Review

Cytokinins and plant immunity: old foes or new friends?

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Cytokinins are plant growth promoting hormones involved in the specification of embryonic cells, maintenance of meristematic cells, shoot formation and development of vasculature. Cytokinins have also emerged as a major factor in plant–microbe interactions during nodule organogenesis and pathogenesis. Microbe-originated cytokinins confer abnormal hypersensitivity of cytokinins to plants, augmenting the sink activity of infected regions. However, recent findings have shed light on a distinct role of cytokinins in plant immune responses. Plant-borne cytokinins systemically induce resistance against pathogen infection. This resistance is orchestrated by endogenous cytokinin and salicylic acid signaling. Here, we discuss how plant- and pathogen-derived cytokinins inversely affect the plant defense response. In addition, we consider the molecular mechanisms underlying plant-derived cytokinin action in plant immunity.

Cytokinins: growth hormones regulate plant immunity

Cytokinins are growth control hormones, which promote cell division, nutrient mobilization and leaf longevity [1–8]. Cytokinins can also increase grain yield; for example, by activating inflorescence meristem activity in rice (*Oryza sativa*) [9,10]. Moreover, cytokinin accumulation in tobacco (*Nicotiana tabacum*) leads to tolerance against extreme drought stress [11]. These features highlight the importance of cytokinins in agricultural applications. However, many microbes, mostly plant pathogens, also secrete cytokinin analogs or activate plant cytokinin production to divert nutrients from the host toward dedicated growth of infected tissues, which results in a loss of productivity in agricultural crops [12–16]. For example, biotrophic fungal and bacterial pathogens employ cytokinins to form 'green islands' and gall structures, leading to delayed senescence and enhancement of sink activity [13,14]. It has been assumed that the cytokinin-mediated growth response suppresses plant basal defense mechanisms [12]; however, recent work has revealed that plant-originated cytokinins augment plant immunity together with salicylic acid (SA) signaling [17]. In this review, we discuss the molecular mechanisms underlying distinct actions of plant- and pathogen-derived cytokinins in plant responses, and the role of cytokinins in the priming of plant innate immunity.

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Foe's tales: pathogen-derived cytokinins and pathogenesis

Gall-forming plant pathogenic bacteria such as *Rhodococcus fascians* and *Agrobacterium tumefaciens* and biotrophic fungi such as *Puccinia striiformis* produce auxin and cytokinins to enhance their pathogenicity and modulate the physiology of host plants [12,14,15,18]. *R. fascians*

Glossary

ALD1 (ABERRANT GROWTH AND DEATH 2-LIKE DEFENSE RESPONSE PROTEIN 1): ALD1 encodes aminotransferase and plays important role for SA accumulation during defense response.
AHK (ARABIDOPSIS HISTIDINE KINASE): histidine kinase cytokinin receptors that transduce cytokinin signaling.
ARR (ARABIDOPSIS RESPONSE REGULATOR): type-A ARRs are induced by cytokinins and play negative role during cytokinin signal transduction, whereas type-B ARRs are transcriptional activators that control cytokinin signaling output.
CKX (CYTOKININ OXIDASES/DEHYDROGENASES): CKXs catalyze the degradation of cytokinins.
EIN2 (ETHYLENE INSENSITIVE 2): acts at the downstream of CTR1 and plays key roles for ethylene signaling pathway.
FMO1 (FLAVIN-DEPENDENT MONOOXYGENASE 1): promote cell death at the pathogen infection site to promote resistance to pathogens dependent on ENHANCED DISEASE SUSCPTIBILITY 1 (EDS1).
IAA3/SHY2 (INDOLE-3-ACETIC ACID 3/SHORT HYPOCOTYL 2): induced by auxin and regulates multiple auxin responses in roots.
IPT (ISOPENTENYL TRANSFERASE): cytokinin synthase that mediates a key regulatory step during cytokinin biosynthesis.
KNOX (KNOTTED-LIKE HOMEOBOX): Knotted1-like homeobox gene family that is involved in the activation of shoot apical meristem formation.
NahG: a SA-degrading enzyme of *Pseudomonas putida*. Overexpression of NahG in *Arabidopsis* depletes SA of plants.
NIMIN (NIM1-INTERACTING): interacts with NPR1 to suppress *PR* gene induction. Because SA induces *NIMINs*, they form a negative-feedback loop in SA signaling.
NPR1 (NONEXPRESSOR OF PR GENES 1): also known as NON-INDUCIBLE IMMUNITY 1 (NIM1). A key regulator of the SA-mediated systemic acquired resistance.
PAD4 (PHYTOALEXIN-DEFICIENT 4): a lipase-like gene that plays a critical role in R-protein-mediated defense response.
RAR1 (REQUIRED FOR MLA12 RESISTANCE 1): a protein with isochorismate synthase activity. Knockout of this gene results in salicylic acid deficiency.
RIN4 (RPM1-INTERACTING PROTEIN 4): RIN4 is degraded by *Pseudomonas syringae* effectors and this degradation is recognized by R proteins to trigger defense response.
RPS2 and RPM1 (RESISTANCE TO PSEUDOMONAS SYRINGAE 2 and RESISTANCE TO PSEUDOMONAS SYRINGAE PV. MACULICOLA 1): these two R proteins recognize the degradation of RIN4 and activate the plant defense response.
SID (SALICYLIC ACID INDUCTION DEFICIENT): isochorismate synthase involved in the salicylic acid (SA) biosynthesis.
TIR1 and AFB (TRANSPORT INHIBITOR RESPONSE 1 and AUXIN SIGNALING F-BOX): encode auxin receptors. Interact with ASK1, ASK2 and CUL1 to form SCF ubiquitin ligase complex and degrade IAA proteins.

