pathway is currently being delineated through the use of gain-of-function and loss-of-function mutants. Protein kinases are implicated in early events of jasmonate signaling (*14*). Selective proteolysis may be important in the regulation of jasmonate-dependent gene expression by contributing to the removal of transcriptional regulators from target genes (*15*). Progress in identifying other key regulatory genes is imminent (*16*–*18*). At least five likely developments, from studies currently under way by several groups, should add considerably to interest in the jasmonate pathway. First, the identification of target genes regulated by different jasmonates will clarify how these molecules exert their multiple remarkable biological effects. Second, a better understanding of jasmonate perception will provide vital knowledge on many aspects of regulation. Third, progress on the cell biology of jasmonates will shed light on how these compounds are transported within and between cells and tissues to reach their sites of action. Fourth, more detailed knowledge about how the jasmonate pathway contributes to the coordination of direct defense responses (that is, regulation of defense genes within the plant) and indirect defense responses (for example, control of the behavior of predatory insects in the plant's environment) will permit an understanding of how plants extend their defense umbrella. Finally, further investigation of gas-phase signaling by volatile methyl jasmonate (MJ) within the plant may contribute to the general knowledge of the biology of volatile regulators of gene expression. Aside from answering these basic science questions, advances in the manipulation of the pathway hold promise for future strategies in agriculture. The Jasmonate Biochemical Pathway (http://stke.sciencemag.org/cgi/cm/CMP_7361) (*19*) of the STKE Connections Maps is designed to keep pace with these and other exciting developments. Additionally, the Jasmonate Biochemical Pathway will have interfaces with the Connections Maps of other signaling pathways in plants.

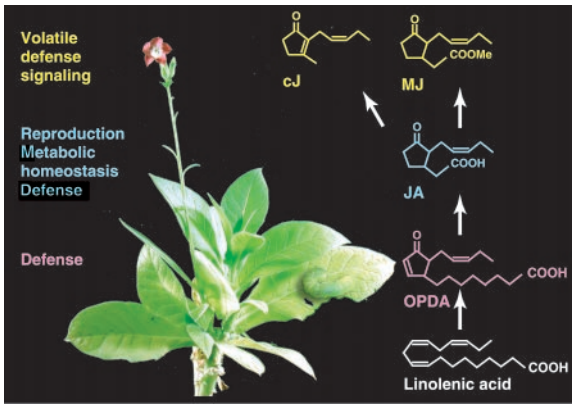

Fig. 1. The jasmonate family of regulators is involved in diverse aspects of plant biology. OPDA is an octadecanoid derived from linolenic acid. It is one of the jasmonates, which, together with jasmonic acid (JA), helps control defense responses. JA also plays key roles in reproduction, and its metabolites methyl jasmonate (MJ) and *cis*-jasmone (cJ) are both volatile and may act as signals both within plants and to associated insects.

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VIEWPOINT

Phosphorelay and Transcription Control in Cytokinin Signal Transduction

Jen Sheen

The past decade has seen substantial advances in knowledge of molecular mechanisms and actions of plant hormones, but only in the past few years has research on cytokinins begun to hit its stride. Cytokinins are master regulators of a large number of processes in plant development, which is known to be unusually plastic and adaptive, as well as resilient and perpetual. These characteristics allow plants to respond sensitively and quickly to their environments. Recent studies have demonstrated that cytokinin signaling involves a multistep two-component signaling pathway, resulting in the development of a canonical model of cytokinin signaling that is likely representative in plants. This Viewpoint outlines this general model, focusing on the specific example of *Arabidopsis*, and introduces the STKE Connections Maps for both the canonical module and the specific *Arabidopsis* Cytokinin Signaling Pathway.

Cytokinins are essential plant hormones that control cell division, shoot meristem initiation, leaf and root differentiation, chloroplast biogenesis, stress tolerance, and senescence. Together with auxin, another plant hormone, cytokinins can reprogram terminally differentiated leaf cells into stem cells and support shoot regeneration indefinitely in plant tissue culture (*1*, *2*). Thus, cytokinins are master regulators of plant growth and development, which are highly plastic and adaptive, as well as remarkably resilient and perpetual. Research interest in the signaling pathways activated by cytokinins has increased recently because of new information arising from studies of *Arabidopsis* and the completion of its genome sequence. However, the importance of this pathway is given additional weight because it represents two-component signaling, a canonical mechanism that mediates diverse biological responses in many taxa. The specific Cytokinin Signaling Pathway (*3*) details the pathway as it has been elucidated in *Arabidopsis*; the canonical Cytokinin Signaling Pathway presents the general view (*4*).

