Molecular mechanisms of cytokinin action

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Cytokinins have been implicated in many apsects of plant development, including a crucial role in regulating cell proliferation. Recent studies indicate that cytokinins may elevate cell division rates by induction of expression of *CycD3*, which encodes a D-type cyclin thought to play a role in the G1→M transition of the cell cycle. Progress has also been made in our understanding of cytokinin perception as homologs of two-component phosphorelay systems have emerged as likely signaling elements.

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Abbreviations

AHP	*Arabidopsis* histidine phosphotransfer protein
CDK	cyclin dependent kinase
SAM	shoot apical meristem

Introduction

Cytokinins, N^6-substituted adenine derivatives, are a class of plant hormones that were first identified as factors that promoted cell division [1,2], and have since been implicated in many other aspects of plant growth and development including shoot initiation and growth, apical dominance, senescence, and photomorphogenic development [3]. Although the physiological effects of cytokinin have been well characterized, the molecular mechanisms underlying cytokinin action remain obscure [3,4]. This review will focus on recent progress made in *Arabidopsis* in defining the role of cytokinin in cell division, and on our current understanding of cytokinin signal transduction.

Cytokinin and the cell cycle

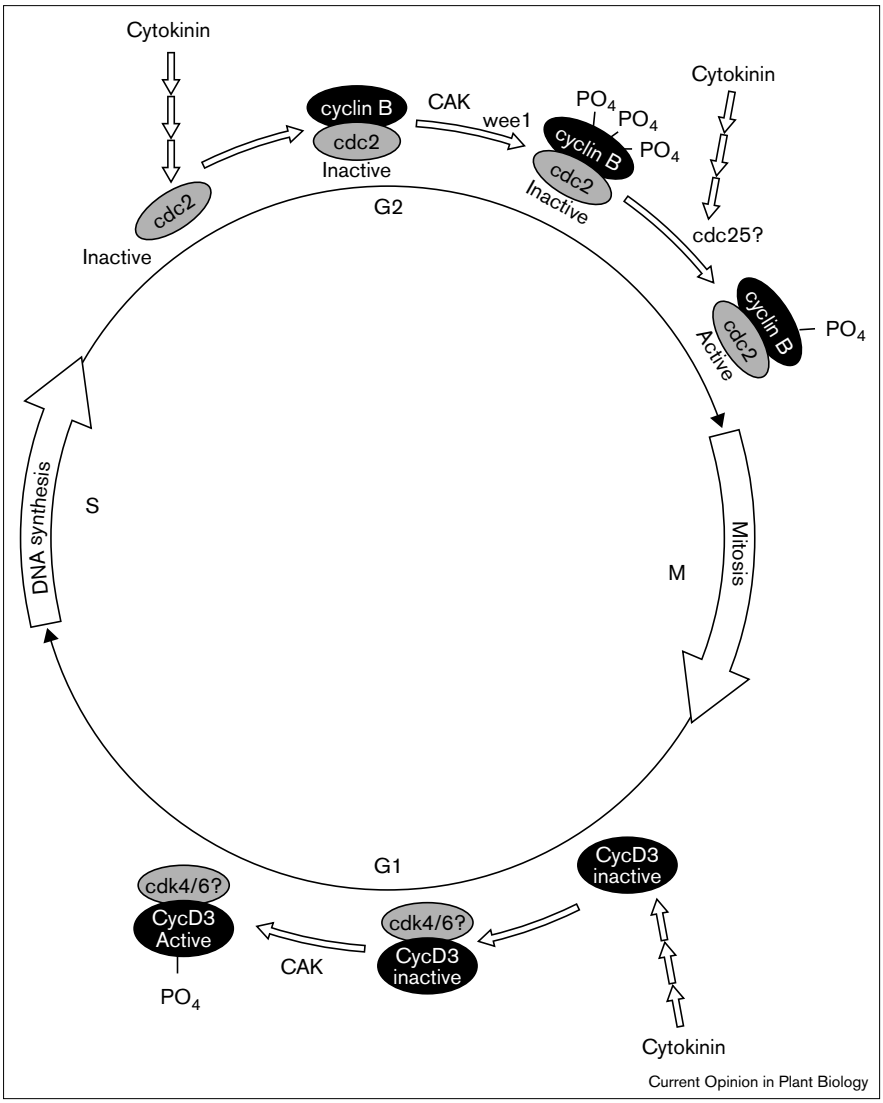
Cytokinins are required, in concert with auxin, for cell division in a wide variety of cultured plant cells. There is also evidence that cytokinin may play a role in stimulating cell division *in vivo*. Immunocytochemistry and direct measurements of cytokinin both reveal high cytokinin levels in mitotically active areas, such as the root and shoot meristems, and very low levels are found in tissues where the cell cycle is arrested [3,5]. Application of exogenous cytokinin to some organs that normally lack this hormone has been shown to induce cell division. Cytokinins have been linked to virtually all stages of the cell cycle, but there has been little definitive evidence that any particular event in the cell cycle plays a role in cytokinin's induction of cell proliferation.

