine and methionine, elevates hydrogen sulfide (H_2S) levels in the gut. This increase in H_2S promotes sulfhydration, a post-translational modification, of the *E. coli* tryptophanase enzyme. Consequently, this reduces tryptophanase activity, thus inhibiting the production of indole, a precursor of uremic toxin, thereby lessening its accumulation in the gut (Figure 1) [23].

Lysine acetylation is another frequently occurring post-translational modification of bacterial proteins that is dependent on the formation of acetyl-phosphate (AcP) [51]. AcP is an intermediate in a reversible pathway in the conversion of acetyl-coenzyme A to acetate (Figure 1). In *E. coli*, growing on acetate as the carbon source, the two glycolytic proteins glyceraldehyde 3-phoshate dehydrogenase (GapA) and 2,3-bisphosphoglycerate-dependent phosphoglycerate mutase (GpmA) were inhibited by acetylation, thus reducing the glycolysis flow. Given that acetate represents the most prevalent SCFA produced in the gut, lysine acetylation could potentially impact the metabolic output of gut bacteria through protein acetylation.

Also, bacterial enzymatic activity is likely to be regulated by intestinal environmental factors. Recently, it was shown that low pH enhances the activity of glutamate decarboxylase (GAD) in the common gut bacterium *Akkermansia muciniphila*, leading to increased production of γ-aminobutyric acid (GABA), the main inhibitory neurotransmitter of the central nervous system (Figure 1) [52].

Other known factors that can alter gut metabolites

In addition to fermentation, gut bacteria produce ATP via anaerobic respiration, using a variety of organic substrates as both electron donors and acceptors [53]. Recently, it was shown that gut bacteria such as *Eggerthella lenta* use formate as an electron donor to reduce urocanate, via the electron transport chain (ETC), resulting in the production of imidazole propionate, a molecule positively associated with type 2 diabetes [53,54]. Similarly, quinones, a component of the ETC, produced by *E. coli*, supports the growth of *Faecalibacterium*, a genus for butyrate-producing species, under laboratory conditions [55]. Thus, it is crucial to explore the regulation of anaerobic respiratory pathways and their potential contribution to the production of metabolites important for host health.

Finally, bacteria in their natural habitats compete for nutrients and niches and employ ways to antagonize other species either by producing bacteriocins or by expressing type VI secretion systems [56,57]. These intra- and interspecies interactions have a great potential for shaping the gut microbial community and governing the production of certain metabolites, which remains to be properly investigated [58,59].

Concluding remarks and future perspectives

Here, we have reviewed how regulation of gene expression influences bacterial growth, survival, fermentation processes, and the production of metabolites. It is important to recognize that within a multispecies environment such as the gastrointestinal tract, the regulation of genetic pathways in one bacterial species can influence the metabolism of other bacterial species [36]. Therefore, integrating bacterial genetic regulatory networks into gut microbiome studies is essential. This will allow us to comprehend the molecular microbial responses to dietary interventions and mitigate or explain the inconsistencies observed across studies. Ultimately, it will aid in designing more effective personalized therapeutic strategies targeting the gut microbiota.

Although genomics, metagenomics, metatranscriptomics, and metabolomics have significantly advanced our understanding of the gut microbiota, researchers currently remain unable to explain several observations. The majority of microbiome research studies employ straightforward microbiome–metabolome analyses to identify associations between community composition and metabolic output, assuming a direct link between the abundance of bacterial producer species and a given produced metabolite (Figure 2) [60–62]. The newly emerging powerful computational tools

