# Integration of Plant Responses to Environmentally Activated Phytohormonal Signals

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*Science* **311**, 91 (2006);
DOI: 10.1126/science.1118642

# Integration of Plant Responses to Environmentally Activated Phytohormonal Signals

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Plants live in fixed locations and survive adversity by integrating growth responses to diverse environmental signals. Here, we show that the nuclear-localized growth-repressing DELLA proteins of *Arabidopsis* integrate responses to independent hormonal and environmental signals of adverse conditions. The growth restraint conferred by DELLA proteins is beneficial and promotes survival. We propose that DELLAs permit flexible and appropriate modulation of plant growth in response to changes in natural environments.

Plants integrate multiple environmental signals during growth regulation. We determined whether the DELLA proteins specifically restrain growth in adverse conditions. DELLAs are nuclear proteins that restrain the cell proliferation and expansion that drives plant growth (*1*–*3*). The phytohormone gibberellin (GA) stimulates growth by promoting the destruction of DELLAs (*4*, *5*), and the phytohormones auxin and ethylene also regulate DELLA restraint (*6*–*8*).

High salinity restricts root water uptake, damages cell physiology, and slows growth (*9*, *10*). We found that the growth of *Arabidopsis* "quadruple-DELLA mutant" seedlings lacking GAI, RGA, RGL1, and RGL2 [four of the five DELLAs encoded by the *Arabidopsis* genome (*11*)] was less inhibited by salt than that of the wild type (Fig. 1, A to D). For example, root elongation of quadruple-DELLA mutants was relatively resistant to salt (Fig. 1B), although not completely resistant (perhaps because RGL3 remains in the quadruple-DELLA mutant line, or as a result of DELLA-independent mechanisms). In addition, salt slowed the leaf production rate (Fig. 1C), leaf expansion (*12*), and biomass accumulation (Fig. 1D) of wild-type plants but had a reduced effect on that of quadruple-DELLA mutants. Finally, we found that salt-treated wild-type plants contained reduced levels of bioactive GAs ($GA_1$ and $GA_4$; Fig. 1D). Thus, salt slows growth by means of a DELLA-dependent mechanism that is associated with reduced accumulation of bioactive GAs.

Reduced GA accumulation causes increased accumulation of DELLAs (*13*, *14*) and consequent growth inhibition. We found that DELLA-dependent salt-induced growth inhibition is also associated with DELLA accumulation. A green fluorescent protein–tagged DELLA (GFP-RGA) accumulated to higher levels in salt-treated *pRGA:GFP-RGA* roots (*6*, *7*, *14*) than in controls (Fig. 1E), despite lack of detectable effect on the levels of *RGA* transcripts (fig. S1). Because GA treatment caused a reduction in GFP-RGA levels in nuclei of salt-treated roots (Fig. 1E), it is likely that salt stress [and other kinds of stress (*15*)] inhibits growth by means of the above identified reduction in bioactive GA level, with consequent accumulation of DELLAs.

Plant salt responses are triggered (at least in part) by increased levels of the phytohormone abscisic acid (ABA) and resultant activation of ABA signaling pathways (*9*, *16*). Indeed, *abi1-1* mutant roots [in which a mutant form of the ABI1 serine/threonine protein phosphatase confers reduced ABA signaling (*17*, *18*)] were resistant to the growth-inhibitory effects of both ABA and salt (Fig. 1B) (*17*), indicating that ABI1-dependent ABA signaling is necessary for normal levels of salt-induced root growth inhibition. Furthermore, GFP-RGA accumulated in ABA-treated roots (Fig. 1F) but not in ABA-treated *abi1-1* roots (fig. S2), whereas quadruple-DELLA mutant roots were relatively resistant to the growth-inhibitory effects of ABA (Fig. 1B). These results suggest that salt inhibits growth (at least in part) by means of ABI1-dependent ABA-mediated enhancement of DELLA restraint.

Exposure to high salinity induces rapid increases in the level of "stress-induced" gene transcripts by means of ABA-dependent and -independent signaling pathways (*16*). However, we found no evidence that DELLAs are involved in the salt inducibility of selected stress-inducible transcripts (fig. S3). Thus, DELLAs regulate the plant "growth response" to salt but do not regulate the levels of the salt-induced transcripts tested here.