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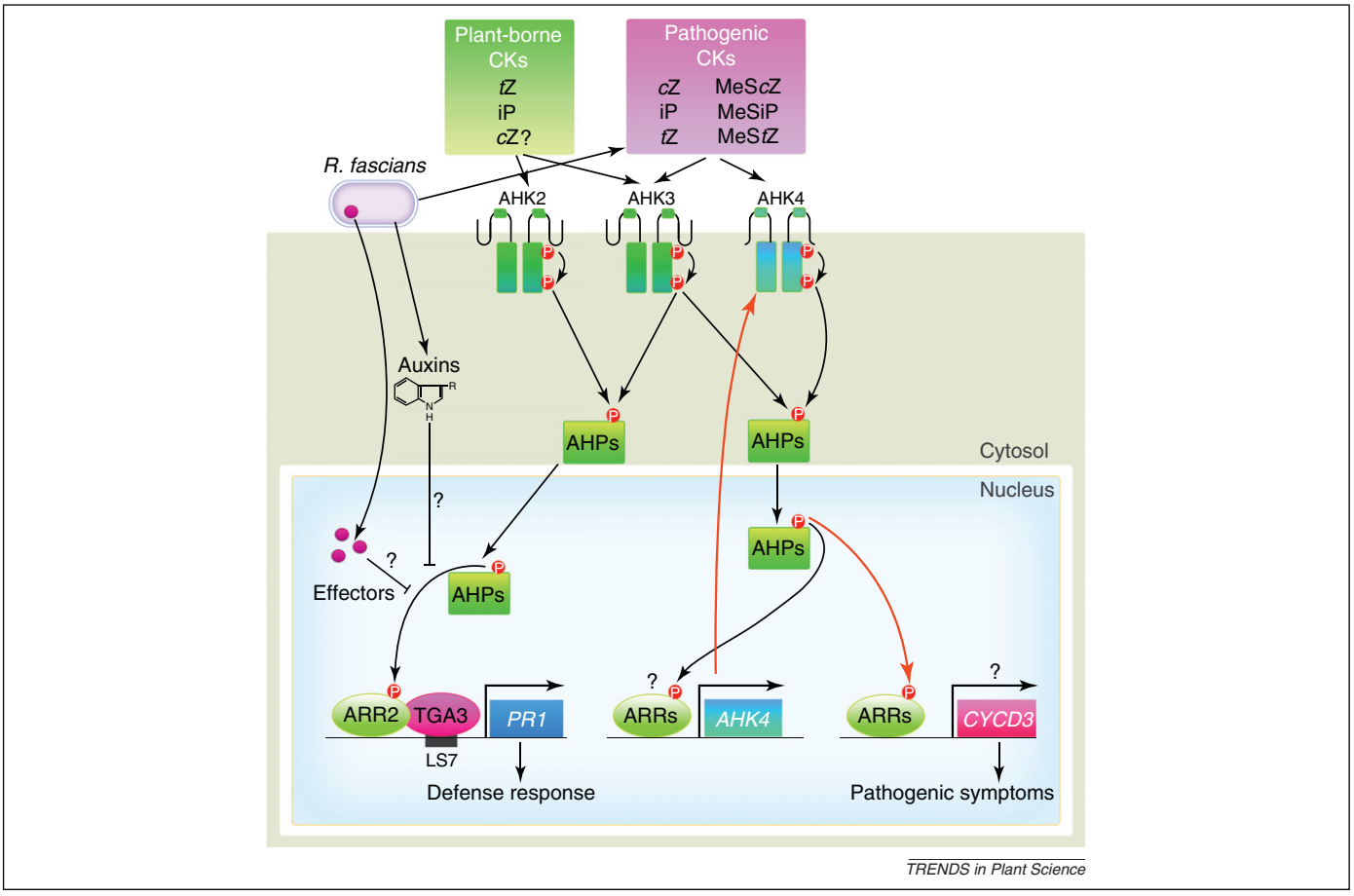

Figure 1. Modulation of plant cytokinin signaling by *R. fascians*-originated cytokinins. *Rhodococcus fascians* produces various cytokinin species, including non-degradable 2-methylthio (2-MeS) derivatives of *cZ*, iP and *tZ* (MeS*c*Z, MeSiP and MeS*t*Z). Perception of this cytokinin mixture from *R. fascians* by a cytokinin receptor AHK3 results in abnormal, constitutive activation of cytokinin signaling, including transcriptional induction of *AHK4*. Accumulation of AHK4 enhances sensitivity to pathogen-derived cytokinins. This results in the de-differentiation of plant cells and leafy gall formation. Although the direct targets of pathogen-derived cytokinins are still elusive, cytokinin-inducible cyclins such as *CYCD3* and expansins might trigger de-differentiation as indicated by ectopic induction of *KNAT1*. It is also possible that *R. fascians* secretes effectors or auxin to specifically suppress AHK2 and AHK3 – and ARR2-mediated defense responses. Red arrows indicate hyper-activated signaling cascades by *R. fascians*-derived cytokinins. Abbreviations: AHP, ARABIDOPSIS HISTIDINE PHOSPHOTRANSFER PROTEIN; *cZ*, *cis*-zeatin; iP, isopentenyladenine; *tZ*, *trans*-zeatin.

and *A. tumefaciens* induce leafy galls and crown galls, respectively. These hyperplasia originate from the abnormal localized accumulation in plant tissues of cytokinins and auxins inducing abnormal cell proliferation and/or delaying the senescence of infected regions to enhance sink activity [15,16,18–21]. After *A. tumefaciens* infection, cytokinins are produced by T-DNA integrated into the plant chromosome to assist crown gall formation. However, bacterial cytokinins might not be essential for the infection of *A. tumefaciens,* as both virulent and avirulent strains produce similar quantities of cytokinins *in vitro* culture [22].

R. fascians induces abnormal hyper-activation of host cytokinin signaling and confers high cytokinin susceptibility on host plants (Figure 1) [16,19]. Depending on the host, various types of cytokinins, such as isopentenyladenine (iP), *cis*-zeatin (*c*Z), and 2-methylthio-*cis*-zeatin (2MeS*c*Z), accumulate in *R. fascians*-infected tissues [16]. *R. fascians* itself produces at least six cytokinin bases: *cis*-zeatin, iP, *trans*-zeatin (*t*Z) and their methylthio-derivatives [15]. In *Arabidopsis thaliana*, pathogen-derived cZ and 2MeS*c*Z are not fully degraded by plant CKXs (see Glossary) and are maintained at high concentrations as long as 35 days after challenged, when the leafy gall is fully developed [16]. Expression of a cytokinin receptor *AHK4* in plants was highly induced by *R. fascians* infection, which can enhance cytokinin sensitivity of host plants. Genetic analysis showed that of the double cytokinin receptor knockout mutants only *ahk3 ahk4* plants failed to induce pathogen-dependent deformations. Accordingly, *R. fascians*-derived cytokinins recognized mainly by AHK3 and AHK4 appear to be major regulators of plant sensitivity to *R. fascians*. It is noteworthy that exogenous application of cytokinin mixtures results in a stronger activation of plant cytokinin signaling compared to the application of a single cytokinin species. Each cytokinin has a different binding affinity for the AHK receptors, therefore the mixture of cytokinins produced by the pathogen should be able to simultaneously and synergistically hyper-activate cytokinin responses downstream of AHK3 and AHK4 which results in responsiveness to *R. fascians*. One of these responses is the induction of *KNOX* genes including *KNAT1* [19]. Ectopic expression of *KNAT1* results in aberrant cell divisions in leaves, which causes leaf serrations, a typical symptom of *R. fascians* infection. Such

