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REVIEW PAPER

The roles of auxin during interactions between bacterial plant pathogens and their hosts

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Abstract

Plant pathogens have evolved several strategies to manipulate the biology of their hosts to facilitate colonization, growth to high levels in plant tissue, and production of disease. One of the less well known of these strategies is the synthesis of plant hormones and hormone analogs, and there is growing evidence that modulation of host hormone signaling is important during pathogenesis. Several plant pathogens produce the auxin indole-3-acetic acid (IAA) and/or virulence factors that modulate host auxin signaling. Auxin is well known for being involved in many aspects of plant growth and development, but recent findings have revealed that elevated IAA levels or enhanced auxin signaling can also promote disease development in some plant–pathogen interactions. In addition to stimulating plant cell growth during infection by gall-forming bacteria, auxin and auxin signaling can antagonize plant defense responses. Auxin can also act as a microbial signaling molecule to impact the biology of some pathogens directly. In this review, we summarize recent progress towards elucidating the roles that auxin production, modification of host auxin signaling, and direct effects of auxin on pathogens play during pathogenesis, with emphasis on the impacts of auxin on interactions with bacterial pathogens.

Keywords: Auxin, bacteria, IAA, plant pathogens, signaling, virulence.

Before discussing the roles of auxin during pathogenesis, it is important to begin with an overview of pathogen–plant interactions and an introduction to the key virulence strategies used by pathogenic bacteria. This provides the biological context necessary to appreciate the multiple roles played by auxin during pathogenesis. As is summarized below, to colonize a plant successfully and cause disease, pathogens must suppress basal host defenses and alter the biology of their hosts in order to render plant tissue suitable for supporting pathogen growth. There is growing evidence that production of auxin and/or modulation of host auxin signaling by the pathogen play an important role in these processes.

Overview of plant–pathogen interactions

Despite the fact that plants are exposed to many different microbes in their surroundings, plant disease is an exception rather than the rule. Disease is relatively rare due, in part, to the fact that plants are able to detect potential pathogens in their immediate vicinity and induce basal host defenses that are effective in preventing most environmental microbes from colonizing and causing disease (Jones and Dangl, 2006; Spoel and Dong, 2012). In turn, plant pathogens have evolved a variety of strategies for evading or suppressing basal host defenses, thus making it possible for them to colonize plant tissue (Dou and Zhou, 2012). Once the initial colonization events have been accomplished, successful pathogens must obtain sufficient water and nutrients from the host to support growth to high levels. Ultimately, for most interactions, high levels of pathogen growth result in tissue damage and the development of disease symptoms.

As a group, phytopathogenic bacteria have evolved the ability to colonize all plant tissues including roots, leaves, flowers, fruits, and, in some instances, the vascular system. Many bacterial pathogens colonize the intercellular space, also known as the apoplast, of plant tissues (Alfano and Collmer, 1996; Dou and Zhou, 2012; Faulkner and Robatzek, 2012; Melotto

and Kunkel, 2013). The apoplast is believed to be a less than optimal place for microbes to grow, as it is a relatively nutrient- and water-poor environment (Beattie, 2011; Xin *et al.*, 2016). In addition, the apoplast may contain antimicrobial compounds that either are constitutively produced or are secreted into the intercellular space as part of an induced defense response (Heath, 2000; Wang and Dong, 2011). Thus, to colonize the apoplast successfully, pathogens must be able to detoxify or exhibit tolerance to these antimicrobial compounds, as well as evade or suppress further induction of host defense responses. Finally, in order to grow to high levels within this space, they must obtain sufficient water and nutrients from the plant cells surrounding the infection site.

Plant defense responses

The ability to evade and/or suppress host defense responses is an essential feature of plant pathogens, as plants can recognize potential pathogens and rapidly activate basal defense responses that inhibit tissue colonization. Plants have evolved the ability to recognize common microbial compounds, often referred to as microbe-associated molecular patterns (MAMPs), including flagellin, lipopolysaccharide (LPS), and peptidoglycan (Jones and Dangl, 2006; Antolín-Llovera *et al.*, 2012). Recognition of these MAMPs results in induction of a series of basal defense responses, including a rapid oxidative burst, stomatal closure, accumulation of elevated levels of defense signaling hormones, induction of defense-related genes, and production of antimicrobial compounds and lytic enzymes that act collectively to inhibit the growth of microbes in plant tissues (Jones and Dangl, 2006; Boller and Felix, 2009).

The plant defense hormones salicylic acid (SA), jasmonates (JAs), and ethylene play important roles in regulating defenses against microorganisms. SA plays a central role in defense against biotrophic and hemi-biotrophic pathogens, which colonize living tissue, whereas JAs and ethylene primarily activate defenses that protect against necrotrophs, which rapidly kill plant cells to obtain nutrients. The regulation of plant defenses is quite complicated, and there is abundant evidence for intricate regulatory interactions between SA, JA, and ethylene defense signaling, resulting in what is more accurately referred to as a signaling network (Katagiri and Tsuda, 2010). To make things even more complex, this signaling network can be further modulated by several other hormones, including auxin, abscisic acid (ABA), and gibberellins (GAs). Not surprisingly, plant pathogens have evolved mechanisms for taking advantage of this regulatory crosstalk as a strategy for promoting pathogenesis, for example by producing hormones or other virulence factors that modulate hormone signaling (Spoel and Dong, 2008; Kazan and Manner, 2009; Robert-Seilaniantz *et al.*, 2011*a*; Kazan and Lyons, 2014; Ma and Ma, 2016).

