Trends in Microbiology

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Review

Roles of Indole as an Interspecies and Interkingdom Signaling Molecule

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A number of bacteria, and some plants, produce large quantities of indole, which is widespread in animal intestinal tracts and in the rhizosphere. Indole, as an interspecies and interkingdom signaling molecule, plays important roles in bacterial pathogenesis and eukaryotic immunity. Furthermore, indole and its derivatives are viewed as potential antivirulence compounds against antibiotic-resistant pathogens because of their ability to inhibit quorum sensing and virulence factor production. Indole modulates oxidative stress, intestinal inflammation, and hormone secretion in animals, and it controls plant defense systems and growth. Insects and nematodes can recognize indole, which controls some of their behavior. This review presents current knowledge regarding indole and its derivatives, their biotechnological applications and their role in prokaryotic and eukaryotic systems.

Intercellular, Interspecies, and Interkingdom Signaling by Indole and Its Derivatives

Bacteria and eukaryotes coexist in the natural environment, and bacterial metabolites modulate eukaryotic immunity. The commensal bacteria colonize the surfaces of animal and plant cells and prevent invasion by pathogenic bacteria [1]. In fact, an imbalance between commensal (or symbiotic) and pathogenic bacteria can result in life-threatening infections. Bacteria produce a wide variety of signaling molecules, siderophores, and antibiotics. Other organisms can sense these signaling molecules, which synchronize multicellular behaviors, such as virulence, biofilm formation, and antibiotic resistance. Also, bacterial signaling molecules can be manipulated and utilized by animal and plant cells [2].

Indole is synthesized from tryptophan by tryptophanase (TnaA) in a large number of Gram-positive and Gram-negative bacterial species (Figure 1). Extracellular indole concentrations in liquid cultures of *Escherichia coli* and *Vibrio cholerae* can reach 0.5 mM [3,4], and indole concentrations of up to 1.1 mM are produced by indole-producing bacteria in the mouse, rat, and human gut [5,6]. Indole is a well-known signaling molecule that modulates spore formation by Gram-positive strains [7,8]; plasmid stability [9], cell division [10], antibiotic tolerance [11,12], and virulence [13,14] in *E. coli;* and biofilm formation by *E. coli* [3,15] and *V. cholerae* [4]. As an interspecies signaling molecule, indole has recently shown diverse roles in various non-indole-producing bacteria (Table 1).

Several plants, including maize, are able to produce indole using indole-3-glycerol phosphate lyases [16] (Figure 1). Furthermore, maize releases volatile compounds, including indole, in response to herbivore attack [17,18]. In contrast, animals cannot synthesize indole but can sense indole using their olfactory systems [19,20]. Insects, such as mosquitoes and butterflies, sense indole, which then controls some of their behavior [21,22]. More recently, several studies

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A variety of bacteria, and some plants, produce large quantities of indole, and thus, indole and its derivatives are widespread in prokaryotic and eukaryotic communities. Recently, indole was shown to be an intercellular, interspecies, and interkingdom signaling molecule.

Indole and its derivatives can suppress the bacterial pathogenesis of several antibiotic-resistant pathogens by inhibiting quorum sensing and virulence factor production.

Insects sense indole, which controls their behavior. Furthermore, indole controls plant defense systems and growth, and modulates oxidative stress, intestinal inflammation, and hormone secretion in animals. Emerging data suggest that indoles may influence human diseases, such as inflammatory, neurological, and metabolic diseases.

