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Unraveling the paradoxes of plant hormone signaling integration

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Abstract

Plant hormones play a major role in plant growth and development. They affect similar processes but, paradoxically, their signaling pathways act nonredundantly. Hormone signals are integrated at the gene-network level rather than by cross-talk during signal transduction. In contrast to hormone-hormone integration, recent data suggest that light and plant hormone pathways share common signaling components, which allows photoreceptors to influence the growth program. We propose a role for the plant hormone auxin as an integrator of the activities of multiple plant hormones to control plant growth in response to the environment.

Plants are sessile organisms and therefore must constantly adapt their growth and architecture to an ever-changing environment. In doing so, plants have evolved mechanisms to discriminate prolonged signals from transient background noise. The correct integration of environmental signals with endogenous developmental programs is therefore a matter of survival.

Phytohormones are small molecules derived from secondary metabolism that take center stage in shaping plant architecture¹ (see Fig. 1 for hormone structures). The presence and effect of some of these hormones have been recognized for more than a century. Analyses of biosynthetic and signaling mutants, in combination with studies of exogenous hormone applications, have shown that gibberellins, auxins and brassinosteroids regulate expansion along longitudinal axes and greatly influence plant stature and organ size. Paradoxically, these three-hormone pathways do not act redundantly, in spite of the fact that plants are renowned for genomes packed with sequence redundancy. Loss-of-function mutations in either biosynthesis or signaling genes in any one of these hormone pathways cause dwarfism. In contrast, ethylene acts primarily to increase cell expansion along transverse axes and greatly reduces the stature of dark-grown seedlings. Meanwhile, cytokinins control growth mainly through the regulation of cell division and differentiation and act antagonistically to auxins in controlling meristem activities. Absciscic acid in contrast antagonizes growth promotion by both gibberellins and brassinosteroids. Jasmonic acid is mainly involved in plant defense against pathogens but has been involved in growth, and strigolactone is a recently discovered small-molecule hormone that regulates branching¹. These examples together illustrate the complexities of growth regulation by different phytohormones.

In the past decade, our knowledge of the molecular mechanism underlying perception and signal transduction of these small molecules has greatly increased¹. Phytohormones are

perceived by a variety of receptors, including receptor kinases in the plasma membrane (brassinosteroids), histidine kinases similar to bacterial two-component receptors localized in the endoplasmic reticulum (ethylene) or plasma membrane (cytokinins), and novel receptors of different classes found in the cytosol and nucleus (abscisic acid, gibberellins and auxins). For the most part, the major signaling components downstream of these receptors are known, identified almost exclusively through *Arabidopsis thaliana* genetics. Although the details of the precise mechanisms of signaling are just being revealed, in all cases, the output of signaling involves changes in the expression of hundreds of genes, many of which play a role in cell expansion and division.

Plant hormone transduction pathways rarely show cross-talk according to the biological definition of the word (cross-talk being defined as specific components shared between more than one signaling pathway)². It is possible that shared signaling components will be found; however, it is conceivable that there are only a few examples of cross-talk because plant hormone signaling pathways are often very short and use noncanonical signaling modules. In metazoans, signal integration is achieved through the convergence of many pathways in a signaling hub (for example, nuclear factor- κ B) or the use of second messengers such as calcium. In plants, the bulk of signal integration during hormone signaling occurs at the gene-network level, downstream of signal transduction³. For most hormones, there is very little or no overlap in the target genes. Rather, different downstream gene family members are regulated by different hormones. One exception is brassinosteroids and auxin, which have been shown to share signaling components (ARF2 and BIN2)⁴ and to act synergistically on the transcription of a shared set of genes (~40% of the total auxin-regulated genes are also coregulated by brassinosteroids⁵). In plants, one hormone signaling pathway often regulates genes for the biosynthesis of a second hormone or signaling pathway component so that integration also occurs at this level (Fig. 1).

A major challenge now is to understand how these different signaling pathways are integrated to give dynamic changes in growth rates during development. Here, we will use two examples to show how phytohormone response pathways are integrated to coordinate growth. First, we will examine the concerted action of multiple hormones, each acting in different cell types, to coordinate growth of cells within the primary root. Second, we will use the example of the shade-avoidance response to illustrate the dynamics of plant hormone signaling for adaptation to a changing, often suboptimal environment.