There is extensive literature regarding regulation of the cell cycle in yeast and animal cells (reviewed in [6,7]). Cell cycle progression is controlled at the G1→S and G2→M checkpoints, primarily by two classes of proteins: cyclins, and cyclin dependent kinases (CDKs) (Figure 1). Passage through the checkpoints requires the activation of CDKs, and this is achieved by association with cyclins and by altering the phosphorylation state of the CDK [8,9]. By associating with different cyclins, cdc2, the first CDK identified, controls both G1→S and G2→M transition in yeast. Animal cells have families of CDKs, some similar to cdc2, that act at G2→M, and others that are distinct and act exclusively at G1→S. B-type cyclins are the major class of mitotic cyclins, which act at the G2→M transition, and D-type cyclins are the major class involved in G1→S transition. cDNAs encoding CDKs and G1 and mitotic cyclins have been isolated in plants, and CDK inhibitors have been shown to block cell cycle progression at both G1→S and G2→M in *Arabidopsis* and *Petunia* cells [10–12]. Therefore, it is likely that these proteins also mediate the cell cycle in plants. Given the link between cytokinins and cell division, a natural question that arises is whether cytokinins affect the expression or activity of these cell cycle regulatory proteins.

Several observations suggest that cytokinins may play a role in the G2→M transition (Figure 1; reviewed in [13]), though a decisive link is lacking. For example, cytokinins induce the expression of the *cdc2* gene in a number of plant tissues, including intact *Arabidopsis* roots [14], and they have been demonstrated to influence the activity, via the phosphorylation state, of a cdc2-like kinase in tobacco protoplasts [15]. Recently, compelling evidence that cytokinin regulates the G1→S transition in the cell cycle has been obtained by Murray and co-workers [16••]. This group previously identified three different *Arabidopsis* genes encoding D-type cyclins by complementation of a yeast strain deficient in G1 cyclins, and found that one, *CycD3*, was induced in cultured cells by exogenous cytokinin application [17]. Their recent work [16••] demonstrates that cytokinin increases cell proliferation, at least in part, via an increase in *CycD3* expression.

To examine *CycD3* gene expression in response to cytokinin, *Arabidopsis* suspension culture cells were starved of cytokinin for 24 hours [16••]. Within one hour of cytokinin treatment, *CycD3* transcripts began to accumulate to higher levels. The steady-state level of *CycD3* mRNA was also found to be responsive to cytokinin application in intact seedlings. To examine *CycD3* expression in response to endogenous cytokinin, the authors employed the *Arabidopsis* mutant *altered meristem program 1* (*amp1*) [18]. The *amp1* mutant contains endogenous cytokinin levels six-times higher than that of wild-type. In addition,

Figure 1



Abbreviated model of the cell cycle and potential roles for cytokinin. Progression through the cycle occurs principally by the successive activation of a series of protein kinases, two sets of which are shown. The activation of the cyclin-dependent kinases (cdc2, cdk4, and cdk6 are depicted) is achieved by association with specific cyclins, followed by phosphorylation. In the case of cdk4 and cdk6, a cyclin activating kinase (CAK) catalyzes this reaction, whereas cdc2 requires an additional phosphorylation by the wee1 protein kinase. Application of exogenous cytokinins elevates the steady-state level of *cdc2* and *CycD3* (a D-type cyclin) transcripts, and overexpression of *CycD3* obviates the cytokinin requirement for division in culture.

the *amp1* mutation results in multiple morphological changes, including an enlarged apical meristem, increased leaf number, altered phyllotaxy, and delayed senescence. Consistent with the notion that endogenous cytokinin regulates *CycD3* gene expression, untreated *amp1* plants displayed a higher steady-state level of *CycD3* transcript relative to comparable wild-type plants.

