Minireview

New approaches to the study of developing wood

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Abstract

Two recent papers illustrate contrasting approaches to studying gene expression during development of the xylem, the tissue that transports water and solutes around higher plants. The two methods used, studying single cells differentiating *in vitro* and collecting samples from across the region around the cambium of poplar trees, have both revealed genes that have altered expression during xylem development.

All plants face the problem of water transport. In the tallest trees, water taken up from the roots must be transported several hundred feet to reach the leaves in the canopy. The xylem is the tissue responsible for the transport of water and solutes around the plant in angiosperms and contains a subset of cells that are highly specialized for long-distance transport of water, known as vessels or tracheary elements (TEs). Formation of TEs is characterized by patterned formation of a thick secondary cell wall, followed by programmed cell death that results in the loss of the contents of the cell and removes the end wall between adjacent TEs. The result is a series of dead cells devoid of cell contents. These cells are connected end-to-end to form a continuous vessel that allows unimpeded water transport. The highly characteristic series of events that occurs during TE formation has made it an attractive system for researchers studying plant-cell differentiation. A potentially more important reason for studying xylem development is its role in wood formation. Each year, trees increase their girth by the activity of the cambial meristem, the stem-cell tissue that lies in a ring close to the outside of the trunk. Cells formed by the cambium may differentiate to form either phloem on the outside, which transports carbon around the plant, or xylem towards the inside (Figure 1). The phloem is eventually crushed by successive years of growth and, consequently, wood is composed almost exclusively of walls from the xylem cells.

Two recent papers [1,2] illustrate contrasting approaches to studying gene expression during xylem formation. Milloni and colleagues [1] have used the established *Zinnia* cell system. Almost uniquely among plants, mesophyll cells from leaves of the ornamental plant *Zinnia* are able to form TEs in culture with remarkable efficiency and in a highly synchronous manner after induction by the hormones auxin and cytokinin (see Figure 2). Consequently, since this experimental system was first described more than 20 years ago [3], *Zinnia* has been a widely studied model system (see [4,5] for reviews), and a number of studies have isolated genes whose transcription is altered during TE formation [6]. In the paper by Milloni *et al*. [1], however, a far more ambitious approach to studying gene expression during *Zinnia* development is described: the authors screened for genes with altered expression patterns after the induction of TE formation using the cDNA-amplified fragment length polymorphism (cDNA-AFLP) technique.

AFLP has been widely used to produce DNA markers, but can also be used to study mRNA expression. The method involves digesting cDNA with two different enzymes and then ligating specific adaptors onto each end. Rather than amplifying the entire population simultaneously, it is possible to select a subset of the cDNA fragments by using primers that add two or three base pairs to the adaptor sequence. For example, by adding a GC at the end of one

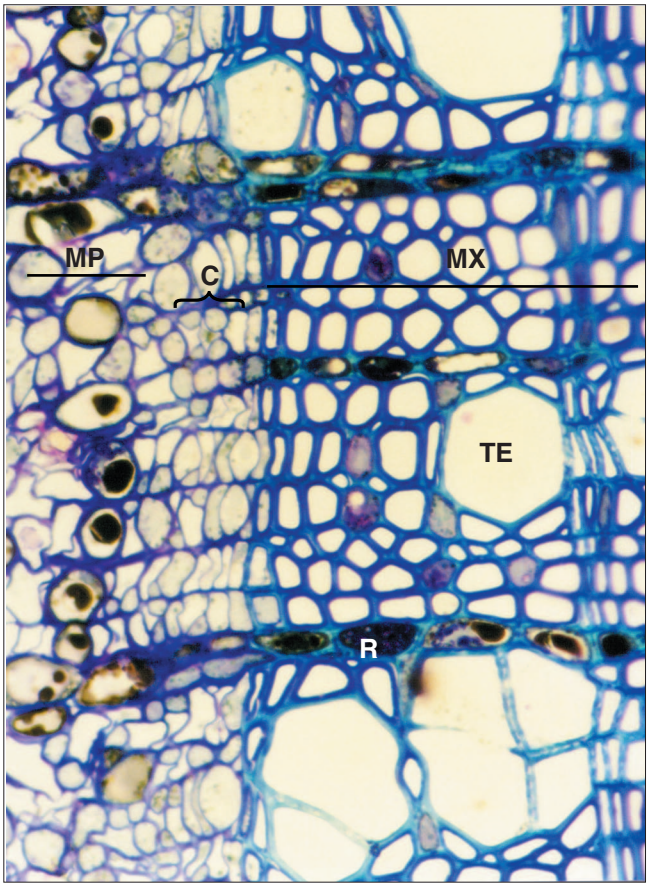

Figure 1
Light micrograph of the cambial region of a silver birch (*Betula pendula*), illustrating the usual organization of the region. C, cambium; MP, mature phloem; MX, mature xylem; R, rays; TE, tracheary elements.