Spatial control of phytohormone signaling within the primary root

The root is organized in a stereotypical pattern of cell types along radial and longitudinal axes (Fig. 2a). Plant hormones have a role in the growth of the primary root, but unexpectedly, the artificial expression of signaling or biosynthesis components of hormone signaling pathways in different root tissues revealed that individual hormone pathways act in discrete cell types. For example, DELLAs are a group of five nuclear proteins that redundantly repress growth and that are degraded by the 26S proteasome upon gibberellin perception¹. Tissue-specific expression of a mutant nondegradable DELLA protein showed that the endodermis is the primary site of DELLA turnover, and by extrapolation, gibberellin action in the root⁶ (Fig. 2b). Furthermore, the growth of this tissue is rate limiting for the elongation of other cells and therefore can drive root growth and control meristem size^{6,7}. Similarly, cytokinins are required at the transition zone to control root cell differentiation and meristem size⁸ (Fig. 2b). Our unpublished data implicate the root epidermis as the site of brassinosteroid action (S. Savaldi-Goldstein and J.C., unpublished data), similar to recently reported results in the shoot⁹. Although the sites of action of all plant hormones have not been fully characterized, at least some of them act in specific tissues that do not

fully overlap, which may partly explain why phytohormones are not redundant in their growth phenotype.

If different hormone pathways act locally in the root, how is the growth of the entire organ coordinated? We propose that auxin is a central factor that allows molecular communication between different tissue layers. Auxin accumulation and fluxes (Fig. 2b) are controlled by specific transporters. This hormone acts in a dose-dependent and cellular context-specific manner and can induce a variety of outputs ranging from cell growth to differentiation or organ initiation. Most hormone pathways heavily affect auxin homeostasis by modifying either auxin transport, biosynthesis or signaling (Fig. 1). For example, cytokinins induce the expression of *SHY2/IAA3*, a negative regulator of the core auxin signaling pathway at the transition zone of the root, thereby providing a frontier for auxin-induced cell proliferation versus differentiation¹⁰ (Fig. 2b). Ethylene, too, controls auxin levels by manipulating the expression of both auxin transporters (from the AUX and PIN families) and biosynthetic enzymes (for example, the auxin biosynthesis enzyme TAA1)^{11–14}. Additionally, auxin feeds back on ethylene biosynthesis¹⁵ in a complicated mechanism that controls the auxin-ethylene level in root cells.

There are many cases of regulation between hormone pathways that do not involve auxin³ (Fig. 1). However, it appears that plants might use auxin as an integrator of hormone activity that otherwise predominantly functions in specific niches within the root. Auxin also has a prominent role in shaping the plant body in response to environmental stimuli.

Hormone signaling integration and phenotypic plasticity in response to the environment: the shade-avoidance response

Plants have to deal with a constantly fluctuating environment due to changes in light levels, temperature, pathogen attack or competition for resources. Perhaps because they are photosynthetic, plants are especially attuned to their light environment. Light not only influences every major developmental transition but also is used by plants to assess seasonal time, time of day and whether they are being shaded by other plants. For shade-intolerant plants, such as tomato or *Arabidopsis*, a reduction in the red/far-red (R:FR) light ratio signals the close proximity of competitors and triggers the shade-avoidance syndrome (SAS). Physiological experiments have defined shade as light with a R:FR ratio less than 1 and nonshade light as having a R:FR ratio above 1 (ref. 16). A common phenotype of the SAS is the reallocation of energy resources from storage organs to stems and petioles so that the shaded plant may outgrow its competitors. Concomitantly, both root and leaf growth are inhibited, and chlorophyll content is reduced (Fig. 3). In cases of prolonged shade, leaf senescence and reproductive development are accelerated, and plants are more susceptible to insect herbivory, leading to decreased biomass and seed yield¹⁶. Shade avoidance thus has had a negative impact on agriculture, despite the fact that it is an adaptive trait of major ecological significance.

