## Scientific Correspondence

# *Zinnia*. Everybody Needs Good Neighbors[1]

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Cells in an organism exist within a social context—the sum of interactions with neighboring cells help to define the nature of each individual. The *Zinnia* system provides a unique opportunity to study in vitro the interactions between different plant cell types and the consequences for commitment to a particular cell fate.

*Zinnia* is a model system in which mesophyll cells, released from young leaves and incubated with auxin and cytokinin, become committed to a new cell fate. Up to 60% or so of the cells eventually transdifferentiate into dead lignified tracheary elements (TEs) over the course of about 4 d (Fukuda, 1996). The emphasis in past work has been on the TEs themselves, but it has become more evident of late that the system also provides a paradigm for studying cell-cell interactions in development, interactions that have been singularly difficult to approach before in plants. In this respect, animal systems have proven to be more amenable to experimental approaches, and the patterns of signaling between cells to determine cell fate are relatively well understood. The competence of animal cells to respond to extracellular signals, provided through paracrine, autocrine, or in the case of distant cells not of the same type, endocrine signaling mechanisms, depends on the prior developmental history of each cell. The polarity of the embryo permits the later patterning of broad domains in which the cell populations express different combinations of homeotic regulatory genes. These domains become progressively refined as groups of cells acquire positional information. Finally, individual cells become determined to specialized cell fates. In both animals and plants, developmental genetics has uncovered key regulatory genes that can broadly define cell fate. Additionally, in animals, experimental embryology has been used to establish the state of commitment to a particular cell fate by microsurgical manipulation of groups of cells into new environments and by observing whether they adopt the fate of their new or original environment. In plants, studies using genetic mosaics have shown that the position of a cell, not its clonal origin, determines its fate (Scheres, 2001). Rare cell division events place daughter cells in neighboring files in the Arabidopsis root. The fate of these daughters is appropriate to their new position (Kidner et al., 2000). Within meristems, cells transit through domains of expression of regulatory genes to their final fate, with only a small number of cells remaining uncommitted to provide a population of "stem" cells that renew the meristem continuously (Haecker and Laux, 2001). Although surgical excision and transplantation of specific cells is rarely feasible in intact plants, cell culture systems offer a useful and appropriate alternative. In particular, the *Zinnia* mesophyll cell system now offers an opportunity to study the consequences of extracellular signaling for developmental fate.

Plant growth factors have been shown in two model systems to be involved in cell commitment. Ethylene is required for the final commitment of cells to produce a root hair in the Arabidopsis root epidermis (Tanimoto et al., 1995). Both mesophyll cells (Fukuda and Komamine, 1980) and epidermal cells (Church and Galston, 1989) of *Zinnia elegans* can be induced, by auxin and cytokinin, to transdifferentiate into TEs in vitro. Although Arabidopsis provides an excellent molecular genetic model, the *Zinnia* system has the advantage of being more amenable to biochemical or in vitro culture studies. Because differentiation is synchronously induced in a large number of the cells in a relatively homogeneous cell population, it is possible to study the biochemistry and molecular biology of xylogenesis free from the complexity of intact plant tissues (Fukuda, 1996; McCann, 1997).

Three factors, wounding, auxin, and cytokinin, are involved in initiating TE formation in *Zinnia*. Mechanically isolated cells may be regarded as wounded cells, and wounding is known to induce TE formation in intact plants. When a vascular bundle in a stem is severed, a connection is reestablished by the transdifferentiation of intervening pith parenchyma into vascular elements (Sachs, 1981). However, many further signals are required to complete TE formation (Fukuda, 1996). Inhibitors of brassinosteroid synthesis block TE formation until a very late stage of differentiation (Yamamoto et al., 1997). Because the competence to differentiate is also highly dependent on cell concentration until a late stage of culture, this strongly suggests that cell-cell signaling mechanisms are involved and, therefore, that commitment and at
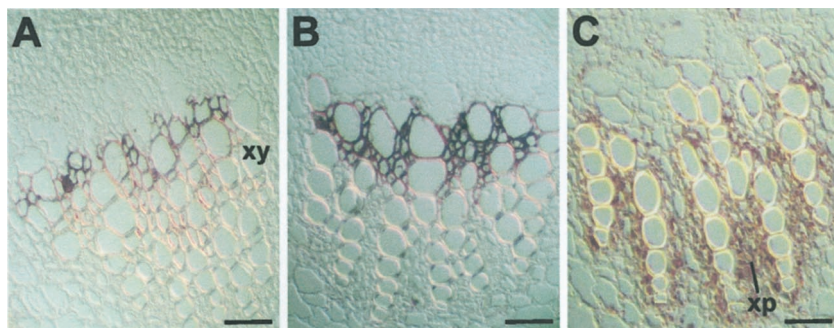
**Figure 1.** In situ hybridization shows the expression patterns in vivo of three *Zinnia* genes that have no homology to any known genes. These were identified from the in vitro system and are expressed in different cellular locations within the xylem in the stem. A and B, Probes localized to slightly different zones of young xylem; C, localization of another probe to xylem parenchyma. Scale bars = 70 μm.

least the early stages of differentiation are not cell autonomous. Because there is an optimal cell concentration for transdifferentiation, both negative and positive regulators of TE formation are likely to be present in the culture medium.