leaf deformations can be partially mimicked by a high dose of exogenous cytokinin, indicating *R. fascians* might induce pathological symptoms by cytokinins *via* KNOX. Although KNOX proteins induce cytokinin biosynthetic *IPT* genes in normal conditions [23,24], *R. fascians*-induced KNOX proteins fail to induce plant cytokinin production, because *IPT* genes are downregulated during *R. fascians* infection, rather than induced [25]. Recently, the effect of *R. fascians* infection on the plant transcriptome has been studied extensively [25]. As *R. fascians*-derived cytokinins play a critical role in its pathogenicity, cytokinin-inducible cell wall-loosening expansins involved in cell proliferation were induced upon *R. fascians* infection [25]. Surprisingly, the expression of cytokinin-induced ROS producers, scavengers and antioxidants, which are critical for stress and defense responses, was reduced by *R. fascians*. For this reason, it is likely that *R. fascians* can activate complex crosstalking of cytokinin-regulated growth responses while suppressing cytokinin-induced defense responses. The same set of cytokinin-induced stress and defense-related genes is also suppressed by auxin secreted from *A. tumefaciens*, which employs auxin for pathogenicity and crown-gall formation [18], thus suggesting that pathogen-derived auxin specifically suppresses the cytokinin-induced defense response during infection (Figure 1).

Cytokinins prompt salicylic acid accumulation and a defense response

In contrast to the cytokinins produced by many biotrophic bacterial and fungal pathogens for their proliferation in host plants, plant-derived cytokinins can be involved in plant resistance to viral infection [26–29]. However, the molecular mechanisms of cytokinin action in disease resistance to a wide spectrum of pathogens and the reason for the inverse effects of cytokinins on plant responses against biotrophic pathogens and viral infection have remained elusive.

S-ADENOSYLHOMOCYSTEINE HYDROLASE (SAHH) mediates intracellular conversion of S-adenoslhomocysteine (SAH) to S-adenosylmethionine (SAM) [26]. Because SAHH could mediate the methylation of the 5′-terminus of viral mRNA, which is a prerequisite for viral replication, antisense inhibition of SAHH is used to suppress viral infection in tobacco plants. The transgenic plants exhibit broad local and systemic resistance to various viruses, including tobacco mosaic virus (TMV), cucumber mosaic virus (CMV), and potato virus X (PVX). It is particularly interesting that these plants also show enhanced resistance to potato virus Y (PVY), which does not require the methylated cap structure for replication. Unexpectedly, about half of the transgenic plants show slightly stunted growth, and root exudates of the transgenic plants contain three times the amount of endogenous cytokinins measured in wild-type plants. These results suggest that the increased cytokinins in the transgenic plants, rather than the direct suppression of viral mRNA cap methylation, are indirectly related to their enhanced resistance to PVY. Similarly, the rice *rgpl* transgenic tobacco plants have enhanced resistance to TMV infection after wound treatment. In rice, rgpl is a Rab/Ypt-related small GTP-binding protein [28]. Introduction of the *rgpl* gene into tobacco results in reduced apical dominance and increased tillering, which is reminiscent of activated cytokinin signaling. Consistent with this finding, the accumulation of endogenous zeatin and zeatin ribose is evident in *rgpl* transgenic plants. The TMV resistance of the transgenic plant results from the accumulation of SA upon wounding in wounded as well as in unwounded systemic leaves. It is apparent that incompatible biotrophic pathogens trigger SA-mediated defense responses, including the induction of acidic pathogenesis-related (PR) proteins such as PR-1a in tobacco. Although it is not clear whether cytokinins directly induce SA accumulation, these studies imply that plant-derived cytokinin accumulation results in an altered morphology and resistance to viral infection, at least in part, through SA accumulation.

Plants possess disease resistance (R) proteins that are able to target avirulent proteins from specified pathogens. The majority of R proteins usually contain a nucleotide-binding (NB) leucine-rich-repeat (LRR) domain with a N-terminal Toll/Interleukin 1 receptor (TIR) region (TIR-NB-LRR) or with a putative coiled-coil region at the N-terminus (CC-NB-LRR) [30]. These R proteins recognize specific avirulence (avr) effector proteins from pathogens and then trigger a defense response such as SA accumulation, leading to induction of *PR* genes and systemic acquired resistance (SAR). UNI1 is a CC-NB-LRR protein that could function as an R protein and elicit the SA-dependent signaling pathway [31–33]. The expression of *PR1* and *PR5* is induced in gain-of-function *uni-1D* mutants; this enhanced expression is diminished by the knockout of a SA biosynthetic gene, *sid2*, or the overexpression of NahG. Moreover, UNI proteins are destabilized in *rar1-28* knockout mutants. RAR1 is a cysteine- and histidine-rich CHORD family protein with two novel zinc-coordinating domains. It interacts with various NB-LRR proteins and stabilizes them to effectively initiate R protein-mediated resistance [34–38]. *uni-1D* plants exhibit phenotypes typical of activated cytokinin signaling, such as loss of apical dominance with ectopic auxiliary meristem formation, delayed senescence and late flowering [33]. In the *uni-1D* mutant, the expression of cytokinin-responsive type-A ARRs, *ARR5* and *ARR6*, is also induced probably by increased endogenous *tZ* and its conjugated forms (Figure 2). Cytokinin depletion by *CKX1* overexpression in a *uni-1D* background suppresses the formation of ectopic meristems and, interestingly, abolishes the activation of both *ARR5* and *PR1*. Therefore, it is likely that UNI is involved in both SA signaling and plant development *via* the modulation of cytokinin levels. RIN4 is degraded by *Pseudomonas syringae* effectors AvrRpm1 and AvrRpt2 [39,40]. Two R proteins, RPS2 and RPM1, recognize the degradation of RIN4 and activate the plant defense response. Activation of RPS2 and RPM1 by *RIN4* knockdown also results in the activation of expression of the primary cytokinin-responsive gene *ARR5* and in ectopic auxiliary meristem formation [33]. Given these findings, it appears that the R protein-mediated defense response employs cytokinin signaling, which augments SA signaling output to reinforce plant immunity.