The plant life-cycle consists of successive embryonic, vegetative, and reproductive (flowering) developmental phases (*19*). DELLAs delay flowering, particularly in short-day (SD) photoperiods (*20*). Exposure of wild-type plants to salt (*21*) delayed flowering (*10*) (Fig. 2A), irrespective of whether flowering was measured as days to flowering (Fig. 2B), or as number of leaves in the vegetative rosette at bolting (Fig. 2C). In contrast, salt-treated quadruple-DELLA mutants flowered earlier than wild-type controls (Fig. 2, A to C). Thus, salt extends the duration of the vegetative phase by means of a DELLA-dependent mechanism. Furthermore, the extreme effect of salt on the growth rates of the GA-deficient *ga1-3* mutant (*12*) and on the *gai* mutant [which contains a mutant DELLA that is relatively resistant to the effects of GA (*2*, *5*)] prevented flowering within the duration of the experiment (fig. S4).

Flowering is induced by changes in the abundance of transcripts encoding proteins that have floral-promotive or floral-inhibitory function (*19*). For example, *FLOWERING LOCUS C* (*FLC*) transcripts repress flowering, whereas *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS* (*SOC1*), *FLOWERING LOCUS T* (*FT*), *CONSTANS* (*CO*), and *LEAFY* (*LFY*) transcripts promote flowering (*1*, *19*, *20*, *22*–*24*). We found that *CO* transcript levels were slightly reduced, *FLC* transcript levels were slightly increased, and *SOC1* and *FT* transcript levels were not detectably affected in plants grown on salt (Fig. 2D). Furthermore, the relatively rapid flowering of the quadruple-DELLA mutant on salt occurred independently of any detectable effect of DELLAs on the levels of *FLC*, *SOC1*, *FT*, or *CO* transcripts (Fig. 2D). In contrast, *LFY* transcripts were at a substantially reduced level in salt-treated wild-type plants (versus controls) but in salt-treated quadruple-DELLA mutants were at a similar level to those in wild-type controls (Fig. 2D). These observations suggest that salt delays flowering by means of two distinct mechanisms. First, salt slows growth by means of DELLA restraint, thus increasing the duration of the vegetative phase. Second, salt acts by means of DELLAs to inhibit flowering by maintaining relatively low levels of *LFY* transcript. It is possible that *LFY*-dependent regulation of flowering (by means of the GA-DELLA pathway) has increased importance (compared with other floral promotive pathways) in salt-treated plants, as it does in SD (*20*, *22*–*24*). Lastly, we found that ABA delays flowering in a DELLA-dependent manner, again affecting both days to flowering and leaf rosette number (fig. S5). This suggests that salt delays flowering through the same mechanism that contributes to inhibition of vegetative growth (Fig. 1B), by means of ABA-dependent enhancement of DELLA restraint (Fig. 1F).

Ethylene is another phytohormone that signals adverse environments. For example, adversity causes rapid increases in the activity of

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1-aminocyclopropane-1-carboxylic acid (AAC) synthase (ACS), a rate-limiting step in ethylene production (*25*). We found that salt caused an increase in the seedling levels of ACS-encoding *ACS2* and *ACS7* transcripts (*25*) (Fig. 3A), an increase in detectable levels of emanated ethylene (Fig. 3B), and an increase in the levels of ethylene-inducible *CHI-B* and *ERF4* transcripts (*26*, *27*) (Fig. 3A). None of the above ethylene-related salt responses were detectably altered in *gai* or *ga1-3*, in mutants lacking GAI and RGA (*gai-t6 rga-24*), or in the quadruple-DELLA mutant (Fig. 3, A and B). Growth of plants in an ethylene-enriched atmosphere delayed wild-type flowering but was less inhibitory of the flowering of both *gai-t6 rga-24* and quadruple-DELLA mutant plants (Fig. 3C). Thus, salt extends the duration of the vegetative phase through activation of both ABA and ethylene signaling, two independent pathways whose effects are integrated at the level of DELLA function.

