Minireview

Integrating transcriptional controls for plant cell expansion

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Published: 28 October 2004

Genome ***Biology*** 2004, **5:**245

The electronic version of this article is the complete one and can be found online at http://genomebiology.com/2004/5/11/245

Abstract

The plant hormones auxin and brassinosteroid promote cell expansion by regulating gene expression. In addition to independent transcriptional responses generated by the two signals, recent microarray analyses indicate that auxin and brassinosteroid also coordinate the expression of a set of shared target genes.

Control of plant cell expansion by auxin and brassinosteroid

Young plants grow upward from the ground, bend towards light when necessary, and alter their growth in other ways to optimize the resources around them. Their rapid growth and tropic responses depend upon the perception of environmental signals in conjunction with endogenous hormones that control the elongation of cells. The small indolic hormone auxin promotes many plant growth processes including cell expansion during elongation of the seedling stem, or hypocotyl. Steroid hormones called brassinosteroids have similar effects on some of the same physiological processes, including hypocotyl elongation. Although some studies have suggested that the two hormones regulate the expression of a small number of genes in common [1], the upstream components of the auxin and brassinosteroid signaling pathways appear to be entirely distinct [2,3]. Recently, nearly genome-wide studies of gene expression have expanded our knowledge of interactions between auxin and brassinosteroid pathways [4,5]. The results of these studies suggest that the response pathways of the two hormones regulate the expression of independent sets of genes and also coordinate the expression of some common target genes. Much of this coordination appears to occur at target-gene promoters. Continuous modulation of gene expression by the two signals may contribute to plasticity in growth responses.

Early achievements in molecular studies of the auxin response were the identification of auxin-response-factor (ARF) proteins and their binding to a *cis*-regulatory element, the auxin-response element (AuxRE; TGTCTC), in primary auxin-response gene promoters [6]. Some ARFs act as transcriptional activators and some as repressors. ARF functions are inhibited by direct binding of members of the AUX/IAA protein family, identified as products of primary auxin-response genes [7]. Auxin influences gene expression by promoting rapid degradation of AUX/IAA proteins, thereby allowing ARFs to regulate transcription. Most mutants impaired in auxin responses are altered in the degradation of AUX/IAA proteins.

The recent isolation of two proteins, *bri1*-EMS-Suppressor 1 (BES1) and Brassinazole-resistant 1 (BZR1), shows that, as for auxin, short-lived nuclear proteins mediate brassinosteroid action. Much brassinosteroid-induced transcription is regulated positively by the functions of BES1 and BZR1, and negatively by their proteolysis [8,9]. The mechanisms of protein turnover in brassinosteroid and auxin responses are likely to have some shared upstream components [10] and to differ in others [5]. Brassinosteroid signaling directs the nuclear accumulation of BES1 and BZR1. These regulators, like the AUX/IAA proteins, contain no known DNA-binding motifs and may contribute to transcriptional regulation by interacting with other factors. Beyond their overlapping

functions, BES1 and BZR1 appear additionally to regulate distinct genes, suggesting targets in putative transcriptional complexes may differ [3].

Interdependent responses to auxin and brassinosteroid

Two recent reports describe direct comparisons of the transcriptional profiles of the auxin and brassinosteroid responses in *Arabidopsis* seedlings. In the first study, Goda *et al.* [4] monitored changes in gene expression after separate applications of auxin and brassinosteroid, using oligonucleotide arrays representing approximately 8,000 genes. Auxin was applied to wild-type seedlings, while brassinosteroid was applied to a brassinosteroid-deficient mutant, called *det2*, to maximize the effects of the hormone [11]. Auxin- and brassinosteroid-regulated genes were divided into two response sets, early (15 and 30 minutes after hormone application) and late (3, 12 and 24 hours after application), together totaling 637 genes. Overall, transcriptional changes occurred much more slowly in response to brassinosteroid than to auxin, and the number of genes activated by each hormone exceeded the number repressed. Of 305 genes upregulated by brassinosteroid in the dataset, 32 were also upregulated by auxin. The effects of the two hormones were separable kinetically and quantitatively. The most common pattern was one of rapid, dramatic activation by auxin and slower, gradual induction by brassinosteroid. This trend was detailed previously in work from the same lab, in which real-time quantitative reverse-transcription PCR (RTQ RT-PCR) was used to quantify transcripts of selected genes [12]. Both studies showed that the accumulation of any given transcript in response to brassinosteroid rarely reached the level of maximal accumulation observed following auxin treatment.