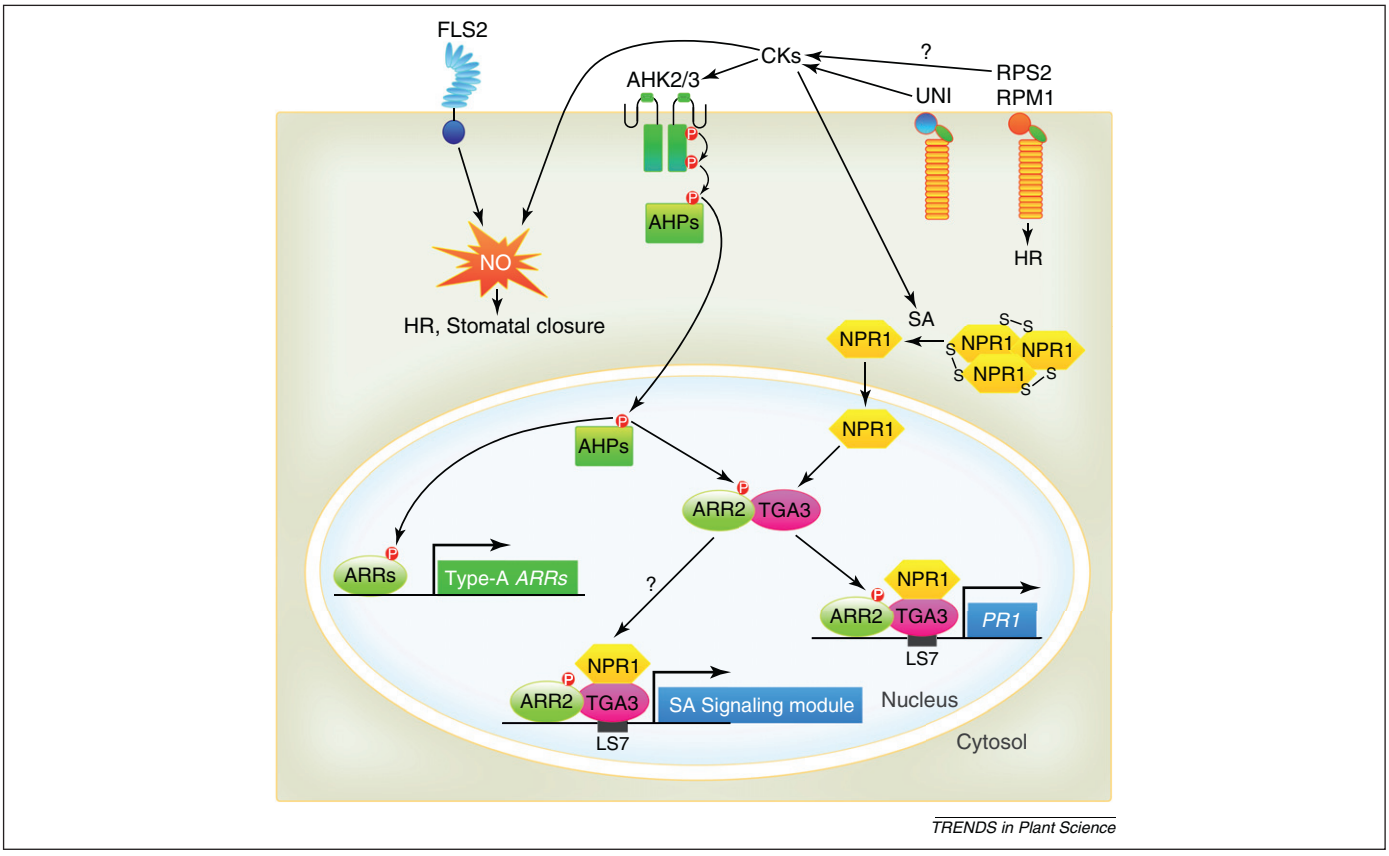

Figure 2. The role of plant-borne cytokinins in plant immunity. A putative R-protein, UNI, induces cytokinin biosynthesis to accumulate salicylic acid, which enhances SA-dependent *PATHOGENESIS-RELATED 1* (*PR1*) expression. Two R proteins, RPS2 and RPM1, also activate the transcription of cytokinin- and SA-responsive genes, *ARABIDOPSIS RESPONSE REGULATOR 5* (*ARR5*) and *PR1*, possibly by inducing cytokinin biosynthesis. Cytokinin perception through AHK2 and AHK3 cytokinin receptors initiates transfer of a phosphoryl group to type-B ARR transcription factors, including ARR2, which results in the activation of type-A ARRs. Upon pathogen perception, SA-activated TGA3 interacts with and recruits ARR2 to the *PR1* promoter to endure hyper-activation of *PR1* by cytokinins. *ARR2* overexpressing plants show enhanced expression of genes involved in SA signaling, including positive and negative feedback regulatory modules, whether dependent or independent of TGA3. Cytokinins also are able to accumulate NO, which plays an important role in the hypersensitive response (HR) and closure of stomata triggered by the perception of bacterial flagellin through the pattern recognition receptor, FLS2.