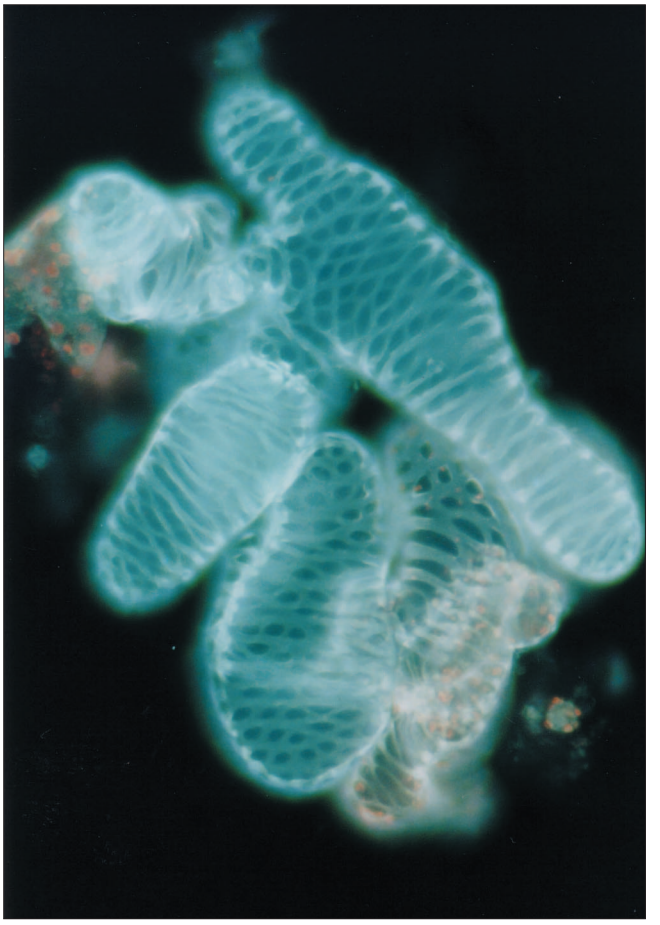

Figure 2
Fluorescence micrograph of *Zinnia* tracheary elements growing in culture.

adaptor sequence, only a sixteenth of the possible cDNA fragments will be amplified. By comparing the intensity of bands derived from mRNA from various stages of TE development it is possible to identify fragments that are differentially expressed. Using this method around 30,000 cDNA fragments were screened and 652 of these exhibited an altered expression pattern during TE formation [1]. In a number of cases, the altered expression pattern detected using AFLP was confirmed using reverse-transcriptase PCR and northern-blotting analysis. The effectiveness of the study was further validated by the fact that the genes corresponding to the 652 differentially expressed fragments included a number that had previously been identified as having altered expression during TE formation in *Zinnia* or other plants. Nearly half of the fragments isolated, however, showed no similarity to any genes of known function. This relatively high figure may mean that many novel genes required for xylem formation have been identified, or it may reflect the relatively short (50-450 base-pair) cDNA fragments used for the analysis. Identification of full-length cDNA clones corresponding to these short fragments should facilitate assignment of a function to some of the corresponding genes.

This study [1] helps to validate the *Zinnia* system as a method for studying vascular differentiation. *In situ* hybridization analysis confirmed that at least some of the genes identified as being expressed during TE formation *in vitro* were also expressed in the developing vascular tissue of intact *Zinnia* plants, suggesting a role in vascular development *in vivo*. The study also identified a *Zinnia* homolog of the *Arabidopsis* gene *MONOPTEROS,* which has previously been identified as being essential for the regulation of vascular patterning by auxin [7]. One advantage of the *Zinnia* system is the highly synchronized manner in which cells differentiate. Milloni *et al.* [1] were able to identity 68 fragments that were expressed within only 30 minutes of induction of TE formation; the genes corresponding to these fragments may be important in the initial stages of TE differentiation. Interestingly, these include those homologous to known components of the auxin signaling pathway such as the *AUX/IAA* genes involved in transcriptional repression

and a number of other genes known to be induced by auxin. Some further analysis is required to determine whether these genes are strictly involved in TE formation or whether they are always induced by either auxin or cytokinin.