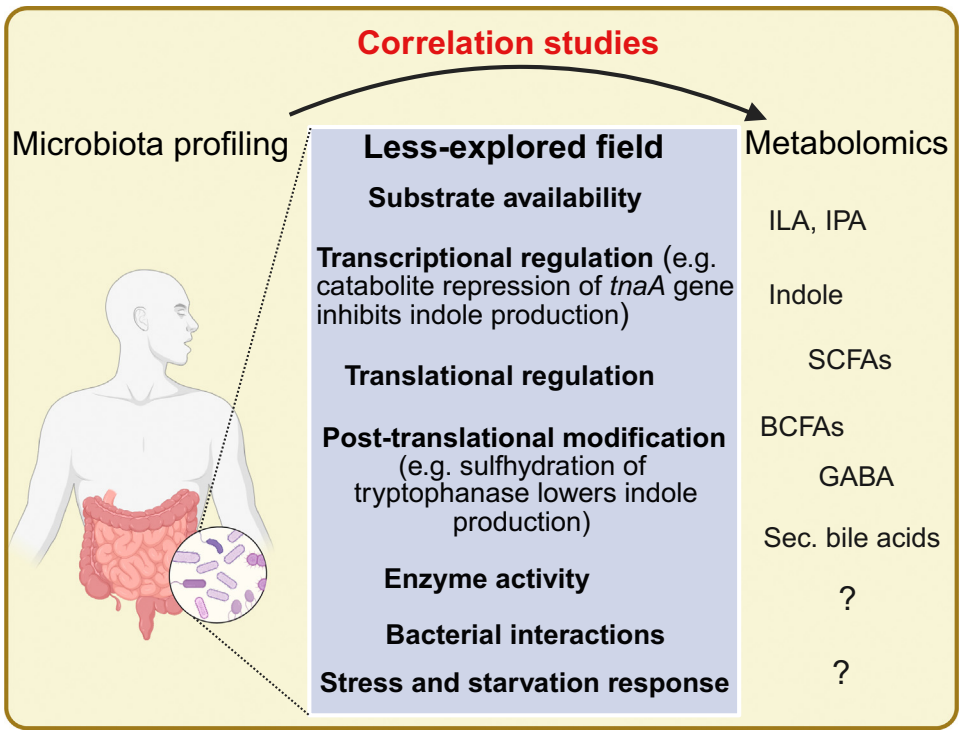

Figure 2. The gap in gut microbiome research. Gut microbiome research primarily employs sequence-based microbiota profiling or metagenomics to monitor the composition of the gut community and correlates that with the abundance of metabolites. However, the regulation of microbial gene expression has the potential to significantly impact the metabolic output of the gut microbiome. A few examples are shown here to emphasize how multiple factors other than bacterial abundance can impact the gut metabolome. We emphasize that the regulatory pathways that produce specific gut microbial metabolites need to be taken into consideration in future microbiome studies to explain observations from intervention studies and enable the design of personalized, targeted treatments aimed at modulating microbial metabolites for therapeutic purposes. The question marks depict unknown metabolites that may be influenced by specific regulations. Abbreviations: BCFAs, branched chain fatty acids; GABA, γ-aminobutyric acid; GAD, glutamate decarboxylase; ILA, indolelactic acid; IPA, indolepropionic acid; SCFAs, short-chain fatty acids; *tnaA*, gene for tryptophanase enzyme. Created with BioRender.com.

Outstanding questions

The gut microbiome comprises numerous bacterial species that remain largely unstudied at the molecular level. Investigating the genetics of a single bacterial species requires years of research. How can we convince and encourage microbiome researchers to collaborate with bacterial geneticists in microbiome studies to study specific bacteria of interest at the molecular level? Are we ready to delve deeper?

Bacterial cells may respond differently to dietary or environmental components under laboratory conditions from the way they do in the intestinal environment *in vivo*. How can we best design our experiments to reduce this ambiguity?

In the gut, typically multiple bacterial species are able to produce the same metabolite. Is correlation analysis sufficient to identify the real producer species in any specific individual?