The perception of canopy shade is mostly mediated by phytochromes, a class of red/far-red light-absorbing photoreceptors¹⁶. Phytochromes monitor the change in light quality under vegetative shade, mainly the reduction of red light (absorption maxima ≈ 660 nm) due to chlorophyll absorption by canopy leaves and a relative increase in far-red light (absorption maxima ≈ 730 nm) caused by increased reflection from neighboring plants. Under red light, phytochrome B (phyB) is converted from a red-absorbing form (P_r) to a long-lived far red-absorbing conformation (P_{fr}), which promotes its translocation from the cytosol to the nucleus (Fig. 3b). phyB(P_{fr}) interacts with a class of basic helix-loop-helix (bHLH) transcription factors called PHYTOCHROME INTERACTING FACTORS (PIFs, here PIF4 and PIF5) that promote growth^{16,17} (Fig. 3b). phyB(P_{fr}) limits stem growth by

promoting the degradation of PIFs by the 26S proteasome (Fig. 3b). However, in a low-R:FR light environment, phyB is mostly in its inactive P_r form¹⁶. Therefore, PIFs are not targeted for turnover, and stems grow¹⁷ (Fig. 3b). Despite considerable agricultural interest, information is scant on how light and growth hormone signaling are integrated to mediate the shade-avoidance response. The problem is complex because it involves multiple, interconnected growth responses of several organs.

Recent studies are beginning to implicate auxin as having a central role in both regulating the growth of individual organs and orchestrating the response between different parts of the plant. Under shade conditions, auxin biosynthesis is rapidly induced in leaves in a process involving TAA1 (ref. 18). Auxin accumulates in leaves, where it positively regulates a cytokinin oxidase, *AtCKX6* (ref. 19) (Fig. 3b). *AtCKX6* breaks down cytokinin, which inhibits leaf growth by arresting cell division¹⁹. At the same time, auxin is actively routed by specific transporters to the stem¹⁸. The local increase in auxin concentration in this tissue has the opposite outcome from that in leaves. Microarray studies indicate that, out of 80 genes with at least a two-fold induction after 1 h of shade treatment, 36 require new auxin synthesis¹⁸. Auxin induces the expression of a number of growth-associated genes including gibberellin biosynthesis enzymes²⁰.

More than half of shade-induced genes act independently of auxin. Among the genes that are auxin-independent are some known shade-upregulated genes that are involved in light signaling, such as LONG HYPOCOTYL IN FAR-RED LIGHT 1 (HFR1), an atypical bHLH protein. HFR1 is a negative regulator of the shade-avoidance response²¹. Following exposure to shade, *HFR1* is induced, and the HFR1 protein dimerizes with PIFs (here PIF4 and PIF5), which inhibits their binding to DNA²². This mechanism provides a negative feedback loop that prevents stem hyperelongation in response to shade²² (Fig. 3b). In addition, gibberellin signaling directly cross-talks with this pathway through the DELLA proteins, which accumulate in the stem in a high-R:FR light environment²³. Similar to HFR1, DELLAs interact with PIFs, which inhibits PIF binding to DNA, and therefore inhibit cell elongation²⁴. Under shade conditions, gibberellin synthesis is stimulated, perhaps by the action of auxin²⁰. This promotes the proteasome-based degradation of DELLA proteins²⁵, thus allowing PIFs to promote stem growth (Fig. 3b). Therefore, DELLA proteins and phyB have a concerted action on PIFs under white light to repress growth. Both repressive effects are relieved under shade light, allowing the stem to grow (Fig. 3b).

Growth-promoting pathways appear to be integrated, at least in part, at the level of the PIF transcription factors. This is true for other responses to environmental changes such as high temperature or diurnal-regulated growth^{26,27}. Moreover, the circadian clock gates hormone gene expression to fine-tune appropriate seasonal and shade growth regulation²⁸. Indeed, a set of plant hormone-associated genes (genes for brassinosteroids, gibberellins, auxins, cytokinins and ethylene biosynthesis and signaling) are coexpressed at the time of day when stems grow at their fastest rate, and phasing of the brassino-steroid pathway to a different time of day causes a growth defect²⁸. Thus, signal integration during the shade-avoidance response involves auxin as both an effector of growth and a messenger between organs (Fig. 3b). However, it is still unclear how light signaling pathways mediate the rapid modification in hormone homeostasis, which lead to the rapid increase of auxin levels during early shade avoidance (Fig. 3b).