In the *Arabidopsis* cytokinin signal transduction pathway, hybrid histidine protein kinases (AHKs) serve as cytokinin receptors and histidine phosphotransfer proteins (AHPs) transmit the signal from AHKs to nuclear response regulators (ARRs), which can activate or repress transcription (*5*–*10*). Similar components are also found in maize,

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suggesting a conservation of the cytokinin signaling mechanism in plants (*11*). There are four major steps to cytokinin signaling: AHK sensing and signaling, AHP nuclear translocation, ARR transcription activation, and a negative feedback loop through cytokinin-inducible *ARR* gene products (Fig. 1). Analyses of mutants and transgenic tissues and plants support the importance of this central signaling pathway in diverse cytokinin responses (*5*–*10*). The multistep two-component phosphorelay mechanism found in *Arabidopsis* is reminiscent of the bacterial two-component signaling system (*12*), but it is linked by AHPs, which shuttle from the cytoplasm to the nucleus in a cytokinin-dependent manner (*6*). Although conserved motifs for two-component phosphorelay systems have been identified in plant hormone ethylene receptors (*13*), phytochrome photoreceptors (*14*), and a putative osmosensor (*15*), until recently the importance of histidine protein kinase activity and phosphorelay had not been demonstrated in plant cells. Functional analyses of AHKs, AHPs, and ARRs in *Escherichia coli*, yeasts, plants, and a leaf protoplast system, and protein-protein interactions in yeast two-hybrid assays, have provided compelling evidence for the importance of multistep two-component phosphorelay in cytokinin signaling (*5*–*10*, *16*–*18*).

In *Arabidopsis*, at least three genes encode cytokinin receptors: *AHK4* [also known as *CYTOKININ RESPONSE 1* (*CRE1*) and *WOODEN LEG* (*WOL*)], *AHK2*, and *AHK3* (*7*, *19*, *20*). Other *Arabidopsis* histidine protein kinases, cytokinin independent 1 (CKI1) and CKI2 (also known as AHK5), can also activate cytokinin responses in the absence of exogenously added cytokinin (*5*, *6*). Quantitative transcription analyses based on cytokinin-inducible *ARR6-LUC* reporter gene activity suggest that CKI1 and AHKs act through different cytokinin perception mechanisms. CKI1 is constitutively active, but AHK4, AHK2, and AHK3 require extracellular cytokinin for their activation (*6*). The function of AHK4 has been thoroughly demonstrated by direct cytokinin binding (*21*) and by the isolation of *cre1* and *wol* mutants that exhibit defects in cytokinin-mediated shoot induction from callus and root vascular morphogenesis, respectively (*7*, *19*). The lack of shoot phenotypes in *cre1* and *wol* suggests that the functions of AHK2 and AHK3 may overlap with that of AHK4 (*20*). Further analyses of cellular expression patterns, cytokinin binding, and chimeric AHKs with swapped domains should clarify the underlying mechanism of each AHK action in cytokinin signaling.

The analysis of fusions between green fluorescent protein (GFP) and AHP (AHP-GFP) has provided the first visual, in vivo evidence that AHP1 and AHP2 are translocated into the nucleus in a cytokinin-dependent manner (*6*). In *Arabidopsis*, there are more AHKs, ARRs, and related proteins than there are AHPs (*18*, *22*), suggesting that multiple two-component signaling pathways may share AHPs (*6*, *10*). The cytokinin pathway does not follow the established eukaryotic histidine protein kinase and mitogen-activated protein kinase (MAPK) cascade paradigm (*23*), but rather integrates multiple AHK activities to common AHPs, which then modulate distinct ARRs in the nucleus (*6*).