Which strategies can we employ to develop more dependable methods for direct collection of gut samples, ensuring proper storage to capture bacterial transcription, and the conduct of transcriptomics?

are assisting researchers in annotating previously unidentified genes, predicting their functions, and identifying novel metabolic pathways within the gut microbiome [63]. However, we lack an understanding of the regulatory mechanisms governing the fermentation pathways and thereby metabolite production by the gut microbiota (Figure 2). The repression and activation of specific metabolic pathway genes due to dietary or environmental conditions may lead to correlations that are not caused by abundances of specific microbial species (Figure 3A). Longitudinal sample analysis is one way in which this bias can be partially reduced and can be helpful in identifying the real producer species. Additionally, longitudinal analysis can be helpful in identifying conditions under which a specific metabolic pathway is either repressed or activated in intervention studies (Figure 3A). Therefore, we

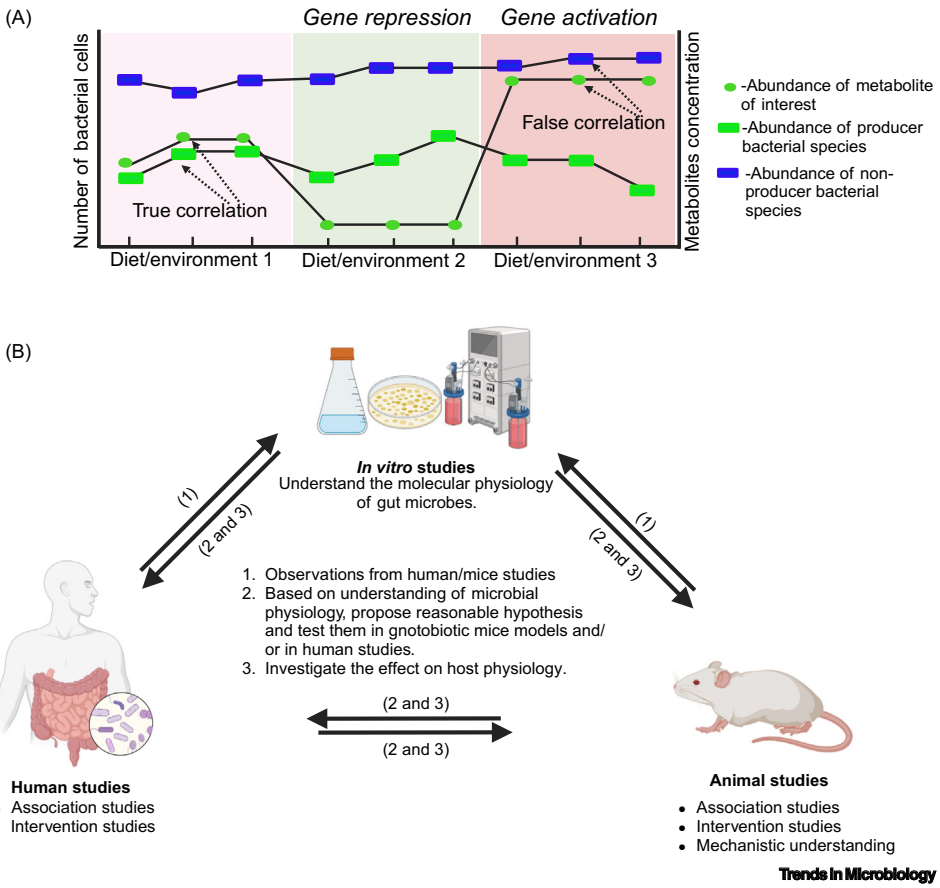

Figure 3. Limitations of correlation studies in microbiome research and strategies to bridge the research gap. Correlation studies are subject to bias due to bacterial gene regulation. (A) The schematic presented here indicates that the regulation of bacterial gene expression caused by diet or environmental changes may introduce false correlations in microbiome–metabolome research. The scheme shows three possible scenarios. Left panel: if the abundance of metabolites (depicted as a green circle) mirrors the abundance of the producer species (depicted as a green rectangle), it implies that there may be insignificant or no gene regulation occurring, thus revealing true correlations between abundance of the producer species and the metabolite. Middle panel: repression of genes aimed at producing a specific metabolite may result in no correlation with the actual producer species. Right panel: activation of genes and subsequent increased metabolite production may lead to 'false' correlation with a highly abundant non-producer species (depicted as a blue rectangle). Longitudinal sample analysis can partially address false correlation bias by taking more samples during each intervention. Moreover, longitudinal studies can shed light on the conditions under which a specific gene/pathway is repressed or activated. (B) A suggested strategy to address the research gap involves interdisciplinary collaboration, incorporating *in vitro* experiments to gain insights into bacterial physiology and refining the design of targeted human and animal studies. Created with BioRender.com.

recommend including longitudinal sample analysis in microbiome analyses whenever possible. However, such studies typically need to be accompanied by *in vitro* methods, as we recently showed for a set of infant microbiotas [6].