Conclusions and future perspectives

It appears that integration between plant hormone signaling predominantly occurs at the level of the transcriptome rather than by crosstalk during signal transduction. Auxin, by its

mobile nature, is used to coordinate hormone action across tissues and organs. An emerging theme is that environmental and hormone pathways can be integrated during signaling by hubs or ‘master regulators’ of growth, such as the PIF transcription factors. However, it is unclear how several environmental inputs are integrated and fed into the growth-regulatory program at the same time. For example, during shade avoidance, the increase in far-red light is not the only input. Indeed, there is also a reduction of the overall light quantity as well as a specific reduction of blue light that is perceived by blue light photoreceptors¹⁶. Because the reduction in blue light can be directional, there might be directional growth of the stem toward the most intense light source. Whether these pathways are integrated at different levels or by the same factors is still an open question.

A major difficulty and motivation for working on an entire organism is that hormone interactions might differ between different organs and tissues. Although tissue-directed expression and cell sorting are now routinely used, there is a crucial need for the development of new non-invasive techniques to visualize both the quantity of small molecules and the activity of signaling pathways. Interactions between hormone signaling have been described at the transcript level (Fig. 1). The challenge is now to decipher how this network is integrated both in space and time. To this end, one needs to monitor the signal itself as well as the input and the output of the signaling pathway (as in Fig. 3b). This is crucial to evaluate the dynamics of events during signaling as well as to distinguish between direct and indirect effects in hormone interaction.

Currently, we have ways to monitor signaling output by the use of transcriptional reporters, such as the synthetic auxin-responsive *DR5* promoter²⁹; however, many of these tools are flawed because they lack hormone specificity. As sets of hormone-specific genes have been identified³ and transcription-factor binding site discovery is greatly facilitated by new sequencing technologies, it is expected that new, more specific transcriptional readouts for plant hormones will be developed in the near future. So far, the only example for which it is possible to track signaling input with tissue resolution is the gibberellin transduction pathway. Using protein degradation as a readout for signaling input is a promising technique, as plant hormone pathways rely heavily on proteasome-based protein degradation¹. However, following protein-protein interactions or post-translational modifications *in planta* might be worth pursuing as well. Finally, monitoring the small molecules directly poses the biggest challenge. FRET-based sensors have been successfully used to monitor sugar quantity *in planta*³⁰, and a similar assay could be used for hormones. Alternatively, the engineering of synthetic signaling pathways or chemical dyes may also allow monitoring of the quantity of small molecules in plants. Altogether, these readouts for hormone signaling would be useful for a deeper understanding of signal integration, which can be used to develop predictive models for plant growth and development.

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Figure 1.

Phytohormone structures and functional interactions. Lines with arrowheads, upregulation of hormone biosynthetic genes or downregulation of genes involved in hormone inactivation; blocked arrows, downregulation of genes involved in hormone biosynthesis or upregulation of genes involved in inactivation of a hormone; diamond arrowheads, changes in gene expression with ambiguous outcome. Figure is adapted from previous work³.

Figure 2.

Tissue-specific action of hormones in the root. **(a)** Schematic representation of the root apex. **(b)** Gibberellin acts in the endodermis to control meristem size and cell elongation in the root, whereas cytokinin acts at the transition zone (gray oval), also to control meristem size. Auxin accumulates in stem cells and the columella root cap. Auxin distribution is ensured by specific carriers that transport auxin through the root (green arrow).

Figure 3. Signal integration during the shade-avoidance response. **(a)** Comparison of a tomato plant grown under sunlight (left) with a high R:FR ratio and a tomato plant grown under vegetative shade (right) with a low R:FR ratio. **(b)** Hormone and light pathway interaction in response to white light (high R:FR, left) and shade (low R:FR, right).