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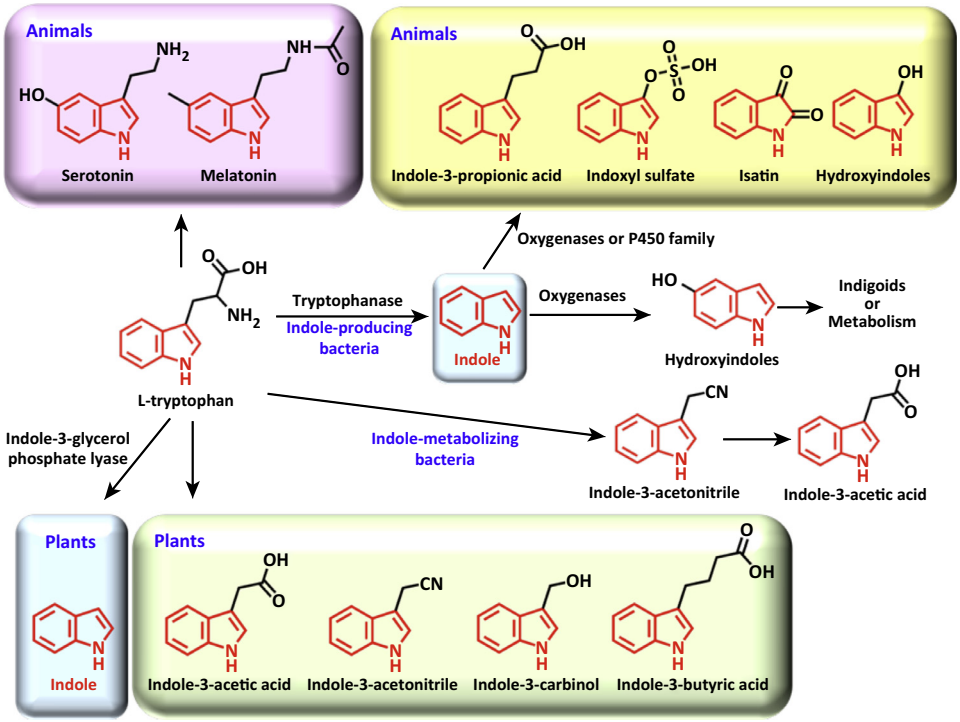

Figure 1. Biosynthesis of Indole and Its Derivatives in Prokaryotes and Eukaryotes. Indole is produced from amino acid L-tryptophan by tryptophanase (TnaA) in various indole-producing bacteria. Some plants, including maize, synthesize indole using indole-3-glycerol phosphate lyases, but animal cells cannot synthesize indole. Indole can be oxidized to hydroxyindoles by diverse oxygenases produced by non-indole-producing bacteria or eukaryotes. Hence, indole and its derivatives are widespread in prokaryotic and eukaryotic systems. In addition, hormone tryptophan-derivatives, such as serotonin, melatonin, indole-3-acetic acid, indole-3-acetonitrile, indole-3-carbinol, and indole-3-butyric acid, possess a core indole moiety.

have suggested that indole may have several beneficial effects on human health by influencing oxidative stresses [23], intestinal inflammation [5,24], and hormone secretion [25].

Indole is stable in indole-producing bacteria, but many non-indole-producing bacteria and eukaryotes can modify or degrade indole using diverse oxygenases, such as monooxygenases, dioxygenases, and P450 family members [23,26]. Hence, indole derivatives are widely present in prokaryotic and eukaryotic communities (Figure 1). However, little is known of the biological role, metabolism, or mechanisms of action of indole derivatives.

A small number of reviews have summarized the biological roles of indole, mostly in indole-producing bacteria [27,28], or the approaches used to synthesize indole-based small molecules [29,30]. In this review, we discuss current knowledge of indole and its derivatives in indole-producing bacteria, in non-indole-producing pathogenic bacteria, and in eukaryotic systems (such as animals, plants, insects, and nematodes); potential biotechnological applications of indoles are also discussed.

Functions and Applications of Indole and Its Derivatives in Indole-Producing Bacteria

Indole biosynthesis and its regulation were well studied during the last century, and its biological functions have been extensively investigated during the last decade, mostly in indole-producing bacteria [3,27]. Indole is considered by some authors to be an intercellular signaling molecule,

Table 1. Roles of Indole and Its Derivatives in Non-Indole-Producing Microorganisms