Bacterial pathogens: strategies and virulence factors

Given that bacterial pathogens grow in the extracellular spaces of plant tissues, the vast majority of virulence factors are secreted, either directly into the plant cell cytosol or into the apoplast. These virulence factors fall into five general classes: (i) protein virulence factors, known as 'effectors', that are secreted directly into the plant cell cytosol via a specialized Type III secretion system (T3SS); (ii) cell wall-degrading enzymes; (iii) polysaccharides; (iv) low molecular weight toxins; and (v) plant hormones and hormone analogs (Block and Alfano, 2011; Dou and Zhou, 2012; Faulkner and Robatzek, 2012; Lindeberg *et al.*, 2012). The latter classes of virulence factors are secreted into the apoplast, and either impact plant cells from the outside, or are transported into the plant cell. Of particular interest for the purposes of this review are Type III secreted effectors that impact hormone biology, as well as plant hormones and hormone analogs produced by pathogens.

Type III-secreted effector proteins

Type III-secreted effector proteins are considered one of the most important classes of bacterial virulence factors, as disruption of the T3SS results in loss of pathogenicity for a large number of bacterial pathogens (Alfano and Collmer, 1996). Most pathogenic strains that rely on the T3SS synthesize a large repertoire (often ≥30) of effectors, which have evolved to function inside plant cells where they modulate various aspects of plant cell biochemistry, signaling, and/or physiology. The functions of many effector proteins have been elucidated, and they carry out diverse activities, including interfering with perception of microbial attack, suppressing host defense responses, activating host gene expression, modulating hormone homeostasis (e.g. synthesis, activity, and metabolism) and signaling, and promoting tissue damage and disease symptom development.

In general, the Type III-secreted effectors that modulate hormone homeostasis or hormone signaling are believed to promote infection and disease development by: (i) manipulating regulatory crosstalk between plant defense hormone (e.g. SA, JA, and ethylene) signaling pathways to suppress host defense; (ii) stimulating physiological processes that may render host tissue more suitable for pathogen growth (e.g. release of water or nutrients into the apoplast); or (iii) promoting plant cell growth to stimulate gall, knot, canker, or pustule formation. In some interactions, manipulation of hormone biology might also result in stimulation of plant cell death and the formation of necrotic disease lesions. There are many effectors that impact host hormone biology, including AvrPtoB and HopAM1 which modulate ABA signaling (de Torres-Zabala *et al.*, 2007; Goel *et al.*, 2008), HopQ1, which influences cytokinin signaling (Hann *et al.*, 2014), HopX1 and HopZ1, which effect JA signaling (Jiang *et al.*, 2013; Gimenez-Ibanez *et al.*, 2014), and XopD, which inhibits ethylene production (Kim *et al.*, 2013). Below we discuss two effectors that modulate auxin biology. A more comprehensive summary of Type III secreted effectors and their functions is provided in several recent reviews (Dou and Zhou, 2012; Lee *et al.*, 2013; Buttner, 2016; Toruno *et al.*, 2016).

One of the first Type III secreted effectors demonstrated to modulate host hormone signaling was AvrRpt2, an effector

found in several *Pseudomonas syringae* strains (Whalen *et al.*, 1991). Expression of AvrRpt2 promotes *P. syringae* virulence, either when delivered into plant cells by the pathogen or when expressed in transgenic plants (Chen *et al.*, 2000). The increase in pathogen growth and symptom production stimulated by AvrRpt2 is correlated with both elevated levels of the auxin indole-3-acetic acid (IAA) in the plant and increased auxin sensitivity (Chen *et al.*, 2007). Biochemical studies demonstrated that AvrRpt2 enhances host auxin signaling by stimulating a reduction in the amount of AUX/IAA transcriptional repressor proteins in the plant cell. However, the mechanism underlying this reduction is not clear, as AvrRpt2, which is a demonstrated cysteine protease (Axtell *et al.*, 2003), does not appear to cleave AUX/IAA proteins directly (Cui *et al.*, 2013). Given the recent findings that modulation of auxin levels and signaling in host tissue promotes *P. syringae* pathogenesis (see below), it may not be surprising that this pathogen has evolved mechanisms for modulating auxin signaling and responses in its hosts.

Many *Xanthomonas* spp. strains express a family of Type III-secreted transcription factors known as transcriptional activator-like effectors (TALEs). TALEs are targeted to the plant cell nucleus, where they bind DNA regulatory elements to regulate gene expression (Boch *et al.*, 2009; Bogdanove *et al.*, 2010). In a recent study, PthA2 and PhtA4, two TALEs of the *X. citri* citrus canker pathogen, were shown to upregulate several citrus genes implicated in auxin synthesis, transport, and signaling (Pereira *et al.*, 2014). These findings are reminiscent of earlier observations in the pepper pathogen *X. campestris* pv. *vesicatoria* (*Xcv*) that the TALE AvrBs3 induced expression of auxin-responsive genes, including a group of auxin-induced *SAUR* genes and genes encoding α-expansins (Marois *et al.*, 2002). Presumably, induction of these auxin signaling and cell wall-modifying genes contributes to the plant cell expansion that gives rise to hypertrophy of mesophyll cells in susceptible pepper leaves and the development of pustules associated with citrus canker (Kay and Bonas, 2009).