Another issue in microbiome research is the limited utility of fecal samples, which are predominantly employed in metatranscriptomic studies to investigate the genetic regulation of bacterial metabolic pathways occurring in the colon. The extremely rapid turnover of bacterial mRNAs renders fecal samples unsuited for transcription analysis [64]. Additionally, oxygen-sensitive organisms might be difficult to recover from feces for *in vitro* studies. Therefore, the use of metatranscriptomics in animal studies where invasive sampling of gastrointestinal content and immediate processing of such samples is feasible currently holds much more potential. Such investigation has already proved useful [65], and the use of similar approaches should be encouraged. In the future, devices for noninvasive sampling of human gut content, which allows fixing the mRNAs at the time point of sampling, may be available [66]. This is likely to increase our ability to understand the gene regulation of the human gut microbiome *in situ*.

Nonetheless, controlled *in vitro* studies remain an essential tool to examine bacterial responses to dietary or environmental factors, either in monoculture, within defined communities, or in undefined communities recovered from feces or intestinal content. An issue with *in vitro* experiments is the fastidious growth requirements and oxygen sensitivity of many gut microbes [67,68]. Some gut species obtain essential nutrients through cross-feeding from other microbes in the gut community, which might be missing in the *in vitro* culture medium [69], underlining the importance of understanding interactions between individual gut microbes. However, with better understanding of the bacterial nutrient requirements and advancements in microbial cultivation techniques, more than 50% of the bacterial diversity in the human gut can now be cultured [70,71].

We find that to enhance mechanistic understanding of gut microbial metabolites production, it is crucial for microbiome researchers to collaborate with microbiologists and bacterial geneticists (see Outstanding questions). The correlations between specific metabolites and microbes observed in human and mouse studies using multiomic techniques by microbiome researchers need to be accompanied by microbiological *in vitro* experiments, and more studies elucidating the regulation of pathways important for metabolite production should be performed (Figure 3B). With knowledge obtained from such studies, we can propose dietary interventions to regulate metabolite production *in vivo* and test them in mice and humans (Figure 3B). As recently shown in our laboratory, such interventions can be designed to increase microbial generation of beneficial metabolites and/or inhibit harmful metabolites [36] and will fill the current gap between microbiome research and clinical applications of personalized microbiome-based therapeutics.

Therefore, we advocate for a comprehensive approach to microbiome research, emphasizing an interdisciplinary environment that incorporates expertise in molecular microbiology, bacterial genetics, microbial ecology, nutrition, bioinformatics, preclinical/animal, and clinical studies. The needed collaboration may be encouraged by interdisciplinary research grants, conferences, and joint training programs.

Acknowledgments

We acknowledge funding from the Novo Nordisk Foundation Challenge programme to T.R.L. (PRIMA, grant NNF19OC0056246) and the Lundbeck Foundation to A.K.S. (LF Experiment, reference number R400-2022-1219).

Declaration of interests

The authors declare no competing interests.