In a related study, Nemhauser *et al.* [5] examined interactions between the auxin and brassinosteroid signaling pathways in both physiological and gene-expression experiments. Assays of mutants with altered hormone biosynthesis or response showed that hypocotyl elongation induced by brassinosteroid varies with the auxin content of seedlings and requires an intact auxin-response pathway, demonstrating that the auxin and brassinosteroid responses operate interdependently in cell-expansion processes. Transcript profiles were determined 2.5 h after brassinosteroid treatment of seedlings, using the more recently developed *Arabidopsis* array representing more than 22,000 genes [5]. Results were analyzed together with those of a previous experiment from the same lab monitoring auxin-induced expression [13]. Transcript levels of 638 genes were affected by brassinosteroid treatment. Of 342 genes upregulated by brassinosteroid, 82 were also upregulated after auxin treatment. Responses were further characterized by quantitative RT-PCR for four of the genes activated individually by brassinosteroid and auxin; for these genes, the two hormones appeared to act synergistically.

Additionally, Nemhauser *et al.* [5] assayed transcriptional responses to brassinosteroid in an *Arabidopsis* mutant with elevated auxin levels, called *yucca* [14]. Surprisingly, approximately 60% of brassinosteroid-responsive genes did not respond to brassinosteroid in the *yucca* mutant [5]. It is possible that the high levels of auxin in *yucca* seedlings saturate transcription of these genes, suggesting that both hormones act in their regulation. Alternatively, however, loss of the brassinosteroid response in *yucca* might be a result of secondary effects of chronically high levels of auxin in these seedlings.

Some of the genes found to be regulated by brassinosteroid were identified previously as auxin primary-response genes. Both groups [5,12] therefore tested the auxin specificity of *DR5::GUS,* a reporter construct used to infer auxin levels in plants. Nemhauser *et al.* [5] used mutant seedlings that differed in hormone content to show that both auxin and brassinosteroid influence *DR5::GUS* expression. Nakamura *et al.* [12] concluded previously that brassinosteroid activation of *DR5::GUS* was weaker and slower than its response to auxin, in agreement with the more gradual brassinosteroid-induction seen in array experiments [4]. Because the promoter of *DR5::GUS* contains repeats of the AuxRE sequence, auxin-controlled regulators of transcription were implicated in brassinosteroid responses.

Signal convergence and coordination

Prior to the development of gene-expression profiling technologies, it was not feasible to determine how broadly a given *cis*-regulatory element functioned. The results of Goda *et al.* [4] and Nemhauser *et al.* [5] suggest that the AuxRE sequence, and by extension ARF proteins, function in the response to both auxin and brassinosteroid. The analysis of each dataset showed that the AuxRE is present in promoters responsive to both auxin and brassinosteroid [4,5]. The element is overrepresented in the set of promoters regulated in common by auxin and brassinosteroid, but contrary to expectations, not in auxin-specific response sets. The core ARF-binding site (TGTC) [15], however, is clustered within promoters of each set of response genes, and enriched most notably in promoters dually activated by auxin and brassinosteroid [5]. It will be interesting to see if these are actually elements of *cis* regulation and if they are bound by ARF proteins. As there are putative ARF-binding sites in promoters affected by both auxin and brassinosteroid, could ARF binding be important in a wider range of promoters than previously thought? If as-yet unidentified accessory proteins modulate ARF occupancy of target promoters or the activities of bound ARFs, the possibility remains that these may be targets for auxin action or signal integration. Little is known about how auxin affects the function of repressive ARFs, which appear to share similar *cis*-binding sites with activating ARFs [16].

Enrichment of other *cis* elements in auxin- and brassinosteroid-responsive promoters suggests that their cognate

transcription factors may be targets of other pathways interacting with those of auxin or brassinosteroid. Elements involved in responses to light, for example, are present in some brassinosteroid-specific promoters. Physiological interactions that occur between light and brassinosteroid may be explained in part by co-regulation of some of the same genes. Even more prominent than AuxREs are the consensus binding sites for transcription factors related to MYC. These are found in 80% of dually regulated promoters [5]. Goda *et al.* [4] observed that 17 genes encoding putative transcription factors were upregulated in response to auxin. What role DNA-binding proteins other than ARFs serve in primary and higher-order auxin responses is an open question. The identification of the set of genes co-regulated by auxin and brassinosteroid led to a model in which the auxin and brassinosteroid signaling pathways converge on a set of gene promoters to regulate their transcription [5]. Depending on the promoter, ARF binding may mediate both auxin responses and interdependent actions of auxin and brassinosteroid (Figure 1).