Synthesis of plant hormones and hormone analogs

Many bacterial plant pathogens synthesize plant hormones, including ethylene (Weingart *et al.*, 2001; Valls *et al.*, 2006), GAs (Lu *et al.*, 2015; Nagel *et al.*, 2017), and auxin (Spaepen and Vanderleyden, 2011; Patten *et al.*, 2013; Duca *et al.*, 2014). In addition, several *P. syringae* strains make coronatine, a structural and functional mimic of the plant hormone jasmonic acid-isoleucine (JA-Ile; Bender *et al.*, 1999; Brooks *et al.*, 2004; Fonseca *et al.*, 2009*a*). In most cases, the ability of pathogenic strains to synthesize these molecules contributes to their virulence.

While this review focuses on auxin, it is helpful to provide a brief overview of the roles of coronatine during pathogenesis, as IAA and coronatine promote virulence via a similar mechanism (suppression of SA-mediated defenses). Coronatine binds to the JA-Ile receptor and activates JA signaling and downstream responses (Fonseca *et al.*, 2009*a, b*; Wasternack and Hause, 2013). JA signaling plays a critical role in many important processes in plants, including growth, development, and defense against herbivores and necrotrophic pathogens (Browse, 2009; Wasternack and Hause, 2013).

Coronatine functions at multiple stages during *P. syrinage* pathogenesis to promote virulence, including: (i) entry into leaf tissue by stimulating the re-opening of stomata that were closed as part of a basal defense response (Melotto *et al.*, 2006); (ii) suppression of SA-mediated defenses to promote colonization of the apoplast (Brooks *et al.*, 2005; Geng *et al.*, 2012; Zheng *et al.*, 2012); and (iii) development of disease symptoms (Brooks *et al.*, 2005; Geng *et al.*, 2014). In all of these roles, the activity of coronatine is dependent upon intact JA perception and signaling in the host (Laurie-Berry *et al.*, 2006; Zheng *et al.*, 2012). Thus, the use of coronatine as a virulence factor to suppress SA-mediated defenses takes advantage of existing antagonistic regulatory crosstalk between the SA and JA defense signaling pathways in the host.

Auxin synthesis by plant-associated bacteria

IAA is the most well-studied form of naturally occurring auxins. In addition to governing many aspects of normal plant development (Woodward and Bartel, 2005; Korasick *et al.*, 2013), IAA plays a role in several plant–microbe interactions. Many plant-associated microbes, including plant growth-promoting rhizobacteria (PGPR), nitrogen-fixing symbionts, and pathogens, produce IAA (Spaepen and Vanderleyden, 2011; Patten *et al.*, 2013; Duca *et al.*, 2014; Yin *et al.*, 2014). Examples of plant-pathogenic bacteria that produce IAA when grown in culture include *X. campestris*, *Pantoea agglomerans* (formerly *Erwinia herbicola*), *Dickeya* spp. (formerly *E. chrysanthemi*), *P. savastanoi*, and several *P. syringae* pathovars (Fett *et al.*, 1987; Glickmann *et al.*, 1998; Manulis *et al.*, 1998; Barash and Manulis-Sasson, 2009; Spaepen and Vanderleyden, 2011; Crepin *et al.*, 2012; Aragón *et al.*, 2014; McClerklin *et al.*, 2017). Here we summarize what is known about the biochemical pathways used by plant-associated bacteria to synthesize IAA, and how genetic analyses of several of these pathways has contributed to our understanding of the role(s) of pathogen-produced IAA during pathogenesis.

Multiple pathways for IAA synthesis utilizing the amino acid tryptophan as a precursor have been described in bacteria (Fig. 1, (Spaepen and Vanderleyden, 2011; Duca *et al.*, 2014). These include the indole-3-acetamide (IAM), the indole-3-acetonitrile (IAN), the indole-3-pyruvate (IPyA), the tryptophan side-chain oxidase (TSO), and the tryptamine (TAM) pathways. As described in more detail below, genetic analyses of the IAM and IPyA pathways have helped elucidate the role of bacterial IAA production in several different plant–microbe interactions (Manulis *et al.*, 1998; Duca *et al.*, 2014). However, genes encoding enzymes that catalyze key steps in several of these pathways have not yet been identified.

The IAM pathway has been mainly observed in plant pathogens that stimulate plant cell growth (e.g. formation of galls and knots), although enzymes involved in this pathway are also encoded in the genomes of some non-pathogenic strains