Using *in situ* hybridization, *CycD3* was found to be expressed in the shoot meristem, leaf primordia and axillary, and its induction was also specific to those tissues. Thus, the expression of this gene correlates with proliferating tissues, as expected if it is an important element regulating cell division. If CycD3 acts downstream of cytokinin in promoting cell division or differentiation, then constitutive expression of *CycD3* should bypass the requirement of cytokinin for cell proliferation in culture. Normally, when explanted into culture, cells require both auxin and cytokinin in the media in order for cell division and callus formation to occur. When leaf explants were obtained from lines that were over-expressing *CycD3*, healthy green calli were formed independently of cytokinin, whereas wild-type controls only formed calli when cytokinin was present. To demonstrate a role for *CycD3* in cell division, the levels of S-phase associated histone H4 mRNA were examined in the leaf explants. Like the callus tissues, wild-type explants only expressed histone H4 in the presence of cytokinin, whereas lines over-expressing *CycD3* expressed histone H4 both in the presence and absence of cytokinin. Finally, the expression of *CycD3* and histone H4 mRNA was observed in parallel with DNA synthesis during synchronous activation of quiescent *Arabidopsis* cells. S phase was found to occur significantly later than the induction of *CycD3*, which implies that CycD3 may be involved in the G1→S transition. These results suggest that cytokinin regulates *Arabidopsis* cell cycle progression at the G1→S transition, at least partially, by inducing *CycD3* transcription.

Cytokinin and the shoot apical meristem

The shoot apical meristem (SAM) is a highly specialized group of cells from which the majority of the aerial portion of the plant is derived by reiterative development [19]. The ability of cytokinins to initiate shoots from callus in tissue culture and the initiation of ectopic meristems in cytokinin overproducing plants suggest a role for cytokinins in SAM development.

One possible mechanism by which cytokinins could influence SAM development is by regulating gene expression. The *knotted1* (*kn1*) homeobox family of genes, which were first identified in maize, is expressed exclusively in the SAM and is involved in its development and maintenance [19–21]. Transgenic plants over-expressing the bacterial cytokinin biosynthetic gene *ipt* have some phenotypes reminiscent of transgenic plants over-expressing *kn1*, such as a delay in senescence, reduced apical dominance, and ectopic shoot formation [19], suggesting that elevated cytokinin levels in these transgenics may induce *kn1* expression. To further address this observation, Rupp *et al.* [22••] examined the expression of *KNAT1* and *STM* (*Arabidopsis* homologs of *kn1*) in transgenic *Arabidopsis* expressing *ipt* under the control of a heat shock promoter. The steady-state mRNA levels of both *KNAT1* and *STM* were elevated following heat shock, and were correlated to elevated cytokinin levels. Elevated *KNAT1* and *STM* transcript levels were also observed in untreated *amp1* plants, implying that endogenous cytokinin can also induce expression of these homeobox genes. These results suggest that cytokinins may act upstream of KNAT1 and STM in regulating SAM development.