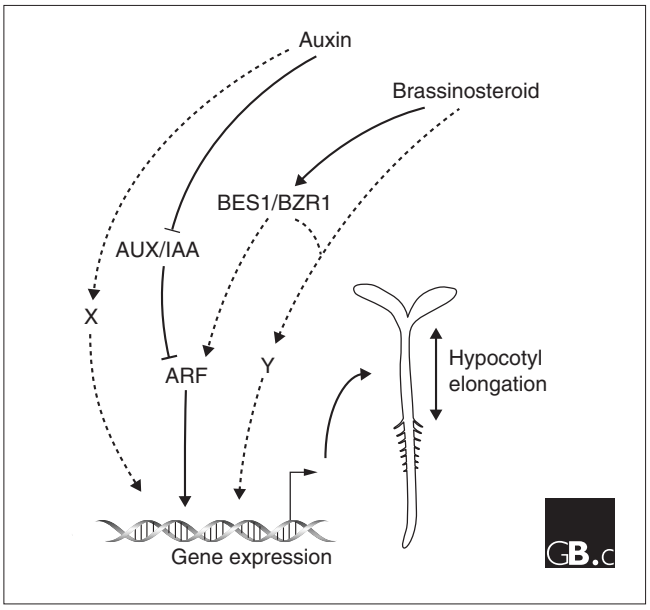

Figure 1
Auxin and brassinosteroid regulate transcription mediating cell expansion by both independent and interconnected mechanisms. Only components known to act in transcriptional regulation of auxin- and brassinosteroid-response pathways are shown. Dashed lines indicate speculative relationships in the model. Auxin promotes the degradation of (AUX/IAA) proteins, which negatively regulate auxin response factor (ARF) function. ARFs are implicated in the regulation of gene expression downstream of both auxin and brassinosteroid. Other regulators of transcription that may bind directly to promoters are proposed (proteins X and Y). The *bri1*-EMS-Suppressor 1 (BES1) and Brassinazole resistant 1 (BZR1) proteins regulate brassinosteroid-induced transcription by unknown mechanisms, possibly involving higher-order transcriptional complexes that include ARFs or other factors. Gene expression regulated by auxin and brassinosteroid coordinates expansion growth of cells and promotes the elongation of hypocotyls in seedlings. Modified from [5].

It is possible that independent auxin and brassinosteroid pathway targets may lead in part to upstream crosstalk. The two recent large-scale studies [4,5] indicate that auxin and brassinosteroid each independently regulate sets of 200-300 genes, which encode products that span the functional spectrum. A small subset of these products might facilitate crosstalk between auxin and brassinosteroid that is separate from gene regulation. Brassinosteroid levels or perception, for example, may be influenced in part by auxin. Goda *et al.* [4] noted that a gene encoding the brassinosteroid catabolic enzyme BAS1 [17] is activated 3 hours after auxin application. Genes encoding brassinosteroid receptors (*BRI1*, *BRI3*) were also slowly upregulated by auxin. It is interesting that brassinosteroid down-regulates genes involved in auxin transport in the results shown by both groups [4,5]. The *PIN* and *PID* genes are required for cellular auxin efflux and have been implicated in the establishment of auxin gradients. It is an intriguing possibility that brassinosteroid may modify growth responses in part by influencing localized auxin levels.

Understanding transcript profiles in terms of their spatial, temporal and genetic contexts is essential for identifying interactions between signaling pathways that target gene regulation. Plant hormones can exert opposite effects on growth and morphogenesis in different tissues. Furthermore, development is often directed by the action of factors with extremely narrow cell-type specificity. Since appropriate genetic circuitry models are likely to depend upon cell-specific, or at least tissue-specific, data, new technologies for obtaining highly localized RNA samples are being perfected [18,19]. Use of regulatory mutants in gene-expression profiling will help define the influence of signaling on transcription. Methods of data analysis must also be chosen carefully, as signal interaction complexities such as feedback mechanisms may not be decipherable when transcript changes are reduced to qualitative response sets (for example, see [20]). Assessments of transcriptional control mechanisms will improve with our ability to discover *a priori* elements of *cis* regulation in genes associated by response. Defining interactions at promoters will be particularly important in attempts to understand hormone pathways as individual and combinatorial modulators of plant responses.

Acknowledgements

Research in the authors' lab is supported by grants from the NIH (GM43644-17), NSF (DBI-0115870), and DOE (DE-FG02-02ER15312).

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