

## Chemical signaling in the gastrointestinal tract

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### Abstract

Chemical signaling via the production of small molecules such as hormones has been studied in detail in higher organisms. These molecules have important functions in maintaining physiological homeostasis as well as allowing organisms to respond to external insults. Virtually every living cell produces hormone-like diffusible small molecules that can be used to convey messages to neighboring cells—a vital step in adaptation, development, and survival within populations. Although most of our knowledge on cellular chemical communication comes from studies of multicellular eukaryotes, it is now understood that bacteria can also communicate using sophisticated signaling systems, in a way analogous to those used by higher organisms. Many of these microbes live in close association with higher eukaryotes, in mutualistic or commensal relationships. We suggest that there may be a wealth of unidentified bioactive small molecules in the human body, originating from both microbial and human cells and that have important biological functions. Because chemical signaling has important roles for the biology of both microbes and humans, detecting, identifying, and studying these chemical signals can further our understanding of the chemical interplay between microbiota and their hosts and provide us with an unexplored source of molecules that could be used for human benefit.

### Microbial chemical signaling

Following the landmark discovery of the structure of DNA in 1953, much of biological research shifted from an organismal to a molecular perspective. In the past few decades, a great deal has been learned about the role played by macromolecules—not only DNA, but also RNA and proteins—in biological systems. Coincidental to these studies was the discovery that many important biological functions are associated with molecules that are not categorized in any of the above molecular groups [1-5]. Such compounds have been referred to as “small molecules”, a general term that is usually associated with any compound of molecular weight around or under 3000 Da and with chemical characteristics that preclude their description as DNA, RNA, or proteins. Small molecules have critical biological functions in humans: they control immune functions, the development of sexual characteristics, stress responses, metabolism, and mineral balance, amongst others [6-9].

In higher organisms, these small molecules are called hormones—from the Greek for “excite” or “arouse”—a term coined in 1905 by Ernest Starling [10]. They are produced by one organ of the body and travel to distant organs to exert physiological effects.

Although much of our knowledge about chemical signaling comes from the study of mammalian and plant hormones, it is now known that bacteria can also produce, sense, and respond to small-molecule signals that allow them to act coordinately. Studies conducted throughout the 1960s by the research groups of Alexander Tomasz on the acquisition and incorporation of foreign DNA by *Streptococcus pneumoniae* and Woodland Hastings on *Vibrio fischeri* luminescence led to the discovery that self-produced diffusible molecules played important roles in the lifestyle of these microbes [11-14]. Although the consequences of such discoveries were not fully appreciated at the time, they formed the foundation

for studies of bacterial communication. It is now widely accepted that many bacterial species use small chemical compounds to communicate with each other and their hosts. These hormone-like molecules are usually produced at a low level and exert their effects when they reach a threshold concentration, allowing bacteria to sense and respond to their populational density [15-18]. Because this phenomenon is dependent upon a threshold cell density, bacterial communication has been termed quorum sensing [19]. Nowadays, there are many known classes of bacterial signaling molecules, such as the acyl-homoserine lactones, peptides, quinolones, and  $\alpha$ -hydroxyketones, among others [15-18,20]. The chemical repertoire used by bacteria to communicate is diverse and new signaling molecules continue to emerge.

### Chemical signaling in complex environments

Studies of bacterial signaling have focused mostly on laboratory-grown, pure cultures of microorganisms. However, this is an artificial setting; in the environment and in their hosts, microbes live in association with a multitude of other species and are constantly presented with opportunities for competition and cooperation. For example, at elevated "unnatural" concentrations, we know that microbial signaling molecules can have antimicrobial properties. However, when these "antibiotics" are produced by microbes in the environment, they are unlikely to be present at concentrations high enough to exert antimicrobial activity, so it is probable that their main biological function is to modulate bacterial gene expression rather than to poison [21,22]. Indeed, chemical signaling has been shown to be an important facet of microbial interactions in the soil environment, and examples of signaling between different microbial species as well as between microbes and plants, both in symbiosis and pathogenesis, exist. In the  $N_2$ -fixation-driven symbiosis between *Rhizobium* and its legume host, many chemical signals act to promote the establishment of a mutually beneficial relationship [23-26]. The bacteria can sense plant-produced small molecules; the root exudates containing flavonoids induce microbial migration to the root surface. Here, quorum sensing occurs through the production of acyl-homoserine lactones, culminating in the production of nodulation factors (made up of lipochito-oligosaccharides) by the bacteria, which induce nodule formation in the plant host [23-26]. Although there is a wealth of information about chemical signaling in soil, many other complex microbial populations exist in nature, and it is certain that microbial signaling plays important roles in these communities.