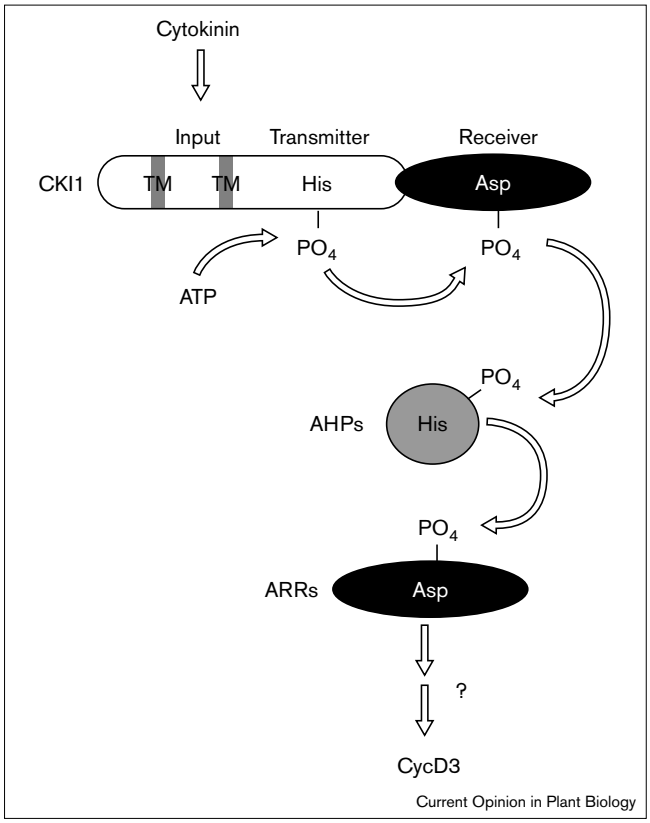
A seemingly converse relationship between cytokinin and the maize *kn1* gene was observed when *kn1* was over-expressed in tobacco [23••]. Expressing *kn1* under the control of a senescence specific promoter (SAG12) resulted in a delay of senescence, similar to the phenotype seen in plants expressing *ipt* under control of the SAG12 promoter [23••]. Intact and detached leaves stayed greener longer and displayed higher chlorophyll content than control plants. Remarkably, older SAG:*kn1* leaves had cytokinin levels 15 times higher than wild-type plants, suggesting that *kn1* may inhibit senescence by increasing cytokinin levels. These results suggest that the levels of cytokinin and *kn1* may positively regulate each other in an interdependent fashion. Alternatively, the elevation of cytokinin in connection with ectopic expression of *kn1* may not accurately reflect the endogenous relationship between cytokinin and *kn1* homologs, or may simply result from an increase in the amount of meristematic tissue, which is a primary source of cytokinin biosynthesis.

Two-component systems and cytokinin

There is an increasing body of evidence linking two-component phosphorelay homologs to plant signaling pathways, including cytokinin signaling [24]. The multi-step phosphorelay, a derivation of the simple bacterial two-component signaling mechanism, involves the sequential transfer of phosphate between histidine and aspartate residues on distinct protein domains (see Figure 2) [25,26]. Active sensor kinases are dimers that trans-phosphorylate on a conserved histidine residue in the transmitter domain. The phosphate is then transferred to an aspartate residue in a receiver domain, and from there to a histidine in a phosphotransfer domain (HPt). The final phosphorylation event is from that histidine to an aspartate on a receiver domain of a response regulator.

The first indication that cytokinin signaling might employ a phosphorelay was the identification of *CKI1*, a sensor kinase homolog that, when over-expressed, allows for cytokinin independent growth in culture [27]. The predicted CKI1 protein contains, in addition to the sensor and

Figure 2



Model for cytokinin signaling in *Arabidopsis*. CKI1 is similar to two-component hybrid kinases. It consists of an input domain that contains two predicted transmembrane domains (TM). If CKI1 is a bona fide cytokinin receptor, then the input domain is the most likely site of cytokinin binding. Upon stimulation of the input domain, CKI1, by analogy to other sensor kinases, could dimerize and autophosphorylate on a histidine residue within the transmitter domain. The phosphate would then be transferred to an aspartate residue on the fused receiver domain of CKI1, and then to a histidine on an AHP protein (an HPt domain-containing protein). Finally, the phosphate may be transferred to an aspartate residue on the receiver domain of an ARR protein. Only the final phosphorylation event has been demonstrated to occur *in vitro*. Cytokinins have been implicated in the regulation of transcription of the CycD3 gene (see text for details).