Bacteria	Indole Compound	Phenotypic Change	Mechanism	Refs
Acinetobacter baumannii, Staphylococcus aureus	Indole-derived flustramine, pyrroloindoline triazole amides	Inhibition of biofilm formation	Unknown	[46]
Acinetobacter oleivorans	Indole	Inhibition of biofilm formation and motility	Inhibition of QS[a] regulator folding	[54]
Agrobacterium tumefaciens	Indole	Increase of biofilm formation and antibiotic tolerance	Change of biofilm-, stress-, and efflux-related genes	[65]
Bdellovibrio bacteriovorus	Indole	Reduction of predation	Downregulation of flagellar and ribosome assembly genes	[60]
Burkholderia unamae	Indole and gallic acid	Induction of biofilm formation	Unknown	[59]
Candida albicans	Indole and 3-indolylacetonitrile	Reduction of biofilm formation and virulence	Stimulation of transcriptional factor	[56]
Chromobacterium violaceum, Pseudomonas chlororaphis, Serratia marcescens	Indole	Inhibition of QS-regulated pigmentation	QS inhibition	[55]
Cyanobacterial *microsystis aeruginosa*	Indole	Inhibition of cyanobacterial blooms	Formation of periphyton biofilms	[58]
Cylindrotheca sp.	Indole and its derivatives	Inhibition of growth and biofilm formation	Induction of cellular Ca^{2+} efflux	[66]
Pseudomonas putida	Indole	Increase of antibiotic tolerance	Activation of efflux pump	[64]
Pseudomonas aeruginosa	Indole, 3-indolylacetonitrile, 7-fluoroindole, indole-based *N*-acylated L-homoserine lactones	Inhibition of virulence factor production	QS inhibition	[42,48, 51–53,69]
Pseudomonas aeruginosa	Indole, 7-hydroxyindole	Increase of antibiotic tolerance	Activation of efflux pump	[48,53]
Pseudomonas aeruginosa	2-Aminobenzimidazoles	Inhibition of biofilm formation	Partly by QS inhibition	[68]
Salmonella enterica serovar Typhimurium	Indole	Increase of antibiotic tolerance	Oxidative stress response	[61]
Salmonella enterica serovar Typhimurium	Indole	Increase of antibiotic tolerance and decrease of motility	Activation of efflux pump and suppression of flagellar genes	[62,63]
Staphylococcus aureus	Indole and 7-benzyloxyindole	Attenuation of virulence	Reduction of staphyloxanthin and hemolysin	[57]
Staphylococcus aureus	Desformylflustrabromine	Inhibition of biofilm formation	Unknown	[45]

[a]QS, quorum sensing.

like a quorum-sensing (QS) signal [11,12,31], and it controls diverse aspects of bacterial physiology [27].

Recently, indole was shown to modulate antibiotic resistance and persister formation in *E. coli*. Indole induces population-based antibiotic resistance in *E. coli* primarily via drug efflux pump and protective mechanisms against oxidative stress [11]. On the other hand, *E. coli* increases the biosynthesis of indole in the presence of antibiotics [32]. Indole also increases the formation of antibiotic-tolerant persister cells, and the process involved is possibly linked to oxidative stress and phage shock pathways [12], which supports previous results that indole increases antibiotic resistance via multidrug transport [11,33,34]. In contrast, it has also been reported that indole decreases persister formation via phosphodiesterase DosP, which reduces cyclic adenosine monophosphate concentrations that are required to activate TnaA [35], and via the toxin protein YafQ, which cleaves TnaA mRNA [36]. The discrepancy in the role of indole and persistence is probably attributable to the different persister assay conditions used in the studies.

Indole at high concentrations (above 1 mM) prevents *E. coli* cell division by modulating membrane potential [10]. This effect is in line with the finding that indole is a proton ionophore, which further suggests that gut epithelial cells might be directly affected by bacterial indole [37]. It was initially believed that the AcrEF-TolC and Mtr transporter proteins are solely involved in the export and import of indole. However, it was recently found that indole passes unassisted across the cell membrane independently of AcrEF-TolC and Mtr in *E. coli* [38]. Interestingly, a transient pulse of intracellular indole (>50 mM) was observed in *E. coli*, which supports its ionophore-mediated effects on *E. coli* cell division and growth [39].

Although *E. coli* and *V. cholerae* strains were the major subjects of indole studies, the roles of indole are now beginning to be revealed in other indole-producing strains; in fact, more than 85 bacterial species are now known to produce indole [27]. The expression of indole-producing tryptophanase has been reported to increase biofilm formation and antibiotic resistance in the marine pathogen *Edwardsiella tarda* [40], whereas indole was found to decrease biofilm formation by, and the virulence of, the marine pathogen *Vibrio anguillarum* [41]. Several Gram-positive bacteria also produce indole, which inhibits antibiotic-resistant spore formation by *Paenibacillus alvei* [8] and *Stigmatella aurantiaca* [7]. It should be noted that the effects of indole on biofilm formation, virulence, and other phenotypes are usually strain-, concentration-, and condition-dependent (e.g., medium and temperature).