### The mammalian gut as an environment for extensive chemical signaling

At birth, humans are colonized by complex communities of microbes. These communities, which are established within the first year of life, have been termed microbiota, microflora, or microbiome and are extremely rich, containing upwards of  $10^{14}$  cells [27-29]. These populations are normally harmless; in fact, they are essential to our health. Microbes colonize our skin, gastrointestinal, genitourinary, and respiratory tracts. It has been estimated that the number of microbes in and on our bodies exceeds our own cells by more than one order of magnitude [27-29]. Even more strikingly, it has been suggested that the collection of microbial genes in our bodies exceeds our own genes by a factor of 100, which means that the human genome is predominantly prokaryotic [30,31]! Although virtually every body surface that is exposed to the environment contains microbes, the gastrointestinal tract is by far the most heavily colonized site. Each individual carries an estimated 1000 distinct bacterial species in their gut [32] and the collective human microbiome has been estimated to contain 35,000 bacterial species or more [33]. This offers a tremendous opportunity for the evolution of multiple microbe-microbe and host-microbe interactions, many of which are conveyed through the activity of small signaling molecules.

Although it has been known for a while that commensal organisms can use diffusible signals to interact with their hosts, as yet, only a few defined examples of such microbe-microbe and host-microbe interactions in the mammalian gastrointestinal tract have been shown to exist. For example, *Bacteroides thetaiotaomicron*, a prominent member of the human gastrointestinal tract microbiome, produces signals that can control host gene expression and epithelial surface glycosylation [34,35]. By doing so, *B. thetaiotaomicron* controls the availability of nutrients in its surroundings to favor its own growth. More recently, this intestinal commensal has also been shown to communicate with pathogens by producing a yet unidentified signal that can control virulence factor production by enterohemorrhagic *Escherichia coli* [36]. This suggests that colonization by this species of bacteria, and potentially others, may be an important tool used by mammals to control infection by virulent bacteria.

In addition to those produced by the microbiota, host-produced small molecules can have profound effects on both commensals and pathogens. The mammalian hormones epinephrine and norepinephrine have been shown to affect commensal microbial populations in the gastrointestinal tract and can also influence the production of

virulence factors by invading pathogens [37-39]. For instance, enterohemorrhagic *E. coli* can sense intestinal epinephrine and norepinephrine, causing it to activate its type-III secretion virulence system [38]. Also, *Campylobacter jejuni* can respond to norepinephrine by upregulating its ability to enter into (invade) host cells [39]. These examples illustrate that microbial signaling does occur in the human body and is required in a number of critical processes.

Although these examples indicate that the intestinal microbiota is involved in numerous biological processes localized to the gastrointestinal epithelium and lumen, it is now well established that the microbiota has an impact on host tissues and organs with which it is not in direct contact. This is exemplified by the role of the intestinal microbiota in several pathological processes of the underlying gastrointestinal immune system, as well as those affecting remote tissues and organs. Intestinal commensals can impact inflammatory bowel disease, diabetes, asthma, obesity, cancer, and even depression [27,40-51]. In most cases, the molecular mechanisms of microbial involvement in these diseases are not known, but because of the important role played by microbial signaling in host functions in the intestinal tract, it is highly likely that microbial chemical signals play a role in some or all of these diseases. The fact that the organs affected by these diseases are not in direct contact with gut commensals suggests that diffusible compounds may be involved (Figure 1). Wikoff *et al.* [52] have recently shown that intestinal microbes can have a significant impact on the levels of certain mammalian blood metabolites, suggesting that the influence of gut microbes on the human body may be largely dependent on the activities of small molecules that are able to act at a distance. Additionally, associations between intestinal small molecules and microbiota-associated pathologies have been described. Through a metabolomics study of the fecal contents of human twins, Jansson *et al.* [53] found that changes in the levels of many microbial small molecules are associated with disease status when they compared healthy subjects with inflammatory bowel disease patients. Additionally, they also found that levels of specific groups of commensal microbes correlated with levels of the metabolites affected. We are only beginning to realize that there is a rich and complex mixture of molecules that exert important effects on all the organisms involved, inside and outside of the intestinal ecosystem. Studying this complex chemical lexicon will be essential to a complete understanding of the relationships between microbes and humans.