References

1. Fan, Y. and Pedersen, O. (2021) Gut microbiota in human metabolic health and disease. *Nat. Rev. Microbiol.* 55–71
2. Koh, A. *et al.* (2016) From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites. *Cell* 165, 1332–1345
3. Agus, A. *et al.* (2021) Gut microbiota-derived metabolites as central regulators in metabolic disorders. *Gut* 70, 1174–1182
4. Liu, J. *et al.* (2022) Functions of gut microbiota metabolites, current status and future perspectives. *Aging Dis.* 13, 1106–1126
5. Pedersen, H.K. *et al.* (2016) Human gut microbes impact host serum metabolome and insulin sensitivity. *Nature* 535, 376–381
6. Laursen, M.F. *et al.* (2023) Key bacterial taxa determine longitudinal dynamics of aromatic amino acid catabolism in infants' gut. *Gut Microbes* 15, 2221426
7. Dekkers, K.F. *et al.* (2022) An online atlas of human plasma metabolite signatures of gut microbiome composition. *Nat. Commun.* 13, 5370
8. Visconti, A. *et al.* (2019) Interplay between the human gut microbiome and host metabolism. *Nat. Commun.* 10, 4505
9. Noecker, C. *et al.* (2019) Defining and evaluating microbial contributions to metabolite variation in microbiome-metabolome association studies. *mSystems* 4, e00579–19
10. Muller, E. *et al.* (2021) A meta-analysis study of the robustness and universality of gut microbiome-metabolome associations. *Microbiome* 9, 203
11. Andreu, V.P. *et al.* (2023) gutSMASH predicts specialized primary metabolic pathways from the human gut microbiota. *Nat. Biotechnol.* 41, 1416–1423
12. Ojala, T. *et al.* (2023) Understanding human health through metatranscriptomics. *Trends Mol. Med.* 29, 376–389
13. Abu-Ali, G.S. *et al.* (2018) Metatranscriptome of human faecal microbial communities in a cohort of adult men. *Nat. Microbiol.* 3, 356–366
14. Turnbaugh, P.J. *et al.* (2010) Organismal, genetic, and transcriptional variation in the deeply sequenced gut microbiomes of identical twins. *Proc. Natl. Acad. Sci. U. S. A.* 107, 7503–7508
15. Gosalbes, M.J. *et al.* (2011) Metatranscriptomic approach to analyze the functional human gut microbiota. *PLoS One* 6, 17447
16. Brauer, M.J. *et al.* (2006) Conservation of the metabolomic response to starvation across two divergent microbes. *Proc. Natl. Acad. Sci. U. S. A.* 103, 19302–19307
17. Moreno, R. and Rojo, F. (2023) The importance of understanding the regulation of bacterial metabolism. *Environ. Microbiol.* 25, 54–58
18. Olson, E.R. (1993) Influence of pH on bacterial gene expression. *Mol. Microbiol.* 8, 5–14
19. Tucker, D.L. *et al.* (2002) Gene expression profiling of the pH response in *Escherichia coli*. *J. Bacteriol.* 184, 6551–6558
20. Unden, G. *et al.* (1994) Oxygen regulated gene expression in facultatively anaerobic bacteria. *Antonie Van Leeuwenhoek* 66, 3–22
21. Carey, J.N. *et al.* (2018) Regulated stochasticity in a bacterial signaling network permits tolerance to a rapid environmental change. *Cell* 173, 196–207.e14
22. Sheridan, P.O. *et al.* (2022) Distribution, organization and expression of genes concerned with anaerobic lactate utilization in human intestinal bacteria. *Microb. Genomics* 8, 000739
23. Lobel, L. *et al.* (2020) Diet posttranslationally modifies the mouse gut microbial proteome to modulate renal function. *Science* 369, 1518–1524
24. Awan, I. *et al.* (2023) A pilot study exploring temporal development of gut microbiome/metabolome in breastfed neonates during the first week of life. *Pediatr. Gastroenterol. Hepatol. Nutr.* 26, 99–115
25. Nguyen, Q.P. *et al.* (2021) Associations between the gut microbiome and metabolome in early life. *BMC Microbiol.* 21, 238
26. Healey, G.R. *et al.* (2017) Interindividual variability in gut microbiota and host response to dietary interventions. *Nutr. Rev.* 75, 1059–1080
27. Guthrie, L. *et al.* (2022) Impact of a 7-day homogeneous diet on interpersonal variation in human gut microbiomes and metabolomes. *Cell Host Microbe* 30, 863–874.e4
28. McOrist, A.L. *et al.* (2011) Fecal butyrate levels vary widely among individuals but are usually increased by a diet high in resistant starch. *J. Nutr.* 141, 883–889