transmitter domains, an attached receiver domain, which is a common arrangement of eukaryotic sensor kinases. A gene family of response regulator homologs, called *ARR1-ARR14*, has recently been identified in *Arabidopsis* and several of these genes are regulated by cytokinin (reviewed in IB D'Agostino and JJ Kieber, unpublished data). The *ARR* genes fall into two classes, type A and type B, on the basis of their sequence similarity and the presence or absence of a carboxy-terminal putative output domain [28••] (IB D'Agostino and JJ Kieber, unpublished data). The ARR genes have been given a variety of names, but for clarity and consistency we will use the nomenclature assigned by Imamura *et al.* [28••] (ARR1–ARR14) in this review. The steady-state mRNA level of the seven type A ARR genes, which lack the putative output domain, are induced by cytokinin, but the type B ARRs are not [28••,29••,30•,31••]. Two ARR genes, *ARR4* and *ARR5* (previously called *IBC6* and *IBC7*), have been shown to display characteristics of cytokinin primary response genes [29••]. The induction of the type A genes by cytokinin, coupled with their similarity to proteins predicted to act downstream of CKI1, suggests that they may act in cytokinin signal transduction.

Homologs of the third protein domain that acts in phosphorelays, the histidine phosphotransfer domain (HPt), were recently identified in *Arabidopsis* [32•,33•]. In contrast to CKI1 and the ARRs, there is little evidence linking these histidine phosphotransfer proteins (AHPs) to cytokinin signaling. However, purified AHP1 proteins that were phosphorylated by crude bacterial membranes were capable of transferring phosphate to purified ARR3, and ARR4 *in vitro* [28••,33•]. The expression of the type B genes was not affected by treatment with plant hormones, including cytokinin.

The type B ARR genes contain large carboxy-terminal extensions that have properties of output domains, which generally act as regulators of transcription. There is a stretch of amino acids that are similar to a Myb-related motif found in some novel plant proteins [28••,34]. The carboxy-terminal domain of *ARR11* has been shown to activate transcription when fused to the GAL4 DNA binding domain [35••]. Further evidence that these proteins are transcription factors is the observation that GFP-fusions to both ARR10 and ARR 11, two type B ARRs, localize to the nucleus in transiently transformed parsley protoplasts [35••].

A model consistent with these observations is presented in Figure 2. This model relies on analogies to bacterial and yeast phosphorelays. Only the phosphotransfer from an AHP to an ARR has been demonstrated *in vitro*, and the evidence linking each module to cytokinin signaling is not definitive. It is possible that the cytokinin-inducible ARR genes, which appear to lack an output domain, act as negative regulators of the constitutive, type B ARR genes. This could explain why exogenous cytokinin induces the type A ARR genes. Further confirmation of this model awaits disruption of the function of these genes *in vivo* and a biochemical analysis of the *in vitro* properties of the purified components.

It is possible that this postulated phosphorelay mediates regulation of the *CycD3* gene by cytokinin. Interestingly, a response regulator called SKN7 has been implicated in expression of a cyclin in yeast [36]. The yeast transcription factors SBF and DCS1/MBF bind to SCB and MCB promoter elements of the G1 cyclin genes, thereby regulating cell cycle progression. SKN7 can also bind to these promoter elements, and when over-expressed, can bypass the requirement for SBF and DCS1/MBF by stimulating G1 cyclin expression [36].

Conclusions

The regulation of expression of the *CycD3* gene appears to be a key mechanism by which cytokinins influence cell proliferation. How this regulation is integrated with other signals regulating cell division, particularly auxin, remains to be determined. Furthermore, the role of cytokinins in other aspects of the cell cycle remains unclear. *In vivo*, most cell division occurs in the meristems, and a second aspect of cytokinin action is its effect on the expression of the *Kn1* gene family, key regulators of meristem function. The role of endogenous cytokinin on the expression of these genes remains to be determined. Various studies have implicated homologs of two-component systems in cytokinin signaling, though the evidence for this link is not conclusive. Given the power of emerging tools in *Arabidopsis*, one would anticipate that these genes will soon be disrupted *in vivo*, which should help elucidate their role in cytokinin action. Other approaches, such as genetic screens for cytokinin-insensitive mutants, may identify additional cytokinin signaling elements [37•]. There are many unanswered questions, but perhaps for the first time we are beginning to glimpse the molecular events underlying cytokinin action.

Note added in proof

The work referred to in the text as IB D'Agostino and JJ Kieber, unpublished data, has now been accepted for publication [38].

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Miller CO, Skoog F, von Saltza MH, Strong F: **Kinetin, a cell division factor from deoxyribonucleic acid.** *J Am Chem Soc* 1955, **77**:1392-1293.