The B-type ARR transcription activators (ARR1, ARR2, and ARR10) carry MYB-like

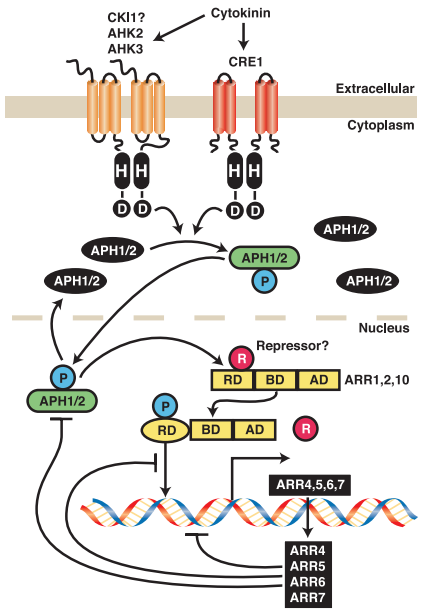

Fig. 1. Model of the cytokinin signal transduction pathway in *Arabidopsis*. Cytokinin signal is perceived by multiple histidine protein kinases at the plasma membrane. After perception of the cytokinin signal, these histidine protein kinases initiate a signaling cascade through the phosphorelay that results in the nuclear translocation of AHPs from the cytosol. Activated AHPs interact with sequestered ARRs or ARR complexes in the nucleus, transfer the phosphate to the receiver domain of their cognate B-type ARRs, and in turn release the transcription activator ARRs from putative repressors in the nucleus. The dephosphorylated AHP shuttles back to the cytosol, where it can be rephosphorylated. The liberated ARRs bind to multiple cis elements in the promoters of target genes. The activation of the transcription repressor *ARR*s as cytokinin primary response genes provides a negative feedback mechanism. RD, response domain; BD, DNA binding domain; AD, transcription activation domain; R, putative repressor.

domains for DNA binding and a glutamine (Q)-rich domain for transcriptional activation (*24*, *25*), and they activate cytokinin-responsive *ARR6* transcription (*6*, *8*). These activators appear to be the evolutionary products of domain shuffling, with ancestral modules originating from both prokaryotic and eukaryotic heritage. Mutation in the conserved aspartate residue of ARR2 does not abolish its function as a transcription activator for a cytokinin early-response gene *ARR6* promoter, suggesting that phosphorylation may not intrinsically activate the transcription factor (Fig. 1) (*6*). Consistently, deletion of the receiver domain of ARR1 results in higher transcription activity in plant cells and constitutive cytokinin phenotypes in transgenic plants (*8*, *24*). Thus, phosphorylation of ARR1 and ARR2 likely eliminates negative regulation (Fig. 1). Ectopic expression in transgenic *Arabidopsis* of ARR2, one of the rate-limiting transcription factors in the response to cytokinin, is sufficient to mimic cytokinin in promoting shoot meristem proliferation and leaf differentiation, and in delaying leaf senescence (*6*). The lack of striking phenotypes in the *arr1* mutant indicates that multiple B-type ARRs may serve similar functions (*6*, *8*). Determining the target genes of these transcription factors using microarrays will add new insight into the molecular basis of cytokinin actions.

The products of the cytokinin-inducible A-type *ARR4*, *ARR5*, *ARR6*, and *ARR7* genes inhibit transcription, which could mediate a negative feedback loop that controls the transient induction of cytokinin primary response genes and allows resetting and/or fine-tuning of the physiological state of the cells (Fig. 1) (*6*, *16*). Although the B-type ARRs with transcriptional activation activities are likely the major regulators of a broad spectrum of cytokinin target genes (*26*), the A-type ARRs could also contribute to the outputs of cytokinin signaling through protein-protein interactions (*16*, *17*).

Two-component elements could potentially be regulated by signals other than cytokinin and provide a cross-talk mechanism in plant signaling networks. For instance, expression of some *ARR*s is regulated by stress (*27*) and sugar signals (*28*). ARR4 also interacts with phytochrome B and modulates light signaling (*29*). Thus, two-component elements could serve as the molecular links in a complex plant signal transduction network that sensitively integrates central growth signals such as plant hormones, sugars, light, and other environmental cues.

The expression analysis of *CYCLIN D* (*30*) and an *ARR5*::*GUS* transgene (*31*) in *Arabidopsis* has shown that root and shoot meristems are major sites of cytokinin actions. However, cytokinin responses can also occur in other cell types (*6*, *31*). This broad cellular competence to cytokinin responses may explain the plasticity of plant development. The emerging short cytokinin signaling circuit could represent a conserved core signaling pathway in different cell types in response to cytokinin. However, additional cell type–specific components are likely to play important roles for cytokinin responses in different cell types and tissues, for example, in dividing and nondividing cells. Elucidation of the expression patterns and subcellular localization of AHKs, AHPs, and ARRs will contribute to a better understanding of their unique or overlapping roles in cytokinin responses and in other two-component signaling pathways in plants. The major challenge is to determine how a con-

served cytokinin signal transduction pathway influences cell cycle, leaf senescence, shoot initiation, and leaf patterning in different cell types at various developmental stages.