29. Salonen, A. *et al.* (2014) Impact of diet and individual variation on intestinal microbiota composition and fermentation products in obese men. *ISME J.* 8, 2218–2230
30. Scott, M. and Hwa, T. (2023) Shaping bacterial gene expression by physiological and proteome allocation constraints. *Nat. Rev. Microbiol.* 21, 327–342
31. Shis, D.L. *et al.* (2018) Dynamics of bacterial gene regulatory networks. *Annu. Rev. Biophys.* 47, 447–467
32. Görke, B. and Stülke, J. (2008) Carbon catabolite repression in bacteria: many ways to make the most out of nutrients. *Nat. Rev. Microbiol.* 6, 613–624
33. Deutscher, J. (2008) The mechanisms of carbon catabolite repression in bacteria. *Curr. Opin. Microbiol.* 11, 87–93
34. Fischbach, M.A. and Sonnenburg, J.L. (2011) Eating for two: how metabolism establishes interspecies interactions in the gut. *Cell Host Microbe* 10, 336–347
35. Feng, J. *et al.* (2022) Polysaccharide utilization loci in Bacteroides determine population fitness and community-level interactions. *Cell Host Microbe* 30, 200–215.e12
36. Sinha, A.K. *et al.* (2024) Dietary fibre directs microbial tryptophan metabolism via metabolic interactions in the gut microbiota. *Nat. Microbiol.*, Published online June 25, 2024. https://doi.org/10.1038/s41564-024-01737-3
37. Pokusaeva, K. *et al.* (2011) Carbohydrate metabolism in bifidobacteria. *Genes Nutr.* 6, 285–306
38. Arzamasov, A.A. *et al.* (2022) Human milk oligosaccharide utilization in intestinal bifidobacteria is governed by global transcriptional regulator NagR. *mSystems* 7, e0034322
39. Tsukuda, N. *et al.* (2021) Key bacterial taxa and metabolic pathways affecting gut short-chain fatty acid profiles in early life. *ISME J.* 15, 2574–2590
40. Egan, M. *et al.* (2014) Metabolism of sialic acid by *Bifidobacterium breve* UCC2003. *Appl. Environ. Microbiol.* 80, 4414–4426
41. Stickland, L.H. (1934) Studies of the metabolism of the strict anaerobes (genus: Clostridium). *Biochem. J.* 28, 1746–1759
42. Neumann-Schaal, M. *et al.* (2019) Metabolism the difficile way: the key to the success of the pathogen *Clostridioides difficile*. *Front. Microbiol.* 10, 219
43. Roager, H.M. and Licht, T.R. (2018) Microbial tryptophan catabolites in health and disease. *Nat. Commun.* 9, 3294
44. Liu, Y. *et al.* (2022) *Clostridium sporogenes* uses reductive Stickland metabolism in the gut to generate ATP and produce circulating metabolites. *Nat. Microbiol.* 7, 695–706
45. Bouillaut, L. *et al.* (2013) Proline-dependent regulation of *Clostridium difficile* Stickland metabolism. *J. Bacteriol.* 195, 844–854
46. Irving, S.E. *et al.* (2021) The stringent response and physiological roles of (pp)pGpp in bacteria. *Nat. Rev. Microbiol.* 19, 256–271
47. Schofield, W.B. *et al.* (2018) The stringent response determines the ability of a commensal bacterium to survive starvation and to persist in the gut. *Cell Host Microbe* 24, 120–132.e6
48. Ontai-Brenning, A. *et al.* (2023) Gut microbes modulate (p)ppGpp during a time-restricted feeding regimen. *mBio* 14, e0190723
49. Carasso, S. *et al.* (2024) Inflammation and bacteriophages affect DNA inversion states and functionality of the gut microbiota. *Cell Host Microbe* 32, 322–334.e9
50. Adams, A.N.D. *et al.* (2021) A novel family of RNA-binding proteins regulate polysaccharide metabolism in *Bacteroides thetaiotaomicron*. *J. Bacteriol.* 203, e0021721
51. Weinert, B.T. *et al.* (2013) Acetyl-phosphate is a critical determinant of lysine acetylation in *E. coli*. *Mol. Cell* 51, 265–272
52. Konstanti, P. *et al.* (2024) Physiology of γ-aminobutyric acid production by *Akkermansia muciniphila*. *Appl. Environ. Microbiol.* 90, e0112123
53. Little, A.S. *et al.* (2024) Dietary- and host-derived metabolites are used by diverse gut bacteria for anaerobic respiration. *Nat. Microbiol.* 9, 55–69
54. Koh, A. *et al.* (2018) Microbially produced imidazole propionate impairs insulin signaling through mTORC1 article microbially produced imidazole propionate impairs insulin signaling through mTORC1. *Cell* 175, 947–961
55. Fenn, K. *et al.* (2017) Quinones are growth factors for the human gut microbiota. *Microbiome* 5, 161