Modulation of salicylic acid signaling by cytokinins *via* ARR2

Although interplay between cytokinin and SA in plant immunity has been suggested based on phenotypic and genetic data, the molecular mechanism determining how cytokinins affect SA signaling and plant responses against pathogen attack is unclear. Recently, it has been suggested that cytokinin signaling through AHK2 and AHK3 receptors activates SA signaling during the interaction with *P. syringae* pv. tomato DC3000 (*Pst* DC3000), a hemibiotrophic bacterial pathogen that causes bacterial speck in *Arabidopsis* (Figure 2) [17]. Exogenous application of *t*Z or increased endogenous cytokinins in *IPT*-overexpressing plants enhances plant resistance against *Pst* DC3000, whereas *ahk2 ahk3* double knockout plants enhance susceptibility. Cytokinins transiently induce the transcription of defense-related genes, including an SA biosynthetic gene *SID2*, a transcription factor *WRKY18*, and an SA signaling marker gene *PR1*. As a result, the endogenous SA level is increased after cytokinin treatment. Cytokinin perception initiates a multiple phosphorelay *via* a two-component signaling cascade, resulting in the phosphorylation of the conserved aspartate residue in ARR transcriptional regulators. ARR2, a type-B ARR that is activated by cytokinins, binds directly to the promoters of *PR1* and *PR2* to induce their transcription, and its activity is directly correlated with plant resistance to the pathogen. As the non-phosphorylable form of ARR2 fails to induce resistance to *Pst* DC3000, it seems that cytokinin-dependent phosphorylation of ARR2 is crucial for the expression of defense-related genes. Although ARR2 binds to *PR* gene promoters, the cytokinin-induced defense response through ARR2 activation depends on SA signaling. The knockout of *NPR1* or NahG transgenic plants fails to induce a cytokinin-mediated defense response. It turns out that ARR2 interacts with the SA response factor TGA3 and the application of SA enhances the binding of ARR2 to the *PR1* promoter. Because SA accumulation stimulated by the perception of microbe-associated molecular patterns (MAMPs) enhances callose deposition [41], *35S:IPT3* with high endogenous cytokinins and *35S:ARR2* plants exhibit elevated callose deposition upon *Pst* inoculation. These findings suggest that TGA3 recruits ARR2 to the promoters of defense genes when SA signaling is activated, and cytokinin-dependent phosphorylation of ARR2 induces hyper-activation of their transcription together with SA-dependent TGA3.

There are 12 type-B ARRs in *Arabidopsis* [42]. Among them, ARR1, ARR10 and ARR12 act redundantly on cytokinin-dependent root development [43–48]. Because an additional knockout of other type-B ARRs does not affect cytokinin sensitivity as much as *arr1*, *arr10* or *arr12*

knockouts, these type-B ARRs are generally considered to be major cytokinin signal transducers [47,49]. However, it is also evident that the AHK3-dependent activation of ARR2 is involved in delaying leaf senescence, and *arr2* knockout plants show insensitivity to ethylene and cytokinin in hypocotyl elongation [2,50], suggesting that ARR2 is important in crosstalks of cytokinins with other developmental signaling cascades. Even so, it is unclear how ARR1 and ARR2, which bind to the same GAT-containing *cis*-element [50–52], can regulate different developmental processes. It has been suggested [17] that the interaction of these type-B ARRs with other transcription factors determines their specific target genes. For example, ARR2 specifically interacts with TGA3 and is recruited to the *PR1* promoter by TGA3, whereas ARR1 cannot interact with TGA3 and fails to induce resistance to *Pst* DC3000. Consistent with this observation, when *Pst* DC3000 is inoculated, it appears that ARR1 and ARR2 transcription factors control different developmental processes (Jaemyung Choi and Daeseok Choi, unpublished data). The SA biosynthetic genes, *SID1* and *SID2*, and the SA-responsive genes, *PR1* and *PR5*, are induced in *35S:ARR2* plants compared to Col-0 controls inoculated with *Pst* DC3000 (Figure 2). *TGA3* and its homolog *TGA5* are induced by *ARR2* overexpression, reflecting a positive feedback loop in the cytokinin-induced defense response. A lipase-like protein, PAD4 functions upstream of SA accumulation [53–55]. The knockout of FMO1 or ALD1 fails to establish SAR to *Pst* [56,57]. NIMIN-1 and -2 induced by SA interact with and inhibit NPR1 to suppress *PR* gene expression, forming a negative-feedback loop [58,59]. The expression of genes encoding these SA signaling components is simultaneously induced in *35S:ARR2* upon *Pst* DC3000 challenge. These data strongly suggest that ARR2 activates a module in the SA-mediated defense response network, not only *PR1*, and possibly elicits SAR, along with negative-feedback regulation of defense responses mediated by NIMIN 1 and 2. By contrast, certain defense-related genes are suppressed, rather than induced, in *ARR1*-overexpressing plants. As ARR1 is involved in the suppression of meristematic cell proliferation at root tips *via* transcriptional activation of *IAA3/SHY2* [43–45,48], ARR1 appears to modulate genes related to the cell cycle such as cyclins (Jaemyung Choi and Daeseok Choi, unpublished data).

The contribution of nitric oxide to cytokinin-modulated disease resistance

Nitric oxide (NO) is known to induce the hypersensitive response (HR), R protein-mediated program cell death, in concert with reactive oxygen intermediates [60], and is also involved in the closure of stomata [61–63]. Plant recognition of a MAMP, such as a conserved peptide from bacterial flagellin, flg22 or lipopolysaccharide (LPS), induced NO production within 10 min at the stomata [64]. Nω-nitro-L-arginine (L-NNA), a nitric oxide synthase (NOS) inhibitor, blocks flg22-, LPS- and non-host pathogen-induced stomatal closure, allowing pathogen entry through stomata. Application of *t*Z induces NO accumulation, but the cytokinin signaling cascade involved in this process is not clear [65]. NO is not likely to be a direct modulator of cytokinin signaling as the expression of a cytokinin-responsive *pARR5:GUS* reporter is not changed by a NOS inhibitor, a NO scavenger, and NO donors [66]. It is still an open question whether cytokinin-induced NO production is involved in the cytokinin-mediated defense response by inducing HR and/or closure of stomata upon pathogen attack (Figure 2).