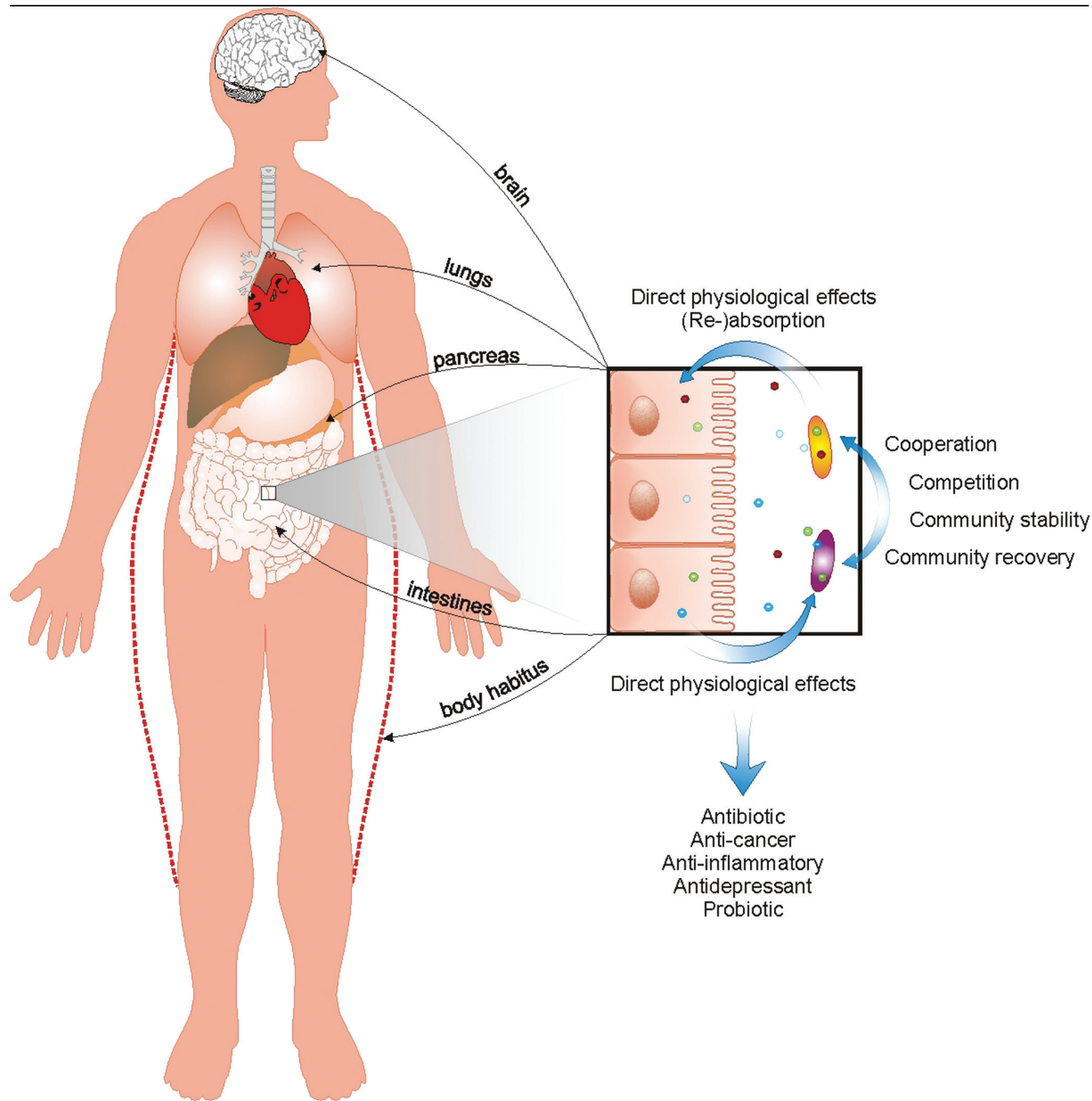
### The intestinal metabolome

The majority of the microbial species present in the mammalian gut cannot be cultured in the laboratory.