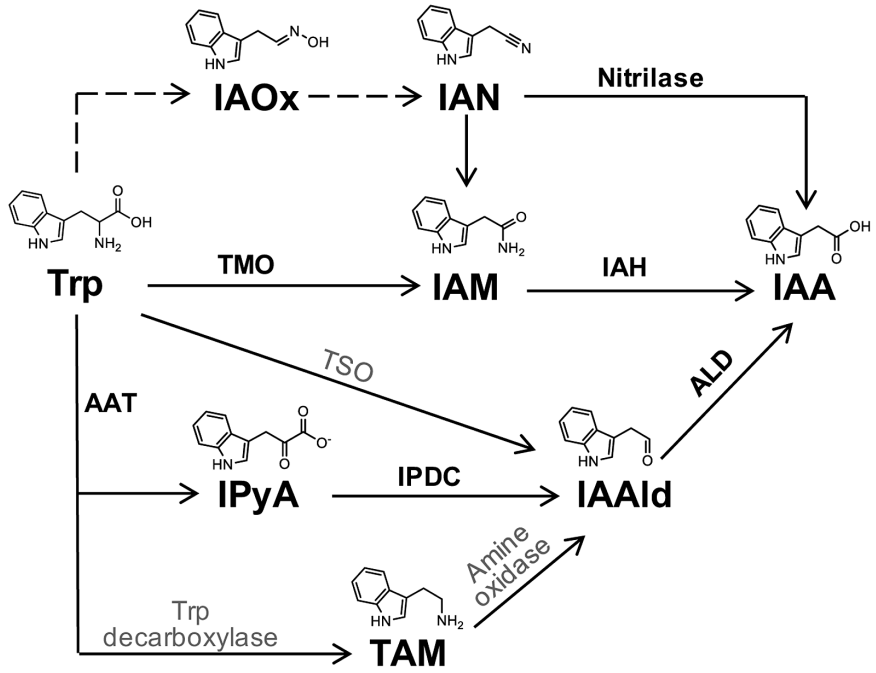

Fig. 1. Overview of IAA biosynthetic pathways found in bacteria. Dashed lines indicate biochemical activities for which microbial enzymes have not been identified. Enzyme activities in bold indicate enzymes for which genes have been identified. Enzyme activities indicated in gray have been detected or proposed, but genes encoding these enzymes have not been reported. Abbreviations for pathway intermediates: Trp, tryptophan; IAOx, indole-3-acetaldoxime; IAN, indole-3-acetonitrile; IAM, indole-3-acetamide; IPyA, indole-3-pyruvate; IAAld, indole-3-acetaldehyde; TAM, tryptamine. Abbreviations for enzymes: AAT, amino acid amino transferases (e.g. *patB*; Shao *et al.*, 2015); TMO, tryptophan 2-monoxygenase; IAH, indole-3-acetamide hydrolase; IPDC, indole-3-pyruvate dehydrogenase; ALD, indole-3-acetaldehyde dehydrogenase [e.g. *aldA* (McClerklin *et al.*, 2017) and *dhaS* (Shao *et al.*, 2015)]; TSO, tryptophan sidechain oxidase. After Spaepen and Vanderleyden (2011) and Patten *et al.* (2013).

that produce IAA (Patten *et al.*, 2013). This is perhaps the most well-known biochemical route for IAA synthesis, as genes encoding the two enzymes for this pathway, *iaaM*/*tms-1* (encoding tryptophan monooxygenase, TMO) and *iaaH*/*tms-2* (encoding IAM hydrolase), are located on the *Agrobacterium* T-DNA that is delivered into the host cell nucleus during genetic transformation (Zupan and Zambryski, 1995) Fig. 1).

Another well-characterized IAA synthesis pathway in plant-associated microbes is the IPyA pathway. Many candidate amino acid amino transferases (AATs), catalyzing the first step of this pathway (conversion of tryptophan to IPyA), have been reported (e.g. *patB*; Shao *et al.*, 2015), and several IPyA decarboxylases (IPDCs) catalyzing the second step (conversion of IPyA to indole-3-acetaldehyde, IAAld), have also been identified and characterized (Patten *et al.*, 2013; Duca *et al.*, 2014). However, genes encoding enzymes for the last step, conversion of IAAld to IAA, have only recently been identified. The *dhaS* gene encoding a potential IAAld dehydrogenase in the PGPR *Bacillus amyloliquefaciens* was identified in a screen for genes potentially involved in IAA biosynthesis (Shao *et al.*, 2015). Transcription of *dhaS* increased 2.5-fold in response to addition of tryptophan to the medium and a *dhaS* deletion mutant produced <25% of wild-type IAA levels. Further, heterologous expression of the *dhaS* gene in a *Bacillus subtilis* strain that normally produces very low levels of IAA resulted in high levels of IAA synthesis. These results suggest that *dhaS* encodes an IAAld dehydrogenase; however, further studies to characterize the biochemical activity of this enzyme have not been reported.

Another IAAld dehydrogenase gene, *aldA*, has recently been identified in the genome of *P. syringae* pv. tomato strain DC3000 (*Pto*DC3000). Mutation of *aldA* in *Pto*DC3000 resulted in a 70–80% reduction in IAA production by cells grown in culture when fed with IAAld (McClerklin *et al.*, 2017). The AldA protein has been purified, and biochemical and structural analyses provide insight into the molecular basis for substrate specificity of this enzyme. The generation of an *aldA* IAA biosynthesis mutant in *Pto*DC3000, a model pathogen strain (Xin and He, 2013), provides a valuable tool for investigating the role of pathogen-derived IAA during pathogenesis (see below).

