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 DELLA 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. DELLA 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 GA1, RGA, RGL1, and RGL2 [four of the five DELLA encoded by the Arabidopsis genome ([1]) 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 (GA1 and GA3; 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 levels, 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, abl-1 mutant roots [in which a mutant form of the ABL1 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 ABL1-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 abl-1 roots (Fig. S2), whereas quadruple-DELLA mutant roots were relatively resistant to the growth-inhibitory effects of ABA (Fig. 1B). These results suggest salt inhibits growth (at least in part) by means of ABL1-dependent ABA-mediated enhancement of DELLA restraint.

Exposure to high salinity induces rapid increases in the level of “stress-induced” gene transcript 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). DELLA delay flowering, particularly in short-day (SD) photo-periods (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 gai-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
1-aminocyclopropane-1-carboxylic acid (ACC), a rate-limiting step in ethylene production (25). We found that salt caused an increase in the seedling levels of ABA-encoding ACS2 and ACS7 transcripts (25) (Fig. 3A), an increase in detectable levels of emananated 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 gai-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.

Extensive salt concentrations kill plants (15), and we found that DELLLAs determine the survival of salt toxicity. For example, the gai-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 DELLLAs enhance survival in saline environments. Furthermore, lack of GAI, RGA, RGL1, and RGL2 suppressed the salt tolerance conferred by gai-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 SCFEBF1/EBF2 (28–30). Mutant plants lacking SCFEBF1/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 abf1-1 mutation...
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 DELLA proteins, at least in part through a reduction in the levels of bioactive GAs. The resultant accumulation of DELLA 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 DELLA 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.

References and Notes
12. P. Achard et al., data not shown.
21. Materials and Methods are available as supporting online material on Science Online.
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 underlyying meristem organization.

The root vascular cylinder has a central axis of xylem cell cylinders 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 post-embryonically by expressing cytokinin oxidase 1–yellow fluorescent protein (YFP) under the