Unraveling the gene network regulating secondary growth in *Arabidopsis thaliana*

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Introduction

Secondary growth is the result of a patterned control of numbers, places and planes of cambial cell division, and a subsequent regulated differentiation of cambial derivatives into tracheary elements, vessels, fibers, parenchyma, and sieve elements. To achieve the patterned growth, every cell must express the appropriate genes in a timely manner upon receipt of various molecular signals that are differentially transduced by cell-to-cell contacts, relative to cell positions, and turn on and off in response to both external and internal stimuli. This complex event of signal perception and sequential processing of the differentiation steps works by changing the global pattern of gene expression in an individual cell. Thus, the genetic control of secondary growth is accomplished by changing the activity of key genes involved in the developmental pathways, which determine the epigenetic state of the vascular cambium.

Even though many aspects of primary growth have been extensively elucidated using various model species, our current understanding of plant biology is glaringly incomplete due to the lack of knowledge on this fundamentally important aspect of plant development that also has significant practical implications. Recently, *Arabidopsis*, the most well studied herbaceous model species, has been shown to undergo secondary growth, when kept from flowering (Lev-Yadun, 1994; Oh et al., 2003; Ko and Han, 2004). Using *Arabidopsis* as a model, we demonstrated that the weight carried by the stem is a primary signal for the induction of cambium differentiation and the plant hormone, auxin, is a downstream carrier of the signal for this process (Ko et al., 2004).

Objectives

The overarching goal of this research is to move forward the frontiers of plant biology in our understanding of secondary growth. Specific objectives to attain this goal are to identify the regulatory gene network and to evaluate their functional roles by determining how the candidate gene products affect secondary growth when they are manipulated.

Approaches

We took a novel approach to unraveling the gene network regulating secondary growth in *Arabidopsis,* which incorporates complementary platforms of comparative-transcriptome analyses such as 'digital northern' and 'digital *in situ*' analysis. This bioinformatics approach exploits a large number of publicly available Affymetrix GeneChip datasets to discover the gene network regulating secondary growth.

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Results and Discussion

We have successfully used thousands of publicly available Affymetrix GeneChip array data to carry out a global comparative transcriptome analysis for the discovery of the gene network regulating secondary growth. The data analysis has been performed by using three complementary filtering platforms. The first platform comprises 1,433 genes that are 3-fold or higher upregulated in the stems undergoing secondary growth when compared to immature stems (with no secondary growth) and 10-day old seedlings (with no inflorescence stem). Although the profiling confirmed various existing insights regarding the genetic regulation of secondary growth, the number of differentially expressed genes is too large to be informative in revealing the networks of genes underlying wood formation. The second platform attempted to identify stem-specific (or preferential) genes by carrying out 'digital northern analysis' using 45 different tissue transcriptome of *Arabidopsis* as templates and six stem-preferential genes as probes. This filtering identified a total of 283 genes whose expression patterns were similar to those of probe genes with a correlation of at least 0.6. Third, 'digital *in situ*' analysis was performed to obtain gene expression profiling at tissue-type resolution by comparing the transcription profiles of secondary xylem, phloem-cambium, non-vascular (cork cambium and cork) cells, and epidermis cells. This step identified 247 xylem genes. Finally, we identified 52 genes that are upregulated in secondary xylem regardless of the organ of origin. A consideration of functions predicted for these genes revealed a surprisingly simple gene network associated with secondary growth. Five of the seven signal transduction-related genes represented in the final gene set encode the essential components of Rho-related GTPase signaling cascade, suggesting that secondary growth may be regulated through small GTPase signaling. Validity of this approach to gene discovery was supported by various independent experimental and bioinformatics methods. The candidate genes discovered in this study will be a focal point of further efforts to unravel the genetic regulations of secondary growth in plants.

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