Less is currently known about the IAN, TSO, and TAM pathways (Fig. 1). A gene encoding a nitrilase that converts IAN to IAA has been identified in *P. syringae* pv. *syringae* strain B728a (*Ps*B728a; Howden *et al.*, 2009), although it is not clear how much this activity contributes to the synthesis of IAA by this strain. *Ps*B728a is also predicted to encode an IAOx dehydratase for generating IAN from IAOx, which the bacteria presumably obtain from their host plant (Howden *et al.*, 2009). The TSO pathway, in which tryptophan is presumably directly converted to IAAld by a monooxygenase, has been reported for several bacteria (Duca *et al.*, 2014), but no specific genes or enzymes have been described or characterized. Tryptophan can also be converted to TAM via the activity of a tryptophan decarboxylase. Enzymes with this activity have been described and characterized in plants, but this activity appears to be rare in bacteria, although it has been observed in some human gut-associated microbes (Williams *et al.*, 2014). The genomes of several *Pseudomaonas putida*

strains are reported to encode enzymes in the TAM pathway, including a putative tryptophan decarboxylase, a putative amine oxidase (converts TAM to IAAld), and several candidate IAAld dehydrogenases (Wu *et al.*, 2011).

Some plant-associated bacteria may also synthesize phenylacetic acid (PAA), a naturally occurring auxin derived from the amino acid phenylalanine (rather than tryptophan). Most evidence points to the biosynthesis of PAA being performed either by the same enzymes that take part in IAA biosynthesis or by enzymes with similar activity, but with specificity for phenyl-based substrates (Patten *et al.*, 2013; Duca *et al.*, 2014). For example, in *Azospirillum brasilense*, PAA is mainly produced from phenylalanine via phenylpyruvate (PPyA) and phenylacetaldehyde (PAAld), in a pathway that parallels the IPyA pathway shown in Fig. 1. In *A. brasilense* the *ipdC* gene is up-regulated by both tryptophan and phenylalanine, and an *ipdC* mutant produces significantly less IAA and PAA when supplied with tryptophan or phenylalanine, respectively (Somers *et al.*, 2005). It is not clear which form of auxin is predominantly produced by this bacterium, as biochemical analysis indicates that although the enzyme has a 10-fold higher binding affinity for IPyA over PPyA, it catalyzes the conversion of PPyA to PPAld with a 100-fold higher turnover rate (Spaepen *et al.*, 2007). Likewise, several bacterial genomes encode enzymes related to those involved in the IAM pathway, but that have been predicted or demonstrated to have specificity for auxin intermediates derived from phenylalanine, rather than tryptophan (Patten *et al.*, 2013; Duca *et al.*, 2014).

Although it is not clear whether PAA plays an important role in plant–microbe interactions, it is worth keeping this possibility in mind. PAA has demonstrated auxin activities, as it can bind the same receptors and induce the same genes as IAA (Sugawara *et al.*, 2015). However, PAA also differs from IAA in important ways; it is not actively or directionally transported out of plant cells, and it does not form a concentration gradient in response to gravitropic stimulation. Further, PAA has been shown to inhibit polar transport of IAA (Morris and Johnson, 1987). Thus, pathogens could synthesize PAA in order to modulate host auxin function and/or localization in the vicinity of infection.

Additional mechanisms for manipulating host auxin biology

The importance of auxin in plant–pathogen interactions is highlighted by the observation that pathogens have evolved multiple strategies for manipulating auxin biology in their hosts. In addition to being able to synthesize auxin, several bacterial pathogens are able to modulate auxin homeostasis and/or auxin signaling in their hosts.

Formation of the IAA-Lys conjugate

Many *P. syringae* and *P. savastanoi* genomes encode the enzyme IAA-lysine synthase (IaaL), encoded by the *iaaL* gene. This enzyme catalyzes the conjugation of the amino acid lysine to IAA, thus converting free IAA into indole-acetyl-ε-L-lysine (IAA-Lys). The IAA-Lys conjugate is believed to be a less active or inactive form of IAA, based on the observation that IAA-Lys has reduced activity in standard auxin response assays in seedlings (Evidente *et al.*, 1985). Although the *iaaL* gene is widespread and highly conserved among *P. syringae* and *P. savastanoi* strains (Glickmann *et al.*, 1998), a role in pathogenesis was only recently demonstrated by the work of two different groups, working in *Pto*DC3000 (Castillo-Lizardo *et al.*, 2015) and *P. savastanoi* pv. *neri* strain Psn23 (Cerboneschi *et al.*, 2016). Surprisingly, their findings revealed that the role of *iaaL* differs between strains and probably depends on the virulence strategy and/or host.

In *Pto*DC3000, a foliar pathogen that causes bacterial speck disease on tomato and several Brassica species (Preston, 2000), *iaaL* mutants exhibited a subtle reduction in growth and symptom development on tomato plants that was most readily detected in competition growth assays and quantification of disease symptom development (Castillo-Lizardo *et al.*, 2015). As free IAA and IAA-Lys levels were not quantified in this study, it is not known whether reduced virulence was due to an increase in free IAA or a decrease in IAA-Lys in infected tissue. Thus, it is not clear from this work whether the reduced virulence implicates a role for the activity of the IAA-Lys conjugate in pathogenesis, or whether the phenotype is due to a change in the concentration of free IAA in infected tissue. A reduction in virulence of *P. syringae iaaL* mutants was not detected in previous studies that investigated the role of *iaaL* in interactions with *Nicotiana benthamiana* and Arabidopsis (Lam *et al.*, 2014; Z. Chen and B. Kunkel, unpublished), suggesting that the importance of *iaaL* during pathogenesis may vary with the host.