Extreme salt concentrations kill plants (*15*), and we found that DELLAs determine the survival of salt toxicity. For example, the *ga1-3* and *gai* mutations conferred increased tolerance of a salt concentration that kills a proportion (~35%) of wild-type plants (*15*) (Fig. 4 and fig. S6), suggesting that stabilized DELLAs enhance survival in saline environments. Furthermore, lack of GAI, RGA, RGL1, and RGL2 suppressed the salt tolerance conferred by *ga1-3* (Fig. 4B), whereas the quadruple-DELLA mutant was less salt tolerant than the wild type (Fig. 4 and fig. S6). Thus, DELLA function promotes salt tolerance. Indeed, as salt concentrations increase, the growth of quadruple-DELLA mutant roots becomes more inhibited than that of the wild type, presumably due to the increased damage susceptibility that reduced DELLA function confers (fig. S7).

We next found that ethylene signaling promotes salt tolerance in a DELLA-dependent fashion. Wild-type plants treated with the ethylene precursor ACC (*25*) displayed increased tolerance of high-salt environments (*12*). In the absence of ethylene, degradation of the EIN3 transcription factor is promoted by the E3 ubiquitin ligase $SCF^{EBF1/EBF2}$ (*28*–*30*). Mutant plants lacking $SCF^{EBF1/EBF2}$ (*ebf1-1 ebf2-1*), or lacking the upstream CTR1 Ser/Thr kinase (*ctr1-1*), exhibit constitutive ethylene responses due to EIN3 accumulation (*28*–*30*). We found that *ein3-1* mutants (lacking EIN3) exhibited reduced salt tolerance and that *ctr1-1* and *ebf1-1 ebf2-1* mutants exhibited increased salt tolerance (Fig. 4, A and B). The increased salt tolerance of *ebf1-1 ebf2-1* mutants was abolished by lack of EIN3 (in *ein3-1 ebf1-1 ebf2-1* mutant plants; Fig. 4). Furthermore, lack of GAI and RGA (in *ctr1-1 gai-t6 rga-24*) substantially suppressed the salt tolerance exhibited by *ctr1-1* (Fig. 4), thus demonstrating that EIN3 promotes salt tolerance by enhancing DELLA function. In addition, we found that the *abi1-1* mutation