2. Miller CO, Skoog F, Okomura FS, von Saltza MH, Strong FM: **Isolation, structure and synthesis of kinetin, a substance promoting cell division.** *J Am Chem Soc* 1956, **78**:1345-1350.

3. Mok DWS, Mok MC: *Cytokinins: Chemistry, Activity and Function*. Boca Raton, FL: CRC Press; 1994.

4. Binns AN: **Cytokinin accumulation and action: biochemical, genetic and molecular approaches.** *Annu Rev Plant Physiol Plant Mol Biol* 1994, **45**:173-196.

5. Dewitte W, Chiapetta A, Azmi A, Witters E, Strnad M, Rembur J, Noin M, Chriqui D, Van Onckelen H: **Dynamics of cytokinins in apical shoot meristems of a day-neutral tobacco during floral transition and flower formation.** *Plant Physiol* 1999, **119**:111-121.

6. Murray AW, Hunt T: *The Cell Cycle, An Introduction*. San Francisco, CA: Freeman; 1993.

7. Norbury C, Nurse P: **Animal cell cycles and their control.** *Ann Rev Biochem* 1992, **61**:441-470.

8. Pines J: **Cyclins and cyclin-dependent kinases – take your partners.** *Trends Biochem Sci* 1993, **18**:195-197.

9. Solomon MJ: **Activation of the various cyclin/cdc2 protein kinases.** *Curr Opin Cell Biol* 1993, **5**:180-186.

10. Tréhin C, Planchais S, Glab N, Perennes C, Tregear J: **Cell cycle regulation by plant growth regulators: involvement of auxin and cytokinin in the re-entry of *Petunia* protoplasts into the cell cycle.** *Planta* 1998, **206**:215-224.

11. Glab N, Labidi B, Qin LX, Tréhin C, Bergounioux C, Meijer L: **Olomoucin, an inhibitor of the cdc2/cdK kinases activity, blocks plant cells at the G1 to S and the G2 to M cell cycle transitions.** *FEBS Lett* 1994, **533**:207-211.

12. Abraham RT, Acquarone M, Andersen A, Asensi A, Belle R, Berger F, Bergounioux C, Brunn G, Buquet-Fagot C, Fagot D *et al.*: **Cellular effects of olomoucine, an inhibitor of cyclin-dependent kinases.** *Biol Cell* 1995, **83**:105-120.

13. Hare PD, van Staden J: **The molecular basis of cytokinin action.** *Plant Growth Reg* 1997, **23**:41-78.

14. Hemerly AS, Ferreira P, de Almeida Engler J, Van Montagu M, Engler G, Inze D: ***cdc2a* expression in *Arabidopsis* is linked with competence for cell division.** *Plant Cell* 1993, **5**:1711-1723.

15. Zhang K, Letham DS, John PC: **Cytokinin controls the cell cycle at mitosis by stimulating the tyrosine dephosphorylation and activation of p34cdc2-like H1 histone kinase.** *Planta* 1996, **200**:2-12.

16. •• Riou-Khamlichi C, Huntly R, Jacqmard A, Murray JAH: **Cytokinin activation of *Arabidopsis* cell division through a D-type cyclin.** *Science* 1999, **283**:1541-1544.
This paper demonstrates that cytokinins may modulate the cell cycle by regulating *CycD3* expression. The authors show that the steady-state level of *CycD3* mRNA is induced both in cultured cells and intact tissue by cytokinin, and *CycD3* expression was found to localize to proliferating tissue. In a crucial experiment, they demonstrate that constitutive overexpression of *CycD3* is sufficient to bypass the requirement for cytokinin in tissue culture, suggesting that cytokinins may increase cell division by up-regulating this gene.

17. Soni R, Carmichael JP, Shah ZH, Murray JAH: **A family of cyclin D homologs from plants differentially controlled by growth regulators and containing the conserved retinoblastoma protein interaction motif.** *Plant Cell* 1995, **7**:85-103.

18. Chaudhury AM, Letham S, Craig S, Dennis E: ***amp1*-a mutant with high cytokinin levels and altered embryonic pattern, faster vegetative growth, constitutive photomorphogenesis and precocious flowering.** *Plant J* 1993, **4**:907-916.

