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Developmental mechanisms regulating secondary growth in woody plants

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Secondary growth results in the radial expansion of woody stems, and requires the coordination of tissue patterning, cell differentiation, and the maintenance of meristematic stem cells within the vascular cambium. Advances are being made towards describing molecular mechanisms that regulate these developmental processes, thanks in part to the application of new genetic technologies to forest trees, and the extension of knowledge about evolutionarily conserved mechanisms from model annuals. New studies demonstrate a central role for developmental mechanisms that involve transcriptional regulators, phytohormones and the cell wall in regulating secondary growth.

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Introduction

The secondary growth of woody stems is a dynamic process that integrates multiple developmental mechanisms and responds to physiological and environmental cues. The stem cell initials of the vascular cambium produce daughter cells that are recruited to differentiate within secondary phloem (bark) or secondary xylem (wood) [1,2]. Initials are identified by their position within secondary vascular tissues, presumably through cell-to-cell communication. Secondary xylem and phloem tissues are comprised of heterogeneous cell types whose positions are also determined during development. They differentiate into cell types appropriate for their tissue context and establish functional relationships with neighboring cells. For example, sieve elements differentiate exclusively within the phloem and work in association with neighboring companion cells to translocate nutrients and information molecules. Cell differentiation and the rate of growth must also respond to environmental and seasonal changes. For many trees, vigorous growth early in the growing season (early wood) is characterized by the production of secondary xylem containing tracheary elements that have large-diameter lumens and thin secondary cell walls, whereas drought stress results in tracheary element lumens of decreased diameter. These and myriad other developmental processes must occur within the context of interacting tissue systems that are responsible for mechanical support, physiological and defense processes, and for the translocation of water, solutes and signaling molecules over long distances.

What are the mechanisms that regulate these developmental processes? In this review, we discuss recent advances in understanding three important types of mechanisms that regulate secondary growth: transcriptional regulators, phytohormones, and information molecules of the cell wall. Discoveries are being driven by the application of new genomic technologies to forest trees and by the extension of information about evolutionarily conserved mechanisms from model annuals.

New technologies and conserved mechanisms

The massive woody bodies of forest trees are amazing examples of secondary growth. Because forest trees have long generation times and suffer from inbreeding depression, the traditional developmental genetic strategies used in model annuals cannot be applied to trees. Many genomic and transgenic technologies can be directly applied to trees, however, and have contributed greatly to our understanding of secondary growth in recent years. Global gene expression during woody growth has been cataloged in both angiosperm [3,4•] and coniferous [5] species by expressed sequence tag (EST) sequencing and microarray analysis. In *Populus*, gene-tagging strategies that produce dominant phenotypes have been used to identify genes on the basis of expression pattern [6] or generation of mutant phenotypes [7]. Forest tree populations are typified by large amounts of genetic variation, and association mapping is now being applied towards understanding natural allelic variation in the genes that regulate secondary growth in pines [8]. Notably, the entire genome of *Populus trichocarpa* has recently been sequenced and is the basis for international efforts to develop new genomic tools for this model tree genus (http://www.ornl.gov/sci/ipgc/).

Genes and mechanisms are often co-opted during the evolution of new developmental processes [9], and there

is ample evidence that this is true for secondary growth [10]. A striking example is that some of the mechanisms that regulate the vascular cambium are co-opted from the shoot apical meristem (SAM), which allows the extension of in-depth knowledge of SAM function in *Arabidopsis* to the study of secondary growth in trees [10]. Indeed, it is likely that woody plants contain gene sets that are similar to those of non-woody plants, as illustrated by comparison of genes expressed in pine stems to the *Arabidopsis* genome [5]. Developmental mechanisms central to plant development that are defined by phytohormones, information molecules from cell walls, and transcriptional regulation are increasingly well understood in model annuals. This information provides inroads into understanding the roles of these mechanisms in regulating secondary growth.