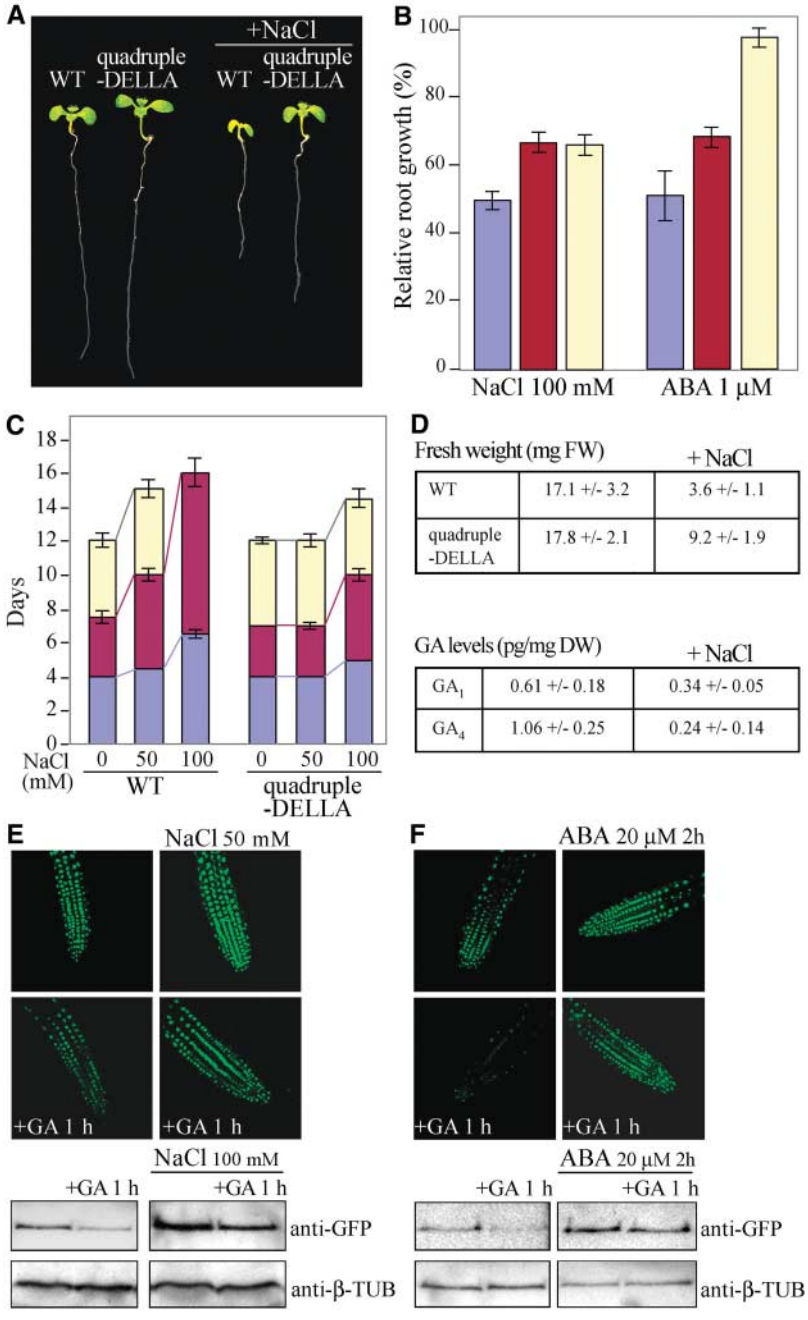


**Fig. 1.** Salt slows vegetative growth by enhancing DELLA function. (**A**) Representative 7-day-old wild-type and quadruple-DELLA mutant (*gai-t6 rga-t2 rgl1-1 rgl2-1*) seedlings grown on 100 mM NaCl (and controls). (**B**) Mean (±SE) relative growth of primary roots of 7-day-old wild-type (blue), quadruple-DELLA mutant (red), and *abi1-1* (yellow) seedlings grown in the presence of 100 mM NaCl or 1 µM ABA (expressed as percentage of untreated control). (**C**) Mean (±SE) rate of production of leaves by wild-type and quadruple-DELLA mutant vegetative rosettes grown on 0, 50, or 100 mM NaCl. Colors show the period until appearance of first (blue), second (red), and third (yellow) pair of rosette (true) leaves. (**D**) Mean fresh weights (FW) (±SE) of 14-day-old wild-type and quadruple-DELLA plants grown on 100 mM NaCl (+NaCl) and control; mean GA levels [picograms per milligram dry weight (DW); ±SD; $n = 4$] in 14-day-old wild-type seedlings grown on 100 mM NaCl (+NaCl) and control. (**E**) GFP fluorescence (viewed by fluorescence confocal microscopy) in tips of *pRGA:GFP-RGA* primary seedling roots after a 1-hour treatment with 10 µM $GA_3$ (+GA) (or control) in the presence or absence of NaCl, together with immunodetected (by an antibody to GFP) GFP-RGA in salt-treated (or control) GA-treated (or control) *pRGA:GFP-RGA* roots. β-tubulin (β-TUB) serves as a sample-loading control. (**F**) Same as (E) except that ABA (concentration as shown) was used in place of NaCl.