Indole derivatives are also widespread in nature. Some indole derivatives, such as, 4-fluoroindole, transit lipid membranes faster than does indole [37], and the substitution of functional groups on the indole moiety modulates biofilm formation by *E. coli* [31]. In addition, the plant auxin 3-indolylacetonitrile [42], indole-3-acetaldehyde (from a *Rhodococcus* sp. strain) [43], and skatole [44] inhibit biofilm formation by enterohemorrhagic *E. coli* O157:H7 and spore maturation in *P. alvei* [8] more so than indole. Also, indole, indole-3-carboxaldehyde, and indole-3-acetic acid in combination in enteropathogenic *E. coli* are killing factors for nematodes [13]. Moreover, various synthetic indole-based molecules, such as desformylflustrabromine [45] and pyrroloindoline triazole amides [46], may inhibit *E. coli* biofilm formation more than indole, which suggests that indole derivatives affect bacterial pathogenicity and antibiotic resistance.

Antivirulence Activities of Indoles against Non-Indole-Producing Microorganisms

In the natural environment, most bacteria survive in multispecies communities, and thus, compete for resources and space, but they are also able to interfere with, or utilize, signaling molecules from other bacteria. Indole is an interspecies signaling molecule; in fact, it was initially reported that indole stimulates biofilm formation by *Pseudomonas aeruginosa* [3], which is

probably due to the degradation of anthranilate [47]. Indole also downregulates *P. aeruginosa* virulence and QS-regulated phenotypes by altering gene expression in a manner contrary to that of acylhomoserine lactones (AHLs) [48]. Many studies have since reported that indole controls various bacterial phenotypes and that it possesses antivirulence properties against non-indole-producing pathogenic bacteria (Table 1). Possible antivirulence approaches include the inhibition of bacterial QS, biofilm formation, toxin production, and of diverse bacterial adhesive factors [49].

Bacteria produce a variety of QS molecules, such as AHLs, autoinducers (AIs), and oligopeptides, and use these QS molecules for social adaptation, virulence production, biofilm formation, and antibiotic resistance [50]. The inhibition of AHL-mediated quorum signaling by indole is widespread in bacteria, including *P. aeruginosa* [48,51–53], *Acinetobacter oleivorans* [54], *Chromobacterium violaceum*, *Pseudomonas chlororaphis*, and *Serratia marcescens* [55] (Table 1). These results suggest that indole has considerable potential as a QS-quenching agent and as an antivirulence compound against pathogenic bacteria.

Indole can attenuate the virulence of non-indole-producing bacteria by inhibiting cell attachment and toxin production. For example, indole inhibits the attachment of the fungal pathogen *Candida albicans* to intestinal epithelial HT-29 cells [56] and reduces the production of the virulence factor staphyloxanthin in *Staphylococcus aureus* [57]; both of these studies demonstrated virulence reduction in a *Caenorhabditis elegans* model [56,57]. Indole also inhibits cyanobacterial blooms caused by the formation of periphyton biofilms [58], induces biofilm formation by rhizospheric *Burkholderia unamae* [59], and delays predation by *Bdellovibrio bacteriovorus* [60]. It would be interesting to determine whether indole affects the virulence of other pathogens as well as those of environmental bacteria.

Indole is known to affect the antibiotic resistance of indole-producing *E. coli*, and it can also modulate the antibiotic resistance or tolerance of other non-indole-producing pathogens. For example, *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) intercepts indole signaling to enhance its antibiotic tolerance by increasing its oxidative stress response [61]. Alternatively, the drug tolerance phenotype of *S.* Typhimurium has been proposed to be due to the increased production of one or more multidrug efflux pumps [62,63]. In fact, indole has been shown to increase the antibiotic tolerances of *P. aeruginosa* [48], the soil bacterium *Pseudomonas putida* [64], and the plant pathogen *Agrobacterium tumefaciens* [65] by modulating efflux pumps. Noticeably, unlike indole, 7-fluoroindole, did not increase antibiotic tolerance but diminished *P. aeruginosa* virulence by repressing hemolytic activity, protease activity, and biofilm formation [53].

Indole is a highly stable chemical in indole-producing bacteria, but it can be degraded or metabolized by many pathogens, such as *P. aeruginosa* [48] and *A. tumefaciens* [65], which represents a limitation to the potential use of indole as an antivirulence drug. It is likely that non-indole-producing pathogens have developed defense systems against indole, which can prevent QS (Table 1). Hence, more potent, stable, non-toxic indole derivatives than indole have been proposed as potential antivirulence compounds. For example, plant-derived indolylacetonitrile reduces virulence factor production in *P. aeruginosa*, in which the compound is stable [42], indolylacetonitrile reduces the virulence of *C. albicans* more effectively than indole [56], 7-benzyloxyindole attenuates the virulence of *S. aureus* more than indole [57], and 6-chloroindole inhibits the growth of, and biofilm formation by, algal *Cylindrotheca* sp. by inducing Ca^{2+} efflux [66].