Pseudomonas savastanoi pv. *neri* is a pathogen of oleander that stimulates plant cell proliferation to cause galls or 'knots'. This strain encodes enzymes in the IAM pathway for IAA synthesis, as well as IAA-Lys synthase (Cerboneschi *et al.*, 2016). A *P. savastanoi iaaL* mutant was generated and plants infected with this mutant accumulated elevated levels of free IAA compared with plants infected with the wild-type strain. In contrast to the reduced virulence observed for the *Pto*DC3000 *iaaL* mutant, the *P. savastanoi iaaL* mutant was found to be hypervirulent on oleander, growing to higher levels and causing more severe knots (Cerboneschi *et al.*, 2016). Thus, the elevated levels of free IAA in plants infected with the *iaaL* mutant promoted virulence. This suggests that, at least during *P. savastanoi* pathogenesis, production of IAA-Lys might be a mechanism for regulating free IAA levels in infected tissue. As discussed below, IAA has been shown to regulate virulence gene expression in several pathogens, thus the modulation of IAA accumulation around the site of infection could play an important role during pathogenesis.

The roles of auxin during plant–pathogen interactions.

An increase in auxin levels and/or auxin signaling in infected host tissue promotes many different processes associated

with pathogenesis, including epiphytic colonization, stimulation of host cell division (e.g. gall formation), inhibition of host defenses, and promotion of pathogen growth in plant tissue (Barash and Manulis-Sasson, 2009; Spaepen and Vanderleyden, 2011; Melotto and Kunkel, 2013; Duca *et al.*, 2014; Kazan and Lyons, 2014; McClerklin *et al.*, 2017). In several cases, the pathogen itself produces auxin, and in these interactions auxin can be viewed as a virulence factor. However, in other interactions, the pathogen stimulates auxin accumulation or auxin signaling in the host through the action of virulence factors that have evolved to modulate host auxin biology.

Stimulation of plant cell growth

Given the well-established role of auxins in promoting plant cell division and expansion, it is not surprising that IAA plays an important role in diseases caused by tumorigenic plant pathogens such as *Agrobacterium tumefaciens*, *P. savastanoi* (formerly *P. syringae* pv. *savastanoi*), and *P. agglomerans* (Barash and Manulis-Sasson, 2009; Spaepen and Vanderleyden, 2011; Duca *et al.*, 2014). In the case of *A. tumefaciens*, the main source of the IAA involved in disease development is not synthesized directly by the pathogen, but rather is produced by plant cells that have been genetically transformed by the *A. tumefaciens* T-DNA element (Thomashow *et al.*, 1986). During infection, the T-DNA is delivered into the host cell nucleus via a complex process involving a large number of virulence genes that are regulated by a highly evolved signaling process (Zupan and Zambryski, 1995; Gelvin, 2010). Integration of the T-DNA into the plant cell genome and subsequent expression of the T-DNA-borne *iaaH* and *iaaM* genes by the plant cell ultimately results in IAA synthesis. Production of cytokinin occurs in these cells as well, as cytokinin biosynthetic genes are also located on the T-DNA. The elevated levels of IAA and cytokinin at the site of infection lead to uncontrolled plant cell proliferation and expansion and gall formation. Other genes localized on the T-DNA direct production and secretion of opines, compounds that provide carbon and nitrogen to support growth of the *A. tumefaciens* cells residing in the gall tissue.

In contrast, the uncontrolled plant cell division and growth in gall formation caused by pathogens such as *P. savastanoi* and *P. agglomerans* is stimulated by IAA produced by the pathogen. These bacteria carry *iaaH* and *iaaM* genes, located either on virulence plasmids (e.g. pPATH, (Barash and Manulis-Sasson, 2009) or in their genomes, and mutation of these genes results in reduced gall formation (Patten *et al.*, 2013; Duca *et al.*, 2014). In the case of *P. agglomerans*, IAA production is also associated with epiphytic colonization of plant tissue (Brandl and Lindow, 1998). Manulis *et al.* (1998) demonstrated that some *P. agglomerans* strains can synthesize IAA via two separate pathways, the IPyA and IAM pathways, and that these pathways differentially contribute to distinct aspects of pathogenesis. Disruption of IAA synthesis via mutation of *ipdC* in the IPyA pathway (Fig. 1) results in reduced epiphytic fitness, whereas mutation of the IAM pathway caused reduced gall formation (Manulis *et al.*, 1998). A possible mechanism underlying the different roles of these pathways during pathogenesis is that the IPyA and IAM pathways could be differentially regulated, in response to the physical environment in which the pathogen is growing (e.g. plant surface versus apoplastic space). Presumably, IAA production via the IAM pathway is accompanied by cytokinin synthesis during stimulation of gall formation, and a putative operon encoding cytokinin biosynthesis genes is located in the vicinity of the IAM pathway genes on the pPATH plasmid in *P. agglomerans* (Barash and Manulis-Sasson, 2009).