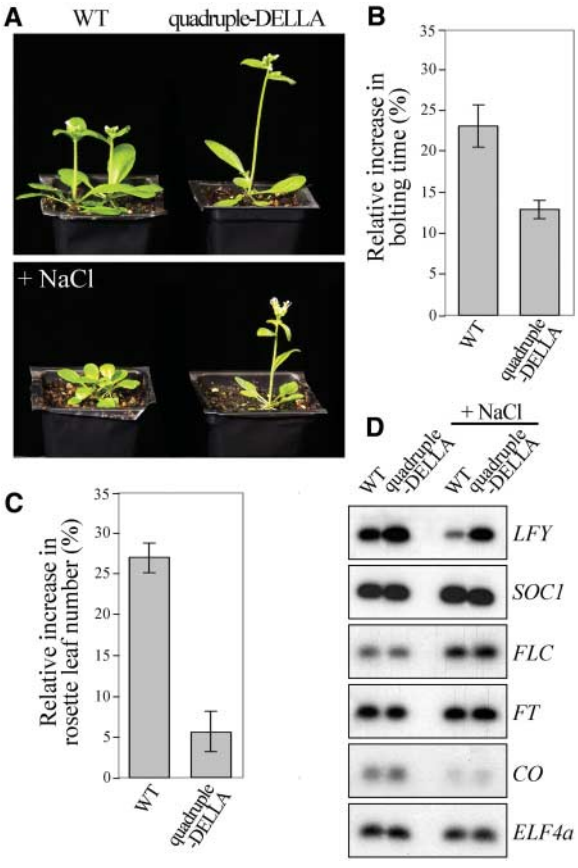


**Fig. 2.** Salt extends the vegetative phase of the life cycle through a DELLA-dependent mechanism. (**A**) Representative (30-day-old) wild type (two plants are shown) and quadruple-DELLA mutants (single plants) grown on soil and watered with saline solution [200 mM NaCl (+NaCl)] or control. (**B** and **C**) Mean (±SE) relative increase in bolting time (B) and number of rosette leaves (C) of wild-type and quadruple-DELLA mutant plants grown on soil and watered with saline solution (200 mM NaCl), expressed as a percentage of the untreated control. (**D**) Levels of floral integrator gene transcripts [determined by reverse transcription polymerase chain reaction (RT-PCR)] in soil-grown NaCl-treated wild-type and quadruple-DELLA mutant plants (and controls). *ELF4a* transcripts provide loading control.

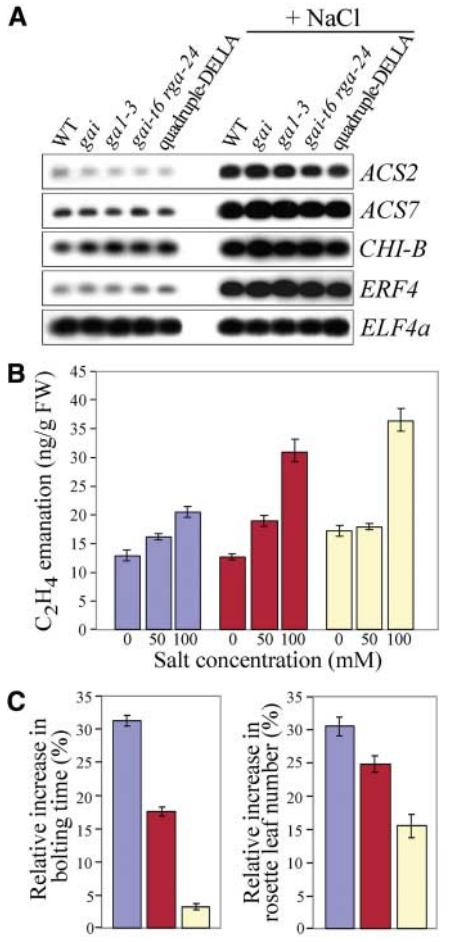


**Fig. 3.** Salt delays flowering by means of a DELLA-dependent ethylene response. (**A**) Levels of selected ethylene biosynthesis and ethylene-responsive gene transcripts (determined by RT-PCR) in 14-day-old plants of the wild type and various genotypes (as indicated) treated with 200 mM NaCl (and controls). *ELF4a* transcripts provide loading control. (**B**) Ethylene gas emanation [nanograms ethylene per gram fresh weight (FW); ±SE; $n = 4$] of 3-week-old wild-type (blue), *gai-t6 rga-24* (red), and quadruple-DELLA mutant (yellow) plants treated with increasing concentrations of NaCl. (**C**) Mean (±SE) relative increase in bolting time and rosette leaf number of wild-type (blue), *gai-t6 rga-24* (red), and quadruple-DELLA mutant (yellow) plants grown in an ethylene-enriched atmosphere (1 part per million), expressed as in Fig. 2, B and C.

conferred reduced salt tolerance (Fig. 4B). This observation, together with our previous ABA-related results, suggests that salt-activated ethylene and ABA signaling pathways integrate at the level of DELLA function to promote salt tolerance.