Priming: a potential source of cytokinin-elicited plant immunity

Once plants have been exposed to a pathogen, they elicit a defense response that is much faster and stronger than in the initial pathogen attack. This phenomenon is referred to as priming [67,68]. There are two types of priming in plants. First, SAR occurs in the distal parts of plants upon localized infection [69]. Second, induced systemic resistance (ISR) is triggered by non pathogenic bacteria such as plant growth-promoting rhizobacteria (PGPR) [70–75]. For example, *Bacillus subtilis* GB03, a PGPR, produces volatile 2,3-butanediol, which promotes plant growth and induces ISR against the soft-rot bacterial pathogen *Erwinia carotovora* subsp. *carotovora* strain SCC1 [72,73]. This occurs through EIN2, in which the knockout induces insensitivity to ethylene and cytokinins [76,77]. Moreover, the *AHK4*-deficient *cre1* mutation abolishes the growth promotion and ISR effect of GB03 in *Arabidopsis*, suggesting a role of cytokinins in these processes (Hyo Bee Park and Choong-Min Ryu, unpublished data). Furthermore, it seems that cytokinins alone can prime the defense response [17]. Pre-treatment of *t*Z 1 day before *Pst* DC3000 inoculation leads to rapid hyper-activation of *PR1*. Because priming does not accompany constitutive activation of the defense response and it specifically hyper-activates defense-related genes when plants encounter pathogens, primed plants can efficiently protect themselves against pathogens with affordable loss of growth [67,78,79]. Although it is still unknown how cytokinins induce priming in the defense response, the activation of cytokinin signaling and activity can be a key step in developing crop plants with enhanced immunity and growth.

Conclusions and further perspectives

Although cytokinins are known to affect plant responses to various pathogens, the underlying molecular mechanism has been obscure. Plant cytokinin receptors, mainly AHK3 in *Arabidopsis*, are able to recognize both plant- and microbe-derived cytokinins, but they elicit different outputs: the activation of a defense response or the development of pathogenic symptoms, respectively [16,17]. The magnitude of cytokinin signaling, described in the first section of this review, cannot solely explain this opposite outcome in the plant defense response, as hyper-activated cytokinin signaling in *uni-1D* led to SA accumulation, not the suppression of defense signaling [33]. Cytokinins can regulate distinct processes such as cell proliferation and the defense response by employing a different downstream subset of signaling components [2,17,46]. Hence, it is possible that unknown effector proteins or pathogen-derived auxins specifically suppress the signaling components of cytokinin-mediated defense responses (Figure 1) [25]. Therefore, a clearer picture of cytokinin-induced defense

responses to a variety of pathogens can be obtained through several lines of investigation. These include the examination of different or opposite cytokinin signaling outputs depending on the effective cytokinin dose on the plant defense response to non-cytokinin-secreting pathogens; identification of pathogenic effectors, if they exist, which specifically disrupt cytokinin-induced immunity through ARR2 and TGA3 interaction or currently unrevealed components; and analysis of auxin signaling mutants, such as auxin receptor *tir1* or *afb* knockouts, for induced susceptibility by pathogen-derived cytokinins.

Maintaining the identity of meristematic cells and protecting meristematic tissues from damage are critical to suppressing pathological phenotypes such as gall formation resulting from aberrant activation of cell division and to sustain growth potential after pathogen attacks to assure proper plant development and pathogen resistance. Cytokinins are enriched in the shoot apical meristem and immature leaves [8]. They are essential to maintain the identity of meristematic cells, and they regulate nutrient sink and source activity for the sustained proliferation of meristematic cells [4]. Cytokinins also promote crop productivity by the activation of inflorescence meristems, as in rice [9]. Moreover, a recent study has indicated that elevated cytokinin levels maintain high cellular redox potentials during drought, thereby reducing irreversible damage from reactive oxygen species to induce drought tolerance in tobacco [11]. These distinct features of cytokinins maintain plant health by simultaneously enhancing cell division activity and resistance to biotic and abiotic stresses. For this reason, cytokinin signaling cascades and homeostasis might be important application targets for generating economically competitive crop plants and increasing plant biomass for biofuel production. In this respect, it is critical to identify the direct regulatory targets of ARR2, an important coordinator of growth and defense, in plant development and defense responses not involving *PR* genes. Cytokinins are likely to be involved in other defense responses in addition to SA signaling [17,27,28]. Accordingly, the identification of other active ARRs and cytokinin-responsive transcription factors responsible for SA-independent cytokinin-regulated immunity through interaction with other defense-related phytohormones, MAMP-triggered immunity, R-protein-mediated defense, and priming can contribute significantly to translating research into agricultural applications.