Hertzberg and colleagues [2] exploited the relatively large size and defined transition of developmental stages across the zone from the cambium to the mature xylem from the hybrid aspen or poplar (*Populus tremula* x *Populus tremuloides*). The ordered nature of the cambial region means that xylem differentiation exhibits a clear spatial pattern: cells in the cambium itself are relatively undifferentiated, but as they differentiate into xylem, they get further displaced from the cambium. Using cryosectioning, Hertzberg *et al.* [2] isolated tissue samples from defined regions between the cambium and the mature xylem that represent the different stages of xylem development. To overcome the problems of using only relatively small regions of tissue, the authors used a PCR-amplification step that allowed the preparation of samples for microarray analysis from very small amounts of RNA [8]. Using a microarray of 3,000 unique expressed sequence tags (ESTs) from a developing xylem library [9], they were able to monitor gene expression through the various stages of xylem development in poplar. More than 1,200 of these ESTs showed a greater than fourfold alteration in expression between different stages. The expression profiles were used to classify the genes into clusters with similar expression profiles; some genes had high levels of expression in the cambium, whereas other genes were expressed at the highest levels during the later stages of xylem development. In results similar to those from the *Zinnia* system [1], only 60% of the genes showed high similarity to *Arabidopsis* genes [2]. Of the 540 genes on the microarray that are homologous to *Arabidopsis* genes of unknown function, a large number (211) were differentially expressed during xylem development.

In contrast to the *Zinnia* system, in which the different stages of xylem development are temporally separated, in poplar the different stages are spatially separated. Genes involved in regulating cell fate and identity would be expected to be expressed in, or close to, the cambium. The findings for two genes, in particular, validate this assertion. The *Arabidopsis* gene *ATHB-8*, which encodes a transcription factor that has been proposed to regulate vascular development, whereas the *KNOTTED-LIKE* genes, which also encode transcription factors, are proposed to maintain cells in an undifferentiated state [10,11]. Consistent with there being a similar role for these genes in poplar, the ortholog of *ATHB-8* is expressed in the cambium and very early in xylem development, whereas the ortholog of *KNOTTED* is strongly downregulated during the later stages of xylem development [2]. The success of the method is also demonstrated by the ability to detect alteration in the expression patterns of genes known to be involved in the synthesis of secondary cell-wall polymers; these include genes involved in lignin biosynthesis, many of which exhibited high levels of expression during xylem development, consistent with a role in secondary cell wall formation [2].

Milloni *et al.* [1] estimate that the 652 genes that they found to be differentially expressed make up around 60% of all the genes that are differentially expressed during xylem development, suggesting that a total of around 1,200 may be differentially expressed. In comparison with the poplar study [2], this figure would appear to be an underestimate. Although poplar xylem is a more complex tissue, containing more cell types, Hertzberg *et al.* [2] were able to identify 1,200 differentially expressed genes using a microarray containing only 3,000 sequences. The number of differentially expressed genes is likely to rise dramatically as larger microarrays are used for these experiments.

Clear similarities exist between the Poplar and *Zinnia* studies [1,2]. In both cases, genes whose function is related to cell-wall biosynthesis and to the cytoskeleton represented a high proportion of the genes exhibiting altered expression levels. This is consistent with the fact that cell-wall biosynthesis and accompanying cytoskeletal rearrangements are among the most important events during xylem development. In particular, both studies found that a number of *CesA* genes, which encode cellulose synthases, were upregulated during the later stages of xylem development; this is consistent with the known role for cellulose in the secondary cell wall. Changes in the expression of genes associated with the cytoskeleton are particularly complex: 14 different tubulin genes were present on the poplar microarray, of which ten were strongly upregulated during the later stages of xylem development whereas the others showed a small decrease in expression over the same period.

The two studies [1,2] discussed here represent the starting point of a project to understand the role of the differentially expressed genes identified during xylem development. It is clear that homology alone will not be enough to assign a function to each of the genes identified. For example, the poplar study identified no fewer than 11 genes with homology to anionic peroxidases that were differentially expressed, but many of these genes had distinct expression patterns, suggesting that their functions in the plant may vary. It is likely to be most difficult to assign a function to genes with no clear homolog in *Arabidopsis*. Despite its size, poplar has a clear advantage in this area, given that one of the initial reasons for selecting it was the relative ease by which genes may be introduced into its cells by transformation. The use of poplar for functional genomic studies has recently received a further boost with the recent announcement by the US Department of Energy that the poplar genome would be sequenced by a consortium of groups within the next 18 months [12]. The genome sequence will greatly help in the development of larger microarrays and in assigning gene function by homology.

Clearly, the future of poplar as a model tree species looks very promising.

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