Although it was previously clear that environmental regulation of plant growth and developmental progression required signal integration, the nature of this integration was unknown. Here, we show that two independent salt-activated phytohormonal signaling pathways (ABA and ethylene) regulate plant development through integration at the level of DELLA function. Because the ABA and ethylene pathways are involved in plant responses to diverse abiotic and biotic inputs, it is likely that DELLA restraint provides a general mechanism for integration of plant growth responses to the environment.

Our results also identify a previously unknown mechanism that permits plant growth response to adversity. Salt-activated signaling pathways enhance the growth-repressing effects of DELLAs, at least in part through a reduction in the levels of bioactive GAs. The resultant accumulation of DELLAs then slows the rate of growth and extends the duration of the vegetative growth phase. This enhanced growth repression is distinct from passive growth rate reductions due to salt-induced perturbation of the physiological and metabolic processes that drive growth. Genetic analysis indicates that of the four *Arabidopsis* DELLA proteins, it is the combined effects of GAI and RGA that predominate in salt-activated growth repression (supporting online material text and fig. S8), the two DELLAs known to play the major role in DELLA-mediated plant growth regulation (*13*).

We show that DELLA-dependent growth restraint is advantageous in adverse environments. Perhaps growth restraint enables the redirection of resources to support mechanisms that promote survival of adversity. Alternatively, smaller plants may be less vulnerable to stress because they have less surface area. Although the nature of these underlying mechanisms remains unknown, it is nevertheless clear that DELLA restraint permits a flexible growth response to environmental variability, thus promoting survival.

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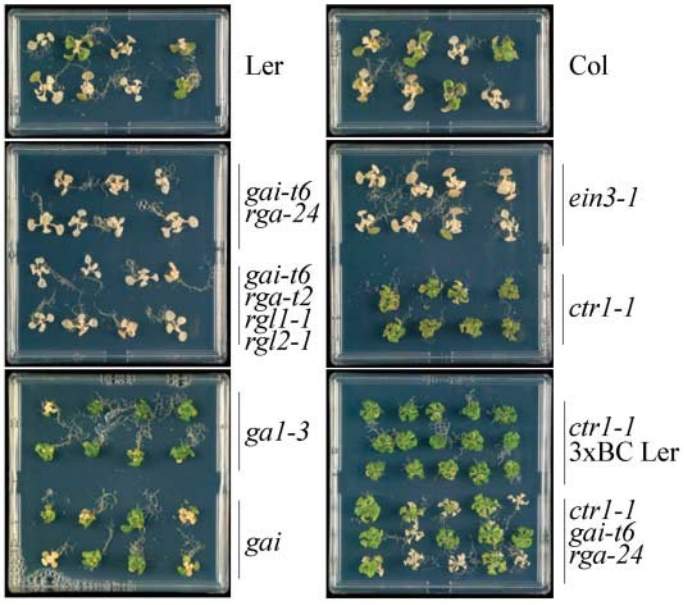
A




B

| Lines | Total plants | Survival | Rate (%) |
|---|---|---|---|
| Ler | 44 | 16 | 36.4 |
| *ga1-3* | 44 | 41 | 93.2 |
| *gai* | 44 | 36 | 81.8 |
| *gai-t6 rga-24* | 44 | 2 | 4.5 |
| *gai-t6 rga-t2 rgl1-1 rgl2-1* | 44 | 3 | 6.8 |
| *ga1-3 gai-t6 rga-24* | 40 | 2 | 5 |
| *ga1-3 gai-t6 rga-t2 rgl1-1 rgl2-1* | 40 | 2 | 5 |
| *abi1-1* | 30 | 6 | 20 |
| Col | 40 | 14 | 35 |
| *ctr1-1* | 40 | 36 | 90 |
| *ein3-1* | 40 | 8 | 20 |
| *ebf1-1 ebf2-1* | 40 | 35 | 87.5 |
| *ebf1-1 ebf2-1 ein3-1* | 32 | 5 | 15.6 |
| *ctr1-1* 3xBC Ler | 30 | 29 | 96.6 |
| *ctr1-1 gai-t6 rga-24* | 30 | 16 | 53.3 |