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References

1 Choi, J. and Hwang, I. (2007) Cytokinin: perception, signal transduction, and role in plant growth and development. *J. Plant Biol.* 50, 98–108
2 Kim, H.J. *et al.* (2006) Cytokinin-mediated control of leaf longevity by AHK3 through phosphorylation of ARR2 in *Arabidopsis*. *Proc. Natl. Acad. Sci. U.S.A.* 103, 814–819
3 Matsumoto-Kitano, M. *et al.* (2008) Cytokinins are central regulators of cambial activity. *Proc. Natl. Acad. Sci. U.S.A.* 105, 20027–20031
4 Sakakibara, H. (2006) Cytokinins: activity, biosynthesis, and translocation. *Annu. Rev. Plant Biol.* 57, 431–449
5 Leibfried, A. *et al.* (2005) *WUSCHEL* controls meristem function by direct regulation of cytokinin-inducible response regulators. *Nature* 438, 1172–1175
6 Hwang, I. and Sakakibara, H. (2006) Cytokinin biosynthesis and perception. *Physiol. Plant.* 126, 528–538
7 Lara, M.E.B. *et al.* (2004) Extracellular invertase is an essential component of cytokinin-mediated delay of senescence. *The Plant Cell* 16, 1276–1287
8 Zhao, Z. *et al.* (2010) Hormonal control of the shoot stem-cell niche. *Nature* 465, 1089–1092
9 Kurakawa, T. *et al.* (2007) Direct control of shoot meristem activity by a cytokinin-activating enzyme. *Nature* 445, 652–655
10 Ashikari, M. *et al.* (2005) Cytokinin oxidase regulates rice grain production. *Science* 309, 741–745
11 Rivero, R.M. *et al.* (2007) Delayed leaf senescence induces extreme drought tolerance in a flowering plant. *Proc. Natl. Acad. Sci. U.S.A.* 104, 19631–19636
12 Robert-Seilaniantz, A. *et al.* (2007) Pathological hormone imbalances. *Curr. Opin. Plant Biol.* 10, 372–379
13 Walters, D.R. and McRoberts, N. (2006) Plants and biotrophs: a pivotal role for cytokinins? *Trends Plant Sci.* 11, 581–586
14 Walters, D.R. *et al.* (2008) Are green islands red herrings? Significance of green islands in plant interactions with pathogens and pests. *Biol. Rev. Camb. Philos. Soc.* 83, 79–102
15 Pertry, I. *et al.* (2010) *Rhodococcus fascians* impacts plant development through the dynamic Fas-mediated production of a cytokinin mix. *Mol. Plant-Microbe Interact.* 23, 1164–1174
16 Pertry, I. *et al.* (2009) Identification of *Rhodococcus fascians* cytokinins and their *modus operandi* to reshape the plant. *Proc. Natl. Acad. Sci. U.S.A.* 106, 929–934
17 Choi, J. *et al.* (2010) The cytokinin-activated transcription factor ARR2 promotes plant immunity via TGA3/NPR1-dependent salicylic acid signaling in *Arabidopsis*. *Dev. Cell* 19, 284–295
18 Lee, C-W. *et al.* (2009) *Agrobacterium tumefaciens* promotes tumor induction by modulating pathogen defense in *Arabidopsis thaliana*. *The Plant Cell* 21, 2948–2962
19 Depuydt, S. *et al.* (2008) Modulation of the hormone setting by *Rhodococcus fascians* results in ectopic *KNOX* activation in *Arabidopsis*. *Plant Physiol.* 146, 1267–1281
20 Goethals, K. *et al.* (2001) Leafy gall formation by *Rhodococcus fascians*. *Annu. Rev. Phytopathol.* 39, 27–52
21 Bari, R. and Jones, J.D.G. (2009) Role of plant hormones in plant defense responses. *Plant Mol. Biol.* 69, 473–488
22 Jameson, P. (2000) Cytokinins and auxins in plant-pathogen interactions – An overview. *Plant Growth Regul.* 32, 369–380
23 Yanai, O. *et al.* (2005) *Arabidopsis* KNOXI proteins activate cytokinin biosynthesis. *Curr. Biol.* 15, 1566–1571
24 Jasinski, S. *et al.* (2005) KNOX action in *Arabidopsis* is mediated by coordinate regulation of cytokinin and gibberellin activities. *Curr. Biol.* 15, 1560–1565
25 Depuydt, S. *et al.* (2009) An integrated genomics approach to define niche establishment by *Rhodococcus fascians*. *Plant Physiol.* 149, 1366–1386
26 Masuta, C. *et al.* (1995) Broad resistance to plant viruses in transgenic plants conferred by antisense inhibition of a host gene essential in S-adenosylmethionine-dependent transmethylation reactions. *Proc. Natl. Acad. Sci. U.S.A.* 92, 6117–6121
27 Sano, H. *et al.* (1996) Regulation by cytokinins of endogenous levels of jasmonic and salicylic acids in mechanically wounded tobacco plants. *Plant Cell Physiol.* 37, 762–769
28 Sano, H. *et al.* (1994) Expression of the gene for a small GTP binding protein in transgenic tobacco elevates endogenous cytokinin levels, abnormally induces salicylic acid in response to wounding, and increases resistance to tobacco mosaic virus infection. *Proc. Natl. Acad. Sci. U.S.A.* 91, 10556–10560
29 Gális, I. *et al.* (2004) Salicylic acid-, but not cytokinin-induced resistance to WClMV is associated with increased expression of SA-dependent resistance genes in *Phaseolus vulgaris*. *J. Plant Physiol.* 161, 459–466