19. Kerstetter RA, Hake S: **Shoot meristem formation in vegetative development.** *Plant Cell* 1997, **9**:1001-1010.

20. Kerstetter R, Vollbrecht E, Lowe B, Veit B, Yamaguchi J, Hake, S: **Sequence analysis and expression patterns divide the maize *knotted1*- like homeobox genes into two classes.** *Plant Cell* 1994, **6**:1877-1887.

21. Jackson D, Veit B, Hake S: **Expression of maize *knotted*1-related homeobox genes in the shoot apical meristem predicts patterns of morphogenesis in the vegetative shoot.** *Development* 1994, **120**:405-413.

22. •• Rupp H-M, Frank M, Werner T, Strnad M, Schmülling T: **Increased steady state mRNA levels of the *STM* and *KNATI* homeobox genes in cytokinin overproducing *Arabidopsis thaliana* indicate a role for cytokinins in the shoot apical meristem.** *Plant J* 1999, **18**:557-563.
Transgenic *Arabidopsis* expressing the *ipt* gene under the control of a heat shock promoter displayed an elevated steady state mRNA levels of *KNAT1* and *STM1*, which was correlated to increased levels of cytokinins. This suggests that cytokinin may influence SAM development, at least partly by activating *KNAT1* and *STM1*, meristem-specific homeobox genes.

23. •• Ori N, Juarez MT, Jackson D, Yamaguchi J, Banowetz GM, Hake S: **Leaf senescence is delayed in tobacco plants expressing the maize homeobox gene *knotted1* under the control of a senescence-activated promoter.** *Plant Cell* 1999, **11**:1073-1080.
Leaves of transgenic tobacco plants over-expressing maize *kn1* under the control of a senescence-specific promoter accumulate elevated levels of cytokinins and display a delay in senescence. This suggests that there may be a complex, interdependent relationship between cytokinin levels and expression of the *kn1* gene family.

24. Chang C, Stewart RC: **The two-component system: regulation of diverse signaling pathways in prokaryotes and eukaryotes.** *Plant Physiol* 1998, **117**:723-713.

25. Appleby JL, Parkinson JS, Bourret BR: **Signal transduction via the multi-step phosphorelay: not necessarily a road less traveled.** *Cell* 1996, **86**:845-848.

26. Perraud A-L, Weiss V, Gross R: **Signaling pathways in two-component phosphorelay systems.** *Trends Microbiol* 1999, **7**:115-120.

27. Kakimoto T: **CKI1, a histidine kinase homolog implicated in cytokinin signal transduction.** *Science* 1996, **274**:982-985.

28. •• Imamura A, Hanaki N, Nakamura A, Suzuki T, Taniguchi M, Kiba T, Ueguchi C, Sugiyama T, Mizuno T: **Compilation and characterization of *Arabidopsis thaliana* response regulators implicated in His-Asp phosphorelay signal transduction.** *Plant Cell Physiol* 1999, **40**:733-742.
Several novel *ARR* genes were identified by *in silica* analysis, and these and the other *Arabidopsis* response regulator genes were classified into the type-A and type-B groups, on the basis of their structural features and their mRNA expression profiles in response to cytokinin. The authors extended their *in vitro* phosphorylation studies with AHP2 using ARR10 (see [33••]), and also demonstrated that at least one type B ARR interacts with the AHPs in the yeast two-hybrid system.

29. •• Brandstatter I, Kieber JJ: **Two genes with similarity to bacterial response regulators are rapidly and specifically induced by cytokinin in Arabidopsis.** *Plant Cell* 1998, **10**:1009-1020.
Using differential display to screen for cytokinin specific messages, two genes, *IBC6* and *IBC7*, with homology to bacterial response regulators were identified in *Arabidopsis*. These genes had characteristics of cytokinin primary response genes; their steady state-level of mRNA was induced rapidly and specifically by cytokinin, and this induction was resistant to cycloheximide treatment. This observation, along with their homology to known signaling proteins, suggests a role for IBC6 and IBC7 in cytokinin signal transduction.