This has forced the use of culture-independent methods to study the composition of microbial communities in and on humans. Most studies rely on metagenomics, the unbiased sequencing of all the DNA fragments isolated from a mixed microbial population [54-56]. More recently, the intestinal environment has been studied through other culture-independent methods such as metatranscriptomics and metaproteomics [57,58], which focus on the unbiased analysis of messenger RNA and proteins, respectively. Altogether, these explorations of the microbial diversity in the gastrointestinal tract suggest that there is significant phylogenetic diversity that remains to be explored [54,59]. Although these studies have provided much information about the composition of microbial communities in the mammalian gut, they tell us very little about the functions of the components of the system or the interactions between them, and it is only recently that we have begun to decipher the molecular functions of these assemblages. However, based on the overwhelming amount of genetic material present in the human gut metagenome (the combination of all genes present in the gastrointestinal tract), we can predict that many biological reactions with the potential to be immensurable sources of bioactive small molecules are yet to be discovered.

In addition, as the mammalian gut is an important component in the host's excretion of metabolic "waste" (i.e., the unwanted products of metabolic reactions), many host-produced small molecules are also found in this environment. Therefore, the gastrointestinal tract is loaded with small molecules, from both the host and the microbiota, that could have a significant impact on the gastrointestinal tract itself and other organs through re-absorption. This chemical lexicon—the metabolome—is mostly unknown, mainly due to the lack of appropriate techniques to study its composition. However, as a result of recent advances in methods of chemical separation and structural elucidation, particularly in methods for high-throughput analysis of complex samples, we now have tools to probe the chemical conversations that we did not even know existed a few years ago. One such technique is the extremely sensitive and accurate Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS), which has been recently used with direct infusion mass spectrometry to study the metabolome of both human and murine plasma [60]. FTICR-MS allows the rapid detection and relative quantification of thousands of molecules in complex biological matrices. In many cases, the accuracy of the mass determination is sufficient to allow metabolite identification based on mass alone. We have used FTICR-MS to study the intestinal metabolome and found that thousands of small molecules are present in the mammalian gut, the majority of which is affected by antibiotic treatment, thus suggesting that the intestinal

Figure 1. Potential roles and applications of small molecules in the intestinal tract



Small molecules are produced in the intestine by both host and microbial cells. Microbial molecules can exert direct effects on host cells and vice-versa. Additionally, the molecules can play an important role in interactions between different microbial components in the intestinal ecosystem; they can be used for cooperation, maintenance of community stability or recovery after an insult, and competition. The molecules can also be absorbed into the intestinal epithelium. This is true for newly synthesized microbial molecules or recycled host molecules, which can be excreted in the intestinal lumen and reabsorbed. Such molecules can reach the bloodstream and exert effects on remote organs such as the brain, lungs, and pancreas, as well as other intestinal sites. They can also affect energy balance and impact obesity and other diseases of the organs mentioned above (autism, depression, allergy, diabetes, inflammatory bowel disease, and so on). Once harvested and studied, these compounds can be used for a multitude of purposes; they can serve several therapeutic roles as antibiotics, anti-cancer therapies, anti-inflammatories, antidepressants, and probiotics, amongst others.



microbiota has a major impact on the chemical composition of the intestinal environment (Antunes and Finlay, submitted for publication). Metabolic studies of samples from healthy and disturbed intestinal ecosystems will allow us to predict causative associations between chemical variations and specific disease states. Hypotheses can then be tested using small molecules (natural or synthetic) to treat conditions in animal models.