The inhibition of QS, and of the virulence of several pathogenic bacteria by indole (Table 1), suggests that natural and synthetic indole derivatives might more potently alleviate bacterial virulence. For example, indole-derived flustramine and pyrroloindoline triazole amides inhibited biofilm formation by *Acinetobacter baumannii*, *E. coli*, and methicillin-resistant *S. aureus* more

efficiently than did indole [46,67] and 2-aminobenzimidazole inhibited biofilm formation by *P. aeruginosa* [68]. In another study, the brominated furanone motif and indole were combined to produce desformylflustrabromine, which inhibited biofilm formation by *S. aureus* and *E. coli* [45], and the *N*-acylated L-homoserine lactone motif and indole were combined to inhibit *P. aeruginosa* QS [69]. Recently, two review papers described indole-containing small molecules that modulate bacterial behaviors and the synthetic approaches they used [29,30]. Since thousands of natural and synthetic indole derivatives have been identified, it would be interesting to investigate indole structure–activity relationships and determine how indole derivatives affect bacterial pathogenicity and antibiotic resistance.

The Impact of Indole on Human Diseases

The human intestinal tract is rich in a diverse range of about 10^{14} commensal bacteria, some of which are crucial for nutrient assimilation and benefit the immune system [70]. Indole has been detected in the mouse and human gut at concentrations ~250–1100 μM, but animal cells cannot synthesize indole [5,6]. Furthermore, gut-bacteria-derived indoles, such as indoxyl sulfate, indole-3-propionic acid, isatin, and 5-hydroxyindole (Figure 2), are present in blood, peripheral tissues, urine, and even brain tissues at concentrations as high as 10–200 μM [23,71,72]. However, their biological roles and targets are poorly characterized. A metabolomic study demonstrated that the production of indoxyl sulfate and the antioxidant indole-3-propionic acid in animal blood completely depended on enteric bacteria [23], and indole and its derivatives may influence human diseases, such as bacterial infections, intestinal inflammation, neurological diseases, diabetes, and cancers (Figure 2).

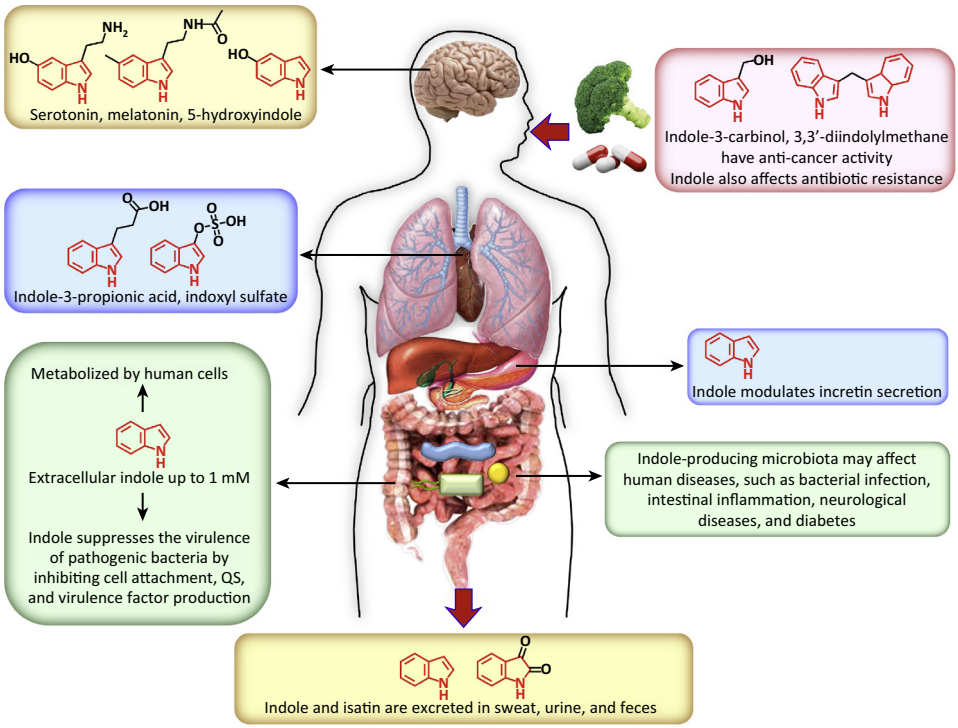

Figure 2. Possible Effects of Indoles on Humans. Indole and its gut-bacteria-derived derivatives, such as indoxyl sulfate, indole-3-propionic acid, isatin, and 5-hydroxyindole, are present not only in the gastrointestinal tract but also in the brain, peripheral tissues, blood, sweat, and urine, and may influence diseases, such as bacterial infections, intestinal inflammation, neurological diseases, and diabetes. Abbreviation: QS, quorum sensing.