30. • Kiba T, Taniguchi M, Imamura A, Ueguchi C, Mizuno T, Sugiyama T: **Differential expression of genes for response regulators in response to cytokinins and nitrate in *Arabidopsis thaliana*.** *Plant Cell Physiol* 1999, **40**:767-771.
This paper extends previous studies [29••,31••] to show that other type A *Arabidopsis* ARR genes are also regulated by cytokinin.

31. •• Taniguchi M, Kiba T, Sakakibara H, Ueguchi C, Mizuno T, Sugiyama T: **Expression of *Arabidopsis* response regulator homologs is induced by cytokinins and nitrate.** *FEBS Lett* 1998, **429**:259-262.
The steady state mRNA levels of five *Arabidopsis* response regulators (including *IBC6* and *IBC7*, which have now been renamed *ARR4* and *ARR5*) were found to accumulate in response to cytokinin treatment. Transcripts also accumulated upon nitrate application to nitrogen starved cells, which suggests that this may reflect an alteration in cytokinin content in response to changing nitrogen levels.

32. • Miyata S-i, Urao T, Yamaguchi-Shinozaki K, Shinozaki K: **Characterization of genes for two-component phosphorelay mediators with a single HPt domain in *Arabidopsis thaliana*.** *FEBS Lett* 1998, **437**:11-14.
Three *Arabidopsis* genes, *ATHP1*, *ATHP2*, and *ATHP3*, encoding histidine phosphotransfer proteins were identified by *in silica* screening of the *Arabidopsis* EST database. The genes were shown to rescue the growth defect of a yeast YPD disruption, confirming that they are functional HPt domains. The pattern of expression of these genes was determined by northern analysis.

33. Suzuki T, Imamura A, Ueguchi C, Mizuno T: **Histidine-containing**
• **phosphotransfer (HPt) signal transducers implicated in His-to-Asp phosphorelay in *Arabidopsis*.** *Plant Cell Physiol* 1998, **39**:1258-1268.
Three *Arabidopsis* genes, *AHP1*, *AHP2*, and *AHP3*, encoding histidine phosphotransfer proteins (identical to *ATHP1-ATHP3*) were identified by searching the *Arabidopsis* EST database, similar to work by Suzuki *et al.* [32•]. A novel aspect of this work is that it was shown that purified AHP1 protein was capable of transferring a phosphate to two ARR proteins *in vitro*. This suggests that the *Arabidopsis* proteins may function in a manner analogous to their bacterial counterparts.

34. Sakai H, Aoyama T, Bono H, Oka A: **Two-component response regulators from *Arabidopsis thaliana* contain a putative DNA-binding motif.** *Plant Cell Physiol* 1998, **39**:1232-1239.

35. Lohrmann J, Buchholz G, Keitel C, Sweere C, Kircher S, Bäurle I,
•• Kudla J, Harter K: **Differentially-expressed and nuclear-localized response regulator-like proteins from *Arabidopsis thaliana* with transcription factor properties.** *Plat Biol* 1999, in press.
This paper provides evidence that the type B ARR proteins are transcription factors. The authors demonstrate that the putative output domain is capable of promoting transcription in yeast when fused to a Gal4 DNA binding domain, and that this domain is capable of directing nuclear localization.

36. Morgan BA, Bouquin N, Merrill GF, Johnston LH: **A yeast transcription factor bypassing the requirement for SBF and DSC1/MBF in budding yeast has homology to bacterial signal transduction proteins.** *EMBO J* 1995, **14**:5679-5689.

37. Vogel JP, Schuerman P, Woeste KW Brandstatter I, Kieber JJ:
• **Isolation and characterization of Arabidopsis mutants defective in induction of ethylene biosynthesis by cytokinin.** *Genetics* 1998, **149**:417-427.
A simple seedling screen based on the ethylene-mediated triple response was used to isolate mutants that fail to elevate ethylene biosynthesis in response to exogenous cytokinin. Four such mutants were analyzed for cytokinin-responsiveness in additional assays, and also for their effects on induction of ethylene biosynthesis by other factors.

38. D'Agostino IB, Kieber JJ: **Phosphorelay signal transduction: the emerging family of plant response regulators.** *Trends Biochem Sci* 1999, in press.