### Exploring the intestinal metabolome for bioactive molecules

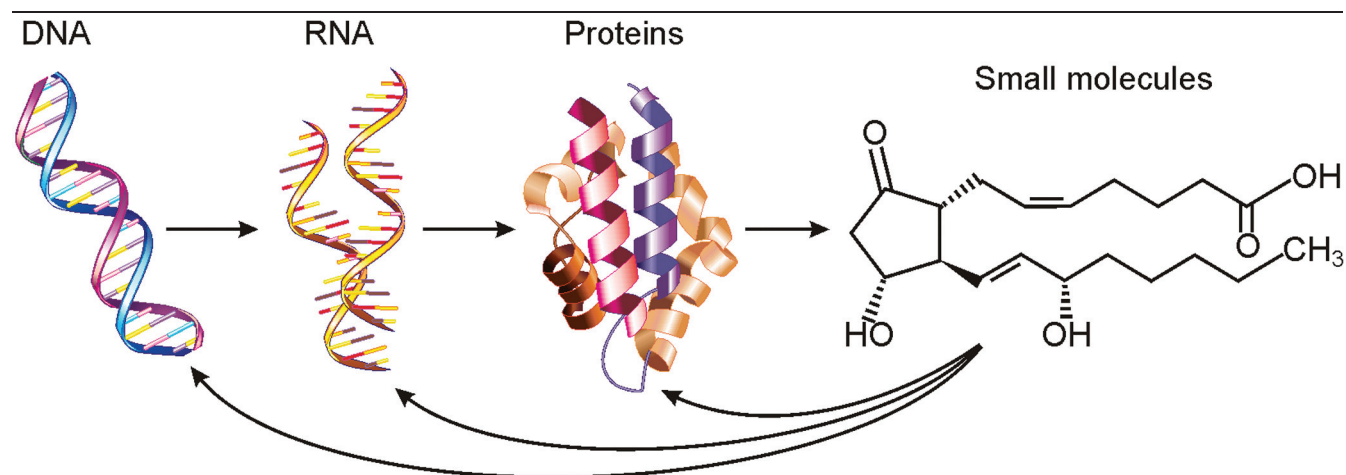
The study of natural biological products from a diverse array of organisms has provided us with a wealth of information on the important roles played by small molecules in biological systems. Additionally, whenever possible, humans have taken advantage of these products for their therapeutic activities (e.g., in antibiotic, anticancer, and anti-inflammatory compounds) [61-63]. Most have been isolated from microorganisms, but some also come from plants and marine organisms. The discovery that the human body is made up of extremely complex ecosystems suggests that it, too, could be used as a rich source of new bioactive molecules. Small molecules in the mammalian gastrointestinal tract are likely to have important functions in the relationship between hosts and their commensal microbes. Since these populations play a key role in human health and disease, the molecules involved may have potential as therapeutics aimed at maintaining or reestablishing homeostasis to prevent or cure diseases. Such activities may involve antibiosis, the capacity to kill other microorganisms,

which could be explored not only in the context of intestinal infections but also for numerous other infections throughout the body. These molecules will also affect host biology, possibly at the interface between microbial populations and the gut-associated immune system. It is known that the microbiota exerts important effects on the maturation of the mammalian immune system, so the small molecules in the intestine could be used to modulate these relationships in controlled ways. An even more daring possibility is that some of these molecules could be used to manipulate the physiology of remote organs and systems. As mentioned previously, the intestinal microbiota can impact diseases such as allergies, diabetes, asthma, and depression, and there is increasing evidence that it is involved in mental development. Therefore, it is possible that the molecules produced by some of the microbes associated with protection against these diseases could be used to remediate or prevent them.

### It's a small-molecule world

Since its discovery, DNA has been considered the foundation of life. Its capacity to store information coupled with its remarkable stability make it the prime candidate for the molecule from which life, as we know it, originated. This concept is imprinted in the "central dogma", which states that DNA holds all genetic information, which is passed on to RNA as a messenger molecule and then translated into proteins, which constitute the machinery and structures that carry out the molecular processes essential for life (Figure 2).

**Figure 2. Small molecules as important messengers of biological information and function**



DNA encodes the genetic information that is passed on to RNA, which acts as the messenger for the synthesis of proteins. Protein enzymatic function can then give rise to a plethora of structurally diverse small molecules. In many cases, these molecules are the primary effectors of biological functions, acting at the DNA, RNA, and protein levels.

Although generally accepted, this viewpoint has been challenged. In 1986, Walter Gilbert suggested that RNA preceded DNA as a self-replicating primitive form of life, giving this molecule a main role in the formation of life [64]. Indeed, RNA molecules with enzymatic functions still exist [65]. Although both DNA and RNA have central functions in the maintenance and decoding of genetic information, the real effectors of these functions are proteins. In the case of structural proteins, they represent the end of the road for a given biological property or function. However, for the majority of proteins, catalytic activity is the main function, thus extending their biological properties to the products of the reactions catalyzed: a plethora of structurally diverse small molecules. It is, therefore, these small molecules that constitute the *raison d'être* of biological function in most cases. Without identifying and studying these molecules, we will not fully understand the functions of metabolic pathways and the interconnections between them. Nor will we be able to fully comprehend the complexities of any biological system. We now have the tools to delve into the unexplored sources of many intriguing molecules in our own bodies. This should be done not only with an intellectual view toward understanding the molecular intricacies of life in more detail but also with a practical view of benefiting from what these molecules may have to offer.

### Abbreviation

FTICR-MS, Fourier transform ion cyclotron resonance mass spectrometry.

### Competing interests

The authors declare that they have no competing interests.

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