Transcriptional regulation of secondary growth

The importance of transcriptional regulation of secondary growth has been underscored by recent studies. Microarray analysis of the cambium region in *Populus* and *Arabidopsis* cataloged the expression of numerous transcription factors, including overlapping expression of transcription factors in both the SAM and the cambium [3,4•]. The Class I KNOX transcription factor SHOOTMERISTEMLESS (STM) is required for stem cell maintenance in the SAM of *Arabidopsis*. The *Populus* ortholog of STM is expressed not only in the SAM but also in the cambium region [4•]. Overexpression of the *Populus* STM leads to alterations in secondary growth, including the delay of daughter cell differentiation (A Groover *et al.*, unpublished). This suggests that STM might play analogous roles in regulating gene expression in the stem cells of both the SAM and the vascular cambium.

Myeloblastosis (MYB)-class transcription factors regulate key aspects of cell differentiation, including the transition from stem cell to terminal cell fates [11]. A gene encoding an R2-R3-class MYB from pine, *Pinus taeda* MYB4 (*Pt*MYB4), is expressed in differentiating xylem where it is associated with lignification and terminal differentiation [12]. *Pt*MYB4 can activate transcription from AC-element promoter sequences that are commonly found in genes encoding lignin biosynthetic enzymes. Overexpression of *Pt*MYB4 in tobacco results in increased overall lignification, including the lignification of normally non-lignified cell types [12]. These results suggest that *Pt*MYB4 directly regulates the transcription of lignin biosynthesis genes during wood formation.

A new type of regulation has recently been discovered in the form of genes that encode microRNAs (miRNAs), small double-stranded RNAs that direct the degradation of targeted mRNAs using a base-pairing recognition [13]. In woody *Populus* stems, miRNAs are expressed that target various transcription-factor transcripts including MYBs, an APETALA2-like protein, a NAC-domain protein, and an Auxin Response Factor [14]. Interestingly, although most of the miRNAs described in plants to date are highly conserved among species, this study provided evidence of *Populus* miRNAs that area not found in *Arabidopsis*. The potential for these miRNAs in regulating secondary growth is illustrated by their upregulation during the formation of tension wood [14], a process of differential growth that occurs in response to mechanical stress on stems.

Phytohormone regulation of secondary growth

New information about phytohormone biosynthesis, perception, and action is clarifying mechanisms through which phytohormones regulate secondary growth. The involvement of multiple phytohormones during secondary growth is illustrated by the expression of genes that are involved in the synthesis of cytokinin, gibberellin, and auxin in *Arabidopsis* root-hypocotyl tissues undergoing secondary growth [15].

Overexpressing the cytokinin synthesis gene *ISOPENTENYL TRANSFERASE* (*IPT*) in *Populus* reduces lignification and xylem formation, as assayed by histological methods (M Robischon, PhD thesis, Cambridge University, UK; 2005), whereas the putative cytokinin receptor BIRCH HISTIDINE KINASE 4 (BHK4) is expressed in the cambial zone in birch (Y Helariutta, pers. comm.). Transgenic tobacco [16] and *Populus* [17] plants that overexpress the gibberellin (GA) biosynthetic enzyme GA-20 oxidase have altered expression of lignin biosynthetic genes, and as a result show changes in the lignification of secondary vascular tissues.

Concentration gradients of auxin within plant tissues are probably involved in tissue patterning, cell differentiation, and response to environmental signals. A radially oriented auxin gradient is present across the cambium region in both pine [18] and *Populus* [19]. Six genes that encode polar auxin influx and efflux carriers in *Populus* were found to be expressed in specific radial positions within stems undergoing secondary growth [20,21], and might play a causative role in establishing auxin gradients. Interestingly, the expression of the same transporters is reduced upon onset of dormancy and is accompanied by a decrease in auxin transport activity, suggesting that auxin transport might link an appropriate developmental response to seasonal changes [20,21].

Cell wall regulation of secondary growth

During secondary growth, the walls of stem cell daughters are extensively modified as they differentiate within secondary phloem and xylem. The most dramatic examples of extracellular modification occur during the differentiation of tracheary elements and fibers, which synthesize an elaborate, lignified secondary cell wall

between the primary cell wall and the plasma membrane. Increasing evidence supports the notion that cell wall modifications and information molecules derived from cell walls participate in fundamental developmental mechanisms. Proteins that are involved in the generation of wall-derived signals or cell wall modifications during secondary growth have been described recently.