The completion of the *Arabidopsis* genome sequence has revealed 54 genes encoding putative AHKs, AHPs, ARRs, and related proteins, suggesting a substantial involvement of this signaling mechanism in many facets of plant cell regulation (*17*, *18*, *32*). The development of the *Arabidopsis* protoplast system has enabled a high-throughput functional genomic analysis of the two-component regulators (*6*). Because pronounced redundancy in the *Arabidopsis* genome is evident (*18*, *32*), cellular analyses of the two-component elements would complement the characterization of a large number of insertion mutants that may not display overt phenotypes. Genetic, genomic, and biochemical experiments will elucidate the details in cytokinin perception, protein-protein interactions, and target gene expression essential in cytokinin signaling.

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VIEWPOINT

Integrin Connections Map: To Infinity and Beyond

Karen H. Martin, Jill K. Slack, Scott A. Boerner, Clifford C. Martin, J. Thomas Parsons*

Integrins are transmembrane proteins that serve as primary sensors of the extracellular matrix (ECM) environment. In response to interactions with the ECM, integrins initiate signaling pathways that regulate cell migration, growth, and survival. Advances in imaging have contributed to the understanding of the dynamic nature of these cell-ECM interactions and the complexes that form at these sites and have provided insights into their regulation and signal organizing functions.

Integrins are primary sensors of the extracellular matrix (ECM) environment and are thus essential for cell migration, growth, and survival. As transmembrane receptors, integrins recognize and bind to specific ECM ligands and transduce signals leading to the activation of intracellular signaling pathways and the assembly of actin-based adhesion structures that propagate cellular forces. Integrin research (described in more than 20,000 literature citations) has enumerated a large number of pathways and proteins thought to be important in integrin function. The Integrin Signaling Pathway (http://stke.sciencemag.org/cgi/cm/CMP_6880) in the STKE Connections Maps highlights the current state of knowledge of integrin function in nonlymphocytic cells and identifies key (but not all) pathways linked to these receptors (*1*).

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Integrins play a central role in organizing the actin cytoskeleton at sites of adhesion to the extracellular matrix (*2*). There has been a growing appreciation of the molecular heterogeneity and dynamic nature of integrin adhesion complexes (*3*, *4*). Although cells form functionally distinct adhesion complexes (for example, focal complexes, which are specific structures localized to the leading edge of migrating cells, and focal adhesions, which are beneath the cell body), a common feature of all adhesion complexes is their linkage to the actin cytoskeleton. Binding of proteins such as α-actinin and talin to integrin cytoplasmic tails, and the subsequent recruitment of the actin-binding protein vinculin and modulators of actin dynamics [such as vasodilator-stimulated phosphoprotein (VASP)], are important steps in linking adhesion complexes to the actin cytoskeleton (Fig. 1, orange). Integrins also regulate signaling pathways to members of the Rho family of small guanosine triphosphatases (GTPases) Cdc42, Rac, and Rho, which are molecular switches that control the dynamics and structure of actin-based processes, such as filopodia, lamellipodia, and stress fiber formation (Fig. 1, orange). Activation of Cdc42 and Rac contributes to the organization of actin networks at the leading edge of migrating cells. Activation of Rho is important for the organization of stress fibers and the regulation of acto-myosin contractility through myosin light chain kinase (MLCK) phosphorylation of myosin regulatory light chains (RLCs). Integrins also contribute to the dynamic turnover and remodeling of adhesion complexes by activating the focal adhesion kinase (FAK) and Src protein tyrosine kinase axis of signaling proteins, which includes the cytoskeletal regulator paxillin (Fig. 1, green). Inhibition or loss of components in this pathway (for example, FAK-null or paxillin-null fibroblasts) severely restricts adhesion complex turnover and inhibits integrin-dependent functions, such as cell migration (Fig. 1, red).

Integrin activation of members of the Ras family of GTPases (Ras, R-Ras, and Rap-1) appears to be important for the downstreamFn1 activation of serine-threonine kinases, such as extracellular signal-regulated kinase (ERK), p21-activated kinase (PAK), and c-Jun NH_2-terminal kinase (JNK), key regulators of gene expression and cell cycle progression (Fig. 1, purple). In addition, both ERK and PAK phosphor-