**Fig. 4.** DELLA-dependent survival of toxic salt concentrations. (**A**) Survival of representative plants of various genotypes (as indicated) on high-salt medium (200 mM). Photograph taken 10 days after transfer of plants to high-salt medium. Live plants are green; dead plants are white. (**B**) Numbers of plants of various genotypes [expressed as number of surviving plants and rate of survival (%)] that survive growth on high-salt medium.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31. We thank T. Sun for *pRGA:GFP-RGA*, C. Lloyd for the antibody to β-tubulin, P. Genschik and J. Leung for mutant *Arabidopsis* lines, and I. Carlson for assistance with the GA analyses. We gratefully acknowledge funding from the European Union (RTN1-2000-00090 INTEGA) and the Biotechnology and Biological Sciences Research Council (Core Strategic Grant to the John Innes Centre and response modes grant 208/P19972), the Belgian Science Policy Office, Fund for Scientific Research (Flanders), and Ghent University. We thank L. Alvey, C. Dean, X. Fu, J. Jones, L. Navarro, D. Richards, H. Sakai, A. Smith, and Y. Yasumura for comments on the manuscript and L. Sakvarelidze for computing assistance during the creation of the figures.


**Supporting Online Material**

www.sciencemag.org/cgi/content/full/311/5757/91/DC1
Materials and Methods
SOM Text
Figs. S1 to S8
References


9 August 2005; accepted 29 November 2005
10.1126/science.1118642


# Cytokinin Signaling and Its Inhibitor AHP6 Regulate Cell Fate During Vascular Development


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The cell lineages that form the transporting tissues (xylem and phloem) and the intervening pluripotent procambial tissue originate from stem cells near the root tip. We demonstrate that in *Arabidopsis*, cytokinin phytohormones negatively regulate protoxylem specification. AHP6, an inhibitory pseudophosphotransfer protein, counteracts cytokinin signaling, allowing protoxylem formation. Conversely, cytokinin signaling negatively regulates the spatial domain of *AHP6* expression. Thus, by controlling the identity of cell lineages, the reciprocal interaction of cytokinin signaling and its spatially specific modulator regulates proliferation and differentiation of cell lineages during vascular development, demonstrating a previously unrecognized regulatory circuit underlying meristem organization.


The root vascular cylinder has a central axis of xylem cell files consisting of protoxylem at marginal positions and metaxylem at central positions. This axis is flanked by phloem and intervening procambial cell files. A proportion of these intervening procambial cell files becomes mitotically active during secondary development and forms the lateral meristem, cambium, through periclinal divisions (*1*, *2*) (Fig. 1A). Cytokinins have been implicated in controlling vascular morphogenesis (*2*–*5*). The *wooden leg* (*wol*) allele of *CRE1* and the triple-knockout mutant for all three genes encoding CRE-family receptors (CRE1/WOL/AHK4, AHK2, and AHK3) display a markedly reduced number of cell files within the vascular bundle, because the periclinal procambial cell divisions required to proliferate the vascular cell files do not occur. This is associated with specification of all the vascular cell files in the root as protoxylem (*2*, *6*, *7*) (fig. S3B). This phenotype can be copied through depleting cytokinins by expressing the *CYTOKININ OXIDASE 2* gene (*8*) under the control of the procambium-specific *CRE1* promoter (fig. S3B), indicating that cytokinin signaling through the CRE-family receptors is required for proliferation and/or maintenance of the procambium.

To investigate whether reduced cell number is a prerequisite for exclusive protoxylem differentiation, we depleted cytokinins postembryonically by expressing cytokinin oxidase 1–yellow fluorescent protein (YFP) under the