In human intestinal epithelial cells, indole and 7-hydroxyindole increase epithelial cell tight-junction resistance and reduce inflammatory cytokine levels [5]. In a germ-free mouse model, oral administration of indole increased the expression of tight and adherens junctions in the colon [24]. These findings suggest that indole and indole-producing bacteria play beneficial roles in the establishment of epithelial barriers and prevent intestinal inflammation. Inflammatory bowel diseases (IBD), such as ulcerative colitis and Crohn's disease, are caused by defects in host immunity and/or alterations in resident bacteria and a weakened epithelial barrier. Furthermore, recent studies have extensively addressed the role of the gut microbiome with respect to IBD [73], increasing the demand for investigatory studies on the roles of indoles and indole-producing bacteria in IBD and the gut microbiome. The chemically induced rat colitis model [74] could be utilized to investigate the anti-inflammatory effects of indole(s) on IBD. Furthermore, metagenomics, metabolomics, metaproteomics, and metatranscriptomics approaches offer the means to extend understanding of the roles of indoles in IBD.

Recently, it was reported that indole modulates the secretion of the incretin peptide GLP-1 by intestinal enteroendocrine L cells [25]; hence, indole or indole-producing microbiota may impact metabolic diseases, such as type 2 diabetes. Notably, glucose repressed indole biosynthesis [75] due to catabolic repression of tryptophanase [76], which suggests that a high sugar diet suppresses indole production and that a high tryptophan protein diet promotes indole production in the human gut [25]. Therefore, we suggest further studies should be undertaken to investigate the roles of indole(s) and indole-producing bacteria *in vivo* with respect to their long-term impacts on metabolic diseases.

Accumulating evidence indicates that gut microbes may be involved in neural development and function [77–79]. Many types of neurotransmitter, such as amino acids, peptides, and monoamines, have been identified, but it is not widely appreciated that commensal bacteria produce neuroactive molecules, such as serotonin, melatonin, γ-aminobutyric acid (GABA), catecholamines, and histamines [77]. Interestingly, serotonin (5-hydroxytryptamine) and melatonin both have indole motifs (Figures 1 and 2), and 5-hydroxyindole and isatin (2,3-indoledione), which are produced by gut bacteria, are present in mammalian brain and peripheral tissues [71,72], though their roles have not been identified. Recently, a few studies have addressed the link between the gut microbiota and the development of neurodegenerative disorders [78,80]. In particular, a metabolomic assessment of plasma showed that the microbiome of germ-free mice exhibited elevated levels of serotonin and the powerful antioxidant indole-3-propionic acid [23]. It would be interesting to investigate the impact of indole(s) and indole-producing bacteria on human behavior and neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases.

Indole-3-carbinol and 3,3′-diindolylmethane from cruciferous vegetables have been shown to be active against several forms of cancer [81], and the National Cancer Institute of the United States has started clinical trials on these two agents in cancer patients. These agents can be administered safely in oral form, in repeated doses, to rodents and humans [82]. Indole-3-carbinol has been shown to regulate cellular signals, including Akt, NF-κB, JNK, MEK, and p53, in various cancer cell lines [83], and 3,3′-diindolylmethane is a potent radioprotector and mitigator that functions by stimulating DNA repair and NF-κB survival signaling [82]. Therefore, indole derivatives are considered to have therapeutic potential for several cancers and should be further investigated.

Roles of Indole in Plants, Insects, and the Soil Environment

Various plants release volatile compounds in response to herbivore insect attack, and some plants, including maize, are able to synthesize indole using indole-3-glycerol phosphate lyases (Figure 1) [16]. In addition, animal feces are indole-rich and indole-producing rhizobacteria are widespread in soils (Figure 3). Herbivore attack activates the expression of indole-3-glycerol