Modulation of defense responses

Auxin also promotes the virulence of several pathogens that do not stimulate gall or knot formation. Free IAA levels increase in plants infected with the fungal pathogen *Puccinia graminis*, as well as bacterial pathogens *P. syringae*, *X. campestris*, and *Ralstonia solanacearum* (O'Donnell *et al.*, 2003; Chen *et al.*, 2007; Ding *et al.*, 2008; Denance *et al.*, 2013; Yin *et al.*, 2014). Further, elevated levels of auxin promote disease susceptibility in several pathogenic interactions (Robert-Seilaniantz *et al.*, 2011*b*; Kazan and Lyons, 2014). For example, IAA and other auxins promote growth of *P. syringae* within host tissue, either when produced by the pathogen (McClerklin *et al.*, 2017), when applied exogenously (Navarro *et al.*, 2006; Chen *et al.*, 2007; Wang *et al.*, 2007), or when endogenous IAA levels are elevated (Mutka *et al.*, 2013).

The availability of mutant strains impaired in auxin biosynthesis has begun to provide insight into the role(s) of pathogen-produced auxins during infection. McClerlkin *et al.* recently demonstrated that the *Pto*DC3000 *aldA* mutant exhibits reduced virulence on *A. thaliana* plants, suggesting that auxin synthesized by the pathogen is a virulence factor. They also observed that SA-mediated defenses were elevated in *A. thaliana* plants infected with the *aldA* mutant, and that growth of the mutant was restored to normal levels in *A. thaliana* mutant plants impaired for SA synthesis. These results suggest that pathogen-derived auxin promotes virulence by suppressing SA-mediated defenses (McClerklin *et al.*, 2017). These findings are consistent with several earlier studies indicating that auxin suppresses defense responses mediated by SA (Park *et al.*, 2007; Wang *et al.*, 2007; Kazan and Manner, 2009; Robert-Seilaniantz *et al.*, 2011*a*).

In other studies, investigating the roles of auxin during *P. syringae* pathogenesis, transgenic *A. thaliana* plants that accumulated elevated levels of IAA due to overexpression of the *YUCCA1* (*YUC1*) IAA biosynthesis gene (Mutka *et al.*, 2013) exhibited increased susceptibility to *Pto*DC3000. However, counter to expectation, neither SA accumulation nor SA-responsive gene expression was suppressed in these plants. Further, plants carrying both a mutation that disrupts SA biosynthesis and the *YUC1* overexpression construct exhibited additive effects of enhanced susceptibility due to both impaired SA-mediated defenses and elevated IAA (Mutka *et al.*, 2013). These results suggest that IAA can also promote pathogen growth through one or more mechanisms that function independently of suppression of SA-mediated defenses.

An example of an SA-independent defense mechanism that appears to be modulated by auxin signaling is production of indole glucosinolates, which have antimicrobial activities. In Arabidopsis, expression of the basal defense-elicited miRNA miR393 down-regulates auxin signaling (Navarro *et al.*, 2006), resulting in increased accumulation of several indole glucosinolates (Robert-Seilaniantz *et al.*, 2011*a*). Although we are not aware of an example of a pathogen stimulating host auxin signaling to counter this defense mechanism, this is a possibility we should keep in mind.

Modulation of host auxin physiology

Additional mechanisms by which auxin might promote pathogenicity could involve altering host physiology and signaling to render the plant tissue more suitable for pathogen growth and disease symptom development. For example, altering source–sink relationships that could result in a re-direction of water or nutrient flow towards the infection site or stimulation of water or nutrient release into the extracellular space colonized by pathogens could support increased numbers of pathogen cells. Likewise, altering the balance between plant cell division, expansion, and cell death could be of benefit to the pathogen at various stages of pathogenesis. Stimulating plant cell division and/or expansion in the vicinity of the infection site could divert limited resources into these processes at the expense of expressing defense responses (Kazan and Manner, 2009). Alternatively, shifting the balance between cell growth and death towards cell death, especially in the later stages of infection, could promote formation of necrotic disease symptoms (Ludwig-Muller, 2015; Naseem *et al.*, 2015).

IAA as a microbial signaling molecule

Although IAA is best known as a regulator of plant growth and development, it can also have a direct effect on microbial organisms by acting as a signaling molecule that regulates gene expression (Spaepen and Vanderleyden, 2011; Duca *et al.*, 2014). IAA regulates bacterial responses likely to be important at different stages during interactions with plants, including regulating virulence gene expression and promoting survival under stress conditions that might be encountered during growth in the vicinity of, on, or within plant tissues. IAA might also regulate processes that govern interactions between microbial cells growing in the plant environment.

In several plant-associated microbes, IAA regulates expression of genes hypothesized to promote interactions with plants. For example, IAA induces large-scale changes in the transcriptome of *A. brasilense*, a PGPR. This includes up-regulation of genes involved in IAA biosynthesis, resulting in a positive feedback loop that reinforces auxin responsiveness (Vande Broek *et al.*, 2005), as well as changes in expression of genes involved in respiration, metabolism, and transport. These observations suggest that IAA promotes physiological and metabolic adjustment for growth in the rhizosphere (Van Puyvelde *et al.*, 2011). IAA was also observed to induce expression of genes predicted to be involved in a Type VI secretion system (T6SS), a secretion apparatus that can inject protein effectors into the cells of other organisms (Ryu, 2015). The role of Type VI secretion in PGPR and other plant-associated bacteria is not well understood, but may help the bacteria gain a competitive advantage in the rhizosphere or elsewhere in the plant environment by injecting toxins into other microbes in the vicinity.