The glycosyl hydrolase superfamily includes chitinase-like enzymes that were first identified on the basis of their ability to degrade chitin in the cell walls of fungal pathogens. Chitinase-like enzymes can potentially regulate developmental processes through the hydrolysis of bonds between carbohydrates, or between carbohydrate and a non-carbohydrate moiety, in the plant cell wall to release signaling molecules. Alternatively, they could sever cell-adhesion or cell-identity molecules. Loss-of-function alleles of the *Arabidopsis thaliana* chitinase-like gene (*AtCTL1*) condition the phenotypes of the *ectopic lignification of pith 1* (*elp1*) and *pom-pom1* (*pom1*) mutants, which have aberrant patterns of lignification [22,23•]. Chitinases have also been shown to be expressed during secondary growth [24]. Although the substrate of most chitinase-like enzymes is not known, attractive candidate substrates include arabinogalactan proteins.

Arabinogalactan proteins (AGPs) are highly glycosylated proteins that are localized to cell walls and regulate various developmental processes. The fasciclin-class AGPs contain the fasciclin domain, which participates in cell adhesion in animals [25]. A recent study in *Populus* found 15 fasciclin-class AGPs proteins that are expressed in differentiating xylem [26]. Interestingly, ten of the *Populus* fasciclin-class AGPs are upregulated during the formation of tension wood [26]. Xylogen, an AGP isolated from cultured *Zinnia* cells, is expressed in developing vascular tissues and directs the interconnected differentiation of tracheary elements into files in vessels through a process of inductive cell–cell interaction [27••].

Interacting regulatory mechanisms

The complex developmental process of secondary growth requires the coordination of multiple regulatory mechanisms, including the ones mentioned here. The current challenge is to not only better define individual regulatory mechanisms but also to understand the interaction among mechanisms. Clues about these interactions are emerging. KNOX homeobox transcription factors act through pathways that involve traditionally studied phytohormones and have been shown to directly regulate genes that encode GA-biosynthetic enzymes [28•]. GA has been shown to influence cell wall lignification in stems [16]. Both KNOX [29] and MYB [12] transcription factors have been shown to regulate genes that encode key cell wall enzymes. Further connections between the regulatory roles of phytohormones and cell wall dynamics are illustrated by the well-characterized increase in cell wall extensibility in response to auxin. miRNAs have been identified that target either auxin-related genes [30] or Class III homeodomain leucine-zipper (HD-ZIP) transcription factors that direct vascular patterning [31]. Additional interconnections among these mechanisms will doubtless emerge from future studies.

Conclusions and future directions

Understanding the interactions among mechanisms that regulate secondary growth will require extensive application of genomics technologies that are capable of comprehensively assaying gene expression, protein function, and metabolic pathways. Such systems are being established by international groups for key tree species, primarily pine and *Populus*. Bioinformatics approaches that extract information and seek correlations among large, complex datasets will become increasingly vital in new studies. In addition, information about mechanisms that are evolutionarily conserved from model annuals, such as *Arabidopsis*, should be fully exploited and incorporated into studies of secondary growth in trees. The detailed functional characterization of individual genes is a limiting factor in the study of secondary growth, and new strategies should be devised for the study of gene function specifically in secondary vascular tissues.

Of course technology is not an end in itself, and the selection of meaningful questions that address key biological processes will remain the driving force behind the study of secondary growth. This article focuses exclusively on recent developments but it should be underscored that seminal findings in the field of vascular development and secondary growth by such notables as Katherine Esau, Marvin Bannan, Claude Brown, Tsvi Sachs, and William Jacobs not only guide current studies, but can now be revisited and extended using new technologies. In addition, results from developmental studies such as those considered here will open new avenues of research. For example, the molecular evolution and natural variation of secondary growth in woody plants should prove to be perennially favorite subjects.

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