30 Bent, A.F. and Mackey, D. (2007) Elicitors, effectors, and R genes: the new paradigm and a lifetime supply of questions. *Annu. Rev. Phytopathol.* 45, 399–436
31 Uchida, N. and Tasaka, M. (2010) Intersections between immune responses and morphological regulation in plants. *J. Exp. Biol.* 61, 2539–2547
32 Chung, K-M. *et al.* (2008) New perspectives on plant defense responses through modulation of developmental pathways. *Mol. Cells* 26, 107–112
33 Igari, K. *et al.* (2008) Constitutive activation of a CC-NB-LRR protein alters morphogenesis through the cytokinin pathway in *Arabidopsis*. *Plant J.* 55, 14–27
34 Shirasu, K. (2009) The HSP90-SGT1 chaperone complex for NLR immune sensors. *Annu. Rev. Plant Biol.* 60, 139–164
35 Botër, M. *et al.* (2007) Structural and functional analysis of SGT1 reveals that its interaction with HSP90 is required for the accumulation of Rx, an R protein involved in plant immunity. *The Plant Cell* 19, 3791–3804
36 Bieri, S. *et al.* (2004) RAR1 positively controls steady state levels of barley MLA resistance proteins and enables sufficient MLA6 accumulation for effective resistance. *Plant Cell* 16, 3480–3495
37 Shirasu, K. *et al.* (1999) A novel class of eukaryotic zinc-binding proteins is required for disease resistance signaling in barley and development in *C. elegans*. *Cell* 99, 355–366
38 Shang, Y. *et al.* (2006) RAR1, a central player in plant immunity, is targeted by *Pseudomonas syringae* effector *AvrB*. *Proc. Natl. Acad. Sci. U.S.A.* 103, 19200–19205
39 Belkhadir, Y. *et al.* (2004) *Arabidopsis* RIN4 negatively regulates disease resistance mediated by RPS2 and RPM1 downstream or independent of the NDR1 signal modulator and is not required for the virulence functions of bacterial type III effectors AvrRpt2 or AvrRpm1. *The Plant Cell* 16, 2822–2835
40 Kim, M.G. *et al.* (2005) Two *Pseudomonas syringae* type III effectors inhibit RIN4-regulated basal defense in *Arabidopsis*. *Cell* 121, 749–759
41 Clay, N.K. *et al.* (2009) Glucosinolate metabolites required for an *Arabidopsis* innate immune response. *Science* 323, 95–101
42 Hwang, I. *et al.* (2002) Two-component signal transduction pathways in *Arabidopsis*. *Plant Physiol.* 129, 500–515
43 Perilli, S. *et al.* (2010) The molecular basis of cytokinin function. *Curr. Opin. Plant Biol.* 13, 21–26
44 Moubayidin, L. *et al.* (2009) Cytokinin-auxin crosstalk. *Trends Plant Sci.* 14, 557–562
45 Ioio, R.D. *et al.* (2008) A genetic framework for the control of cell division and differentiation in the root meristem. *Science* 322, 1380–1384
46 Ishida, K. *et al.* (2008) Three type-B response regulators, ARR1, ARR10 and ARR12, play essential but redundant roles in cytokinin signal transduction throughout the life cycle of *Arabidopsis thaliana*. *Plant Cell Physiol.* 49, 47–57
47 Argyros, R.D. *et al.* (2008) Type B response regulators of *Arabidopsis* play key roles in cytokinin signaling and plant development. *The Plant Cell* 20, 2102–2116
48 Ioio, R.D. *et al.* (2007) Cytokinins determine *Arabidopsis* root-meristem size by controlling cell differentiation. *Curr. Biol.* 17, 678–682
49 Mason, M.G. *et al.* (2005) Multiple type-B response regulators mediate cytokinin signal transduction in *Arabidopsis*. *Plant Cell* 17, 3007–3018
50 Hass, C. *et al.* (2004) The response regulator 2 mediates ethylene signalling and hormone signal integration in *Arabidopsis*. *EMBO J.* 23, 3290–3302
51 Imamura, A. *et al.* (2003) *In vivo* and *in vitro* characterization of the ARR11 response regulator implicated in the His-to-Asp phosphorelay signal transduction in *Arabidopsis thaliana*. *Plant Cell Physiol.* 44, 122–131
52 Sakai, H. *et al.* (2000) *Arabidopsis* ARR1 and ARR2 response regulators operate as transcriptional activators. *Plant J.* 24, 703–711
53 Brodersen, P. *et al.* (2006) Arabidopsis MAP kinase 4 regulates salicylic acid- and jasmonic acid/ethylene-dependent responses via EDS1 and PAD4. *Plant J.* 47, 532–546
54 Jirage, D. *et al.* (1999) *Arabidopsis thaliana* PAD4 encodes a lipase-like gene that is important for salicylic acid signaling. *Proc. Natl. Acad. Sci. U.S.A.* 96, 13583–13588
55 Wiermer, M. *et al.* (2005) Plant immunity: the EDS1 regulatory node. *Curr. Opin. Plant Biol.* 8, 383–389
56 Mishina, T.E. and Zeier, J. (2006) The *Arabidopsis* flavin-dependent monooxygenase FMO1 is an essential component of biologically induced systemic acquired resistance. *Plant Physiol.* 141, 1666–1675
57 Song, J.T. *et al.* (2004) A key role for ALD1 in activation of local and systemic defenses in *Arabidopsis*. *Plant J.* 40, 200–212
58 Weigel, R.R. *et al.* (2005) Interaction of NIMIN1 with NPR1 modulates *PR* gene expression in *Arabidopsis*. *The Plant Cell* 17, 1279–1291
59 Zhang, Z. *et al.* (2008) A lesion-mimic syntaxin double mutant in *Arabidopsis* reveals novel complexity of pathogen defense signaling. *Mol. Plant* 1, 510–527
60 Delledonne, M. *et al.* (2001) Signal interactions between nitric oxide and reactive oxygen intermediates in the plant hypersensitive disease resistance response. *Proc. Natl. Acad. Sci. U.S.A.* 98, 13454–13459
61 García-Mata, C. and Lamattina, L. (2003) Abscisic acid, nitric oxide and stomatal closure - is nitrate reductase one of the missing links? *Trends Plant Sci.* 8, 20–26
62 Desikan, R. *et al.* (2002) A new role for an old enzyme: nitrate reductase-mediated nitric oxide generation is required for abscisic acid-induced stomatal closure in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. U.S.A.* 99, 16314–16318
63 Ma, W. *et al.* (2009) Ca2+, cAMP, and transduction of non-self perception during plant immune responses. *Proc. Natl. Acad. Sci. U.S.A.* 106, 20995–21000
64 Melotto, M. *et al.* (2006) Plant stomata function in innate immunity against bacterial invasion. *Cell* 126, 969–980
65 Tun, N.N. *et al.* (2008) Zeatin-induced nitric oxide (NO) biosynthesis in *Arabidopsis thaliana* mutants of NO biosynthesis and of two-component signaling genes. *New Phytol.* 178, 515–531
66 Romanov, G.A. *et al.* (2008) Does NO play a role in cytokinin signal transduction? *FEBS Lett.* 582, 874–880
67 Hulten, M.V. *et al.* (2006) Costs and benefits of priming of defense in *Arabidopsis*. *Proc. Natl. Acad. Sci. U.S.A.* 103, 5602–5607
68 Jung, H.W. *et al.* (2009) Priming in systemic plant immunity. *Science* 324, 89–91
69 Vlot, A.C. *et al.* (2008) Systemic acquired resistance: the elusive signal(s). *Curr. Opin. Plant Biol.* 11, 436–442
70 Wees, S.C.M.V. *et al.* (2008) Plant immune responses triggered by beneficial microbes. *Curr. Opin. Plant Biol.* 11, 443–448
71 Yang, J. *et al.* (2009) Rhizosphere bacteria help plants tolerate abiotic stress. *Trends Plant Sci.* 14, 1–4
72 Ryu, C-M. *et al.* (2004) Bacterial volatiles induce systemic resistance in *Arabidopsis*. *Plant Physiol.* 134, 1017–1026
73 Ryu, C-M. *et al.* (2003) Bacterial volatiles promote growth in *Arabidopsis*. *Proc. Natl. Acad. Sci. U.S.A.* 100, 4927–4932
74 Yi, H-S. *et al.* (2009) Airborne induction and priming of plant defenses against a bacterial pathogen. *Plant Physiol.* 151, 2152–2161
75 Frost, C.J. *et al.* (2008) Plant defense priming against herbivores: getting ready for a different battle. *Plant Physiol.* 146, 818–824
76 Alonso, J.M. *et al.* (1999) EIN2, a bifunctional transducer of ethylene and stress responses in *Arabidopsis*. *Science* 284, 2148–2152
77 Cary, A.J. *et al.* (1995) Cytokinin action is coupled to ethylene in its effects on the inhibition of root and hypocotyl elongation in *Arabidopsis thaliana* seedlings. *Plant Physiol.* 107, 1075–1082
78 Gust, A.A. *et al.* (2010) Biotechnological concepts for improving plant innate immunity. *Curr. Opin. Biotechnol.* 21, 204–210
79 Beckers, G.J.M. and Conrath, U. (2007) Priming for stress resistance: from the lab to the field. *Curr. Opin. Plant Biol.* 10, 425–431