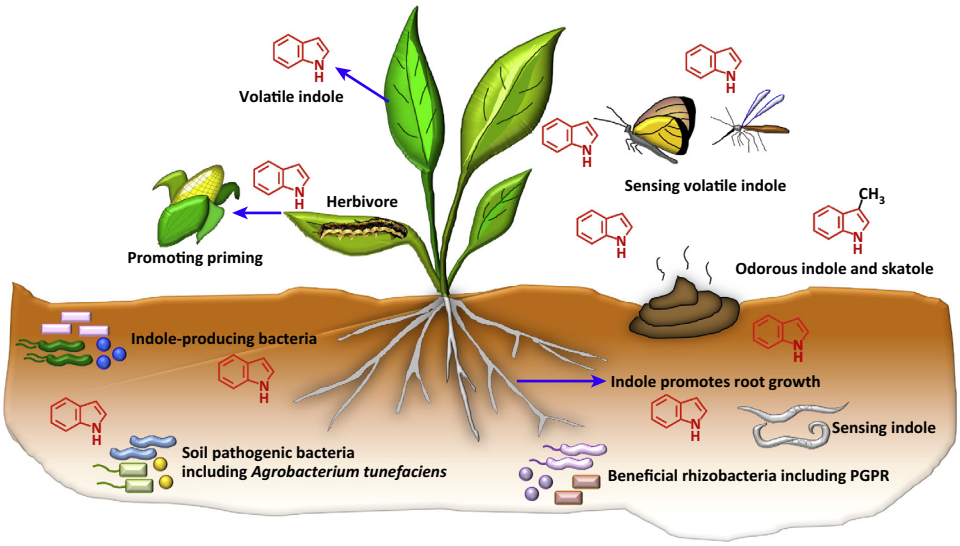

Figure 3. Indole(s) in Plants, Insects, and the Soil Environment. Several plants are able to produce indole, and indole-rich animal feces and indole-producing rhizobacteria are widespread in soils. Indole influences plant defense systems against herbivore attack, and promotes root and plant growth. Insects, nematodes, and animals can sense indole, and indole and skatole are major odorous components in feces. Abbreviation: PGPR, plant-growth-promoting rhizobacteria.

phosphate lyases to enable indole emission by maize [17]. The release of indole is herbivore-specific in maize and is essential for priming maize for further defense against insects [18]. It appears that much remains to be learned of the relationships between plant defense systems and indole(s) and indole-producing bacteria.

Volatile bacterial compounds are important in bacteria and plants. For example, various soil-borne bacterial species were found to produce indole as a potent plant growth modulator [84], and indole was found to promote the root development of *Arabidopsis thaliana* by interfering with auxin signaling that regulates plant growth and behavioral processes [85]. In addition, indole from the rhizobacterium *Proteus vulgaris* increased Chinese cabbage seedling growth [86] and the fresh weight of *A. thaliana* via auxin, cytokinin, and brassinosteroid pathways that are involved primarily in plant growth and differentiation [87]. Since plant auxins, such as indole-3-acetic acid, indole-3-butyric acid, or 3-indolylacetonitrile, possess an indole moiety (Figure 1), it is possible that other indole derivatives might differentially influence plant growth.

Plant-growth-promoting rhizobacteria (PGPRs) promote plant growth, and many microbial plant-growth stimulators are being marketed [88]. To exert their beneficial effects, PGPRs must colonize root surfaces, and simultaneously, colonization by pathogenic bacteria should be prevented. Since indole and its derivatives are well-known bacterial biofilm modulators (Table 1), it would be interesting to investigate the effects of indoles on biofilm formation by PGPRs and plant pathogens. Since some PGPRs probably synthesize indole [84], it would appear that indole might have beneficial effects on PGPRs and plants. However, indole increases biofilm formation and virulence-gene expression by the plant pathogen *A. tumefaciens*, while reducing cell growth and motility [65]. In addition, indole increases the antibiotic tolerance of two rhizobacteria, *P. putida* [64] and *A. tumefaciens* [65]. Therefore, it is important to further understand the role of indoles in the rhizosphere and plants.

Insects, such as mosquitoes and butterflies, are able to recognize indole, which modulates their behavior [21,22,89]. Because indole is a common olfactory cue, indole responsive odorant

receptors are well conserved between species [90]. Furthermore, mosquitoes (e.g., *Anopheles gambiae*) sense human sweat, and indole has been reported to constitute nearly 30% of the volatile headspace of sweat [19]. Indole also plays a role in oviposition for mosquitoes [21]. In the butterfly *Pieris rapae*, indole-3-acetonitrile deters oviposition [22], which suggests that indole and its derivatives influence insect behavior.