56. Peterson, S.B. *et al.* (2020) The central role of interbacterial antagonism in bacterial life. *Curr. Biol.* 30, R1203–R1214
57. Palmer, J.D. and Foster, K.R. (2022) Bacterial species rarely work together. *Science* 376, 581–582
58. Kost, C. *et al.* (2023) Metabolic exchanges are ubiquitous in natural microbial communities. *Nat. Microbiol.* 8, 2244–2252
59. Dolan, J.R. *et al.* (2024) More than the sum of its parts: uncovering emerging effects of microbial interactions in complex communities. *FEMS Microbiol. Ecol.* 100, fiae029
60. Choo, J.M. *et al.* (2017) Divergent relationships between fecal microbiota and metabolome following distinct antibiotic-induced disruptions. *mSphere* 2, e00005–17
61. Melnik, A.V. *et al.* (2017) Coupling targeted and untargeted mass spectrometry for metabolome-microbiome-wide association studies of human fecal samples. *Anal. Chem.* 89, 7549–7559
62. Franzosa, E.A. *et al.* (2019) Gut microbiome structure and metabolic activity in inflammatory bowel disease. *Nat. Microbiol.* 4, 293–305
63. Noecker, C. and Turnbaugh, P.J. (2024) Emerging tools and best practices for studying gut microbial community metabolism. *Nat. Metab.* 6, 1225–1236
64. Deutscher, M.P. (2006) Degradation of RNA in bacteria: comparison of mRNA and stable RNA. *Nucleic Acids Res.* 34, 659–666
65. Nichols, R.G. *et al.* (2020) Metatranscriptomic analysis of the mouse gut microbiome response to the persistent organic pollutant 2,3,7,8-tetrachlorodibenzofuran. *Metabolites* 10, 1
66. Shalon, D. *et al.* (2023) Profiling the human intestinal environment under physiological conditions. *Nature* 617, 581–591
67. Stewart, E.J. (2012) Growing unculturable bacteria. *J. Bacteriol.* 194, 4151–4160
68. Walker, A.W. *et al.* (2014) Phylogeny, culturing, and metagenomics of the human gut microbiota. *Trends Microbiol.* 22, 267–274
69. Strandwitz, P. *et al.* GABA-modulating bacteria of the human gut microbiota. *Nat. Microbiol.* 4, 396–403
70. Lagkouvardos, I. *et al.* (2017) Cultured microbes represent a substantial fraction of the human and mouse gut microbiota. *Gut Microbes* 8, 493–503
71. Lewis, W.H. *et al.* (2021) Innovations to culturing the uncultured microbial majority. *Nat. Rev. Microbiol.* 19, 225–240
72. Laursen, M.F. *et al.* (2021) *Bifidobacterium* species associated with breastfeeding produce aromatic lactic acids in the infant gut. *Nat. Microbiol.* 6, 1367–1382