IAA influences expression of virulence genes in *Dickeya didantii* (formerly known as *Erwinia chrysanthemi)*, a pathogen that causes soft rot and other disease. Yang *et al.* (2007) used both an IAA biosynthesis mutant and exogenous IAA treatment to examine the effects of IAA on virulence gene expression. They observed that an *iaaM* mutant exhibited reduced expression of T3SS-related genes, suggesting that IAA stimulates Type III secretion. They also observed that IAA stimulated production of pectate lyase, a plant cell wall-degrading enzyme that contributes to cell wall maceration and soft rot symptoms. Thus, at least two classes of virulence genes are positively regulated by IAA in this pathogen.

In *A. tumefaciens*, IAA also modulates virulence gene expression during infection. Addition of exogenous IAA to cultures of *A. tumefaciens* resulted in large-scale transcriptional responses. This included significant down-regulation of several virulence (*vir*) gene operons that encode proteins involved in delivering the T-DNA into host cells (Yuan *et al.*, 2008), as well as up-regulation of several genes, the majority of which encode proteins of unknown function (Yuan *et al.*, 2008). One possible role for IAA in regulating virulence gene expression in *A. tumefaciens* may be to provide feedback control to turn off *vir* gene expression and stop the transfer of the T-DNA into host cells once sufficient levels of IAA to stimulate gall formation have been achieved.

IAA also regulates virulence gene expression in *P. savastanoi* and *P. syringae.* Exogenous application of IAA to cultures of *P. savastanoi* was reported to down-regulate expression of genes involved in Type III secretion and to increase transcription of *vgrG*, a gene likely to be involved in Type VI secretion (Aragón *et al.*, 2014). We have obtained similar results in recent work in our lab, demonstrating that IAA down-regulates expression of several Type III secretion-related genes in *Pto*DC3000 growing in culture (G. Harrison and B. Kunkel, unpublished). These results may seem surprising at first, as one might expect that IAA, a molecule produced by the plant, would induce expression of virulence factors, such as the T3SS, which are required for early steps during pathogenesis. However, in this interaction, it is possible that IAA, which may accumulate slowly during the first days of infection, may act as a signal to down-regulate virulence genes once early steps in the infection process have been accomplished. IAA could also then act as a signal to induce virulence genes required in subsequent steps of pathogenesis. We do not presently have sufficient knowledge of *P. syringae* pathogenesis to know the identity of virulence genes involved in later stages of infection. However, we speculate that these genes might be important for growth in the leaf, such as uptake and utilization of nutrients that become available in the apoplast. Given the earlier observations of Aragón *et al.* (2014), induction of a T6SS as a strategy to outcompete other microbes present in the apoplast could also be a component of an IAA-responsive virulence regulon.

Additional insight regarding ways in which IAA could directly affect pathogens to promote virulence and disease development comes from studies on the impact of IAA in *Bradyrhizobium japonicum* and *Escherichia coli* (Bianco *et al.*, 2006; Donati *et al.*, 2013). In both of these organisms, exogenous application of IAA induced expression of genes involved in general stress responses. Treatment with IAA also resulted in increased cell viability when the bacteria were grown under stress conditions, including heat shock, osmotic shock, and oxidative stress. IAA also promoted biofilm production in both *B. japonicum* and *E. coli*. Thus, in addition to regulating virulence genes, IAA may induce changes in gene expression, physiology, and metabolism that promote survival of bacteria when growing under potentially stressful conditions encountered in or on plant tissue.

Although we and others hypothesize that the ability of these pathogens to sense and respond to IAA plays an important role during pathogenesis, this has not been investigated. One approach for exploring this hypothesis is to isolate mutants impaired in IAA perception and/or responses, and to assay these mutants for altered virulence. Such mutants will be critical for identification and characterization of the receptors and signaling pathways used by bacteria to perceive and respond to auxin, and will provide insight into the various roles IAA might play during pathogenesis. For example, one could imagine that IAA is used by the pathogen as a signal that it is has come into contact with a potential plant host, and that it should induce virulence gene expression. Alternatively, or additionally, such studies could reveal that pathogen-produced IAA might function as a cell density factor, for example to regulate gene expression when the bacteria need to shift from one stage of pathogenesis to the next.

Summary and future challenges

The auxin IAA plays multiple roles during interactions between bacterial plant pathogens and their hosts, including suppressing host defenses and stimulating alterations in host physiology to render the host tissue more suitable for pathogen growth. In addition, IAA may also directly impact the pathogen to regulate virulence gene expression, stress responses, and also possibly act as a microbial signal for communicating with other microbes in the environment. The recent findings in this area have given rise to a series of new questions including the following. (i) Do other forms of auxin, such as PAA, play a role in plant–microbe interactions? (ii) Might some IAA–amino acid conjugates have specific functions, rather than being simply less active or inactive forms of auxin? (iii) What other strategies might pathogens use to modulate host auxin biology? (iv) Do microbes produce IAA or other auxins to communicate with or control other microbes in the rhizosphere, phyllosphere, or in non-plant-associated environments? Future studies to address these questions will provide important new insights into the signaling processes that regulate plant–pathogen interactions, as well as possibly uncover new, unexpected roles for IAA and other auxins in the biology of both plants and microbes.

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