Indole also affects the behavior of *C. elegans*, a small free-living soil nematode that feeds primarily on bacteria, such as indole-producing *E. coli*. Furthermore, it has been reported that the virulence of pathogenic *E. coli* toward *C. elegans* requires the bacterial tryptophanase gene to catalyze indole production [91]. Recently, it was confirmed that indole, indole-3-carboxaldehyde, and indole-3-acetic acid in combination can kill *C. elegans* [13]. Also, the neuromodulator melatonin regulates locomotory behavior and homeostatic states [92], and the neurotransmitter serotonin plays an important role in pathogen-induced olfactory learning in *C. elegans* [93]. Because *C. elegans* offers a number of benefits as a model host for studying innate immunity, it is important to understand how *C. elegans* reacts to indoles.

Indole and 3-methylindole (skatole) are major odorous compounds in animal wastes (Figure 3), and tremendous amounts of different antibiotics are administered to farmed animals to prevent bacterial infection and promote growth [94]. Since indole increases the antibiotic resistance of several bacteria [11,48,61,64,65], indole and its derivatives are likely to increase antibiotic use among farmed animals. Furthermore, clean-up technologies are required to remove odorous indoles, and in this context, the use of suitable indole-degrading bacteria is a possibility. Various bacteria have been reported to metabolize indole using dioxygenases and monooxygenases and to produce indole dimers, such as indigoids [26,27]. However, indigoid accumulation is a limitation that prevents the use of such microorganisms to remove the smell of indole *in situ*. Therefore, bacteria capable of utilizing indole as the sole carbon source [95] would provide a better means of completely mineralizing indole so that they can be used in bioremediation of animal and industrial wastes.

Concluding Remarks

Soils and the gastrointestinal tracts of animals contain high levels of indole, and it is now evident that indole and its derivatives are considerably more important as interkingdom signals than was originally believed. As an intercellular and interspecies bacterial signaling molecule, indole plays many roles in bacterial pathogenesis. Recently, studies were commenced on the effects of indole on worms, insects, plants, and animals. Furthermore, indole and its derivatives originating from commensal bacteria may influence human health, but research on these issues is in its initial stages and to date has been limited to cell or animal models.

We are only beginning to understand the effects of indole and indole-producing bacteria in microorganisms and a few eukaryotes and, thus, many more studies on diverse indoles in various prokaryotic and eukaryotic systems can be expected (see Outstanding Questions). Despite the efforts made to elucidate the mechanisms of action of indoles, the genetic and molecular mechanisms of indole signaling remain unclear, partly controversial, and strain specific. Hence, it is important that we understand the mechanisms responsible for the effects of indole and of indole-producing bacteria in different systems. For example, because few indole-binding proteins have been identified [7], the protein, RNA, or DNA sites directly involved in indole signaling in prokaryotic and eukaryotic cells must be identified. Notably, a recent study suggests that the human aryl hydrocarbon receptor has the capacity to bind two indole molecules per ligand binding pocket to facilitate activation [96,97]. In addition, the metabolism and fates of indoles should be investigated in eukaryotic cells, as to date only the hydroxylation and dimerization of indole via oxygenases in bacteria have been studied. Because numerous natural and synthetic indole derivatives and high-throughput screening are readily available,

Outstanding Questions

What are the biological roles of indole and its derivatives in prokaryotic and eukaryotic species, and what amounts of these entities are present in biological systems and in different environments?

What molecular mechanisms are responsible for the effects of indoles? What types of genes and proteins are involved in indole signaling in prokaryotic and eukaryotic cells?

How do the functional groups of indole derivatives modulate bacterial pathogenesis and eukaryotic immunity?

What are the long-term effects of indole and indole-producing bacteria on human health, animals, plants, insects, and the environment?

efforts should be made to identify more potent therapeutic compounds and to determine the nature of the structure–activity relationships. Indole and its derivatives also have potential use for antivirulence agents against bacterial infections and as anticancer agents. However, some indoles may induce antibiotic resistance and biofilm/persister formation of pathogenic bacteria. These contradictory effects should be carefully investigated when one deals with antibiotic-resistant bacteria in medicine and in the environment, aiming to identify beneficial indole compounds without side effects. Also, the toxicity and stability of indoles and their delivery to animal cells should be thoroughly assessed. To date the majority of the studies have focused on the short-term impacts of indoles in single species, and thus, the long-term effects of indoles on eukaryotic species and in different environments require further study. The next few years offer the prospect of substantially expanding the present knowledge of interspecies and interkingdom indole signaling in prokaryotic and eukaryotic systems.

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