The TEs that form in the *Zinnia* system are very easy to distinguish from the starting population of mesophyll cells. Thus, at least two different fates are clearly visible: cells that form TEs (about 60% of the population) and cells that do not. However, our recent results indicate that a more complex state of affairs exists within this apparently simple system.

To obtain a broad set of molecular markers for different stages of xylogenesis, we have recently applied an RNA fingerprinting technology, cDNA-amplified fragment-length polymorphism (Bachem et al., 1996; Durrant et al., 2000), which allows us to detect differentially regulated genes at different time points during TE formation (Milioni et al., 2001). cDNAs are synthesized from mRNA populations isolated from the *Zinnia* cultures at five time points, digested with a pair of restriction enzymes, adaptor ligated, and amplified by PCR to produce the primary template. A subset of this population of fragments is selectively amplified using degenerate primers with two selective nucleotides and then analyzed on polyacrylamide gels. From the 30,000 gene fragments displayed, we selected over 600 genes whose transcription levels show overt changes in abundance over time, and we obtained partial sequences. We have used about 10 of these partial sequences as probes for in situ hybridization experiments both on *Zinnia* stems and on the cell system itself. Most of these hybridize to cells within vascular bundles of the stem. However, we find a variety of cell types are labeled in addition to young xylem tissue. Some sequences are markers for the cambium, xylem parenchyma, or phloem fibers. Figure 1 shows the localization of three probes (for novel genes that have no homology to genes of known function) to different groups of cells within xylem. Within the single cell system, a marker specific for young xylem tissue in vivo is expressed in only a small subset of the population (Fig. 2). These results highlight an important feature of the *Zinnia* system. Patterns of gene expression elicited by auxin or cytokinin in the cell system suggest that at least two functional cell types are

present, one of which is the differentiating tracheid. In vivo, the labeling patterns seen for the genes expressed in the in vitro system are even more complex, hinting that even in the in vitro system there may be more cellular complexity to be uncovered.

Several lines of evidence suggest that the population of living cells that do not differentiate, rather than being simply defective, are instead required to support actively those cells that do differentiate. Dilution of the cells with culture medium at different time points shows that the progress of TE formation does not become fully cell autonomous until a late stage of culture, just before lignification and cell autolysis (N.J. Stacey and M.C. McCann, unpublished data). Some cells may be required to remain as xylem parenchyma cells to act as nurse or feeder cells. If *Zinnia* cells are cultured at too low a cell density, then TE formation is suppressed even in the presence of auxin and cytokinin. However, addition of α-phytosulfokine promotes TE formation in low-density cultures in a concentration-dependent fashion (Matsubayashi et al., 1999). By culturing cells embedded in microbeads of agarose gel and then manipulating the density of cells within each bead, or the density of such beads within a liquid culture, Motose et al. (2001a) were able to demonstrate that the frequency of TE differentiation depended on the local cell density in the beads. A proteinaceous molecule of between 25 and 300 kD could induce TE formation in cells embedded at low density in agarose sheets, but
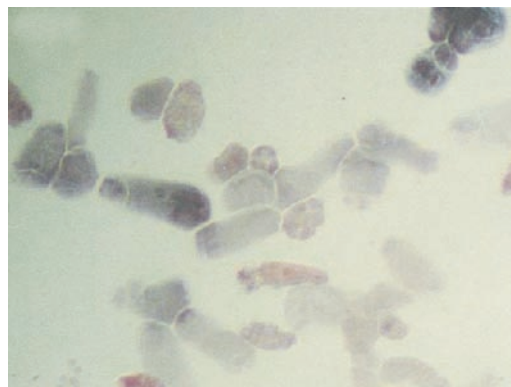


**Figure 2.** A probe that is localized to young xylem tissue in stems is localized in a subpopulation of cells in the in vitro system.

only if the low-density sheet was in contact with a high-cell density sheet and separated by a membrane. A likely candidate for this xylogenesis-promoting factor is an arabinogalactan protein (Motose et al., 2001b). Soybean (*Glycine max*) trypsin inhibitor inhibits both programmed cell death in the *Zinnia* system and the activity of a 40-kD Ser protease secreted during secondary cell wall synthesis in the *Zinnia* system (Groover and Jones, 1999). It was suggested that the Ser protease may act upon a substrate produced in the cell wall during secondary wall formation to produce a signaling molecule that triggers programmed cell death, thus coordinating the cessation of wall deposition with the initiation of the death process.

The importance of living neighbors that may act as nurse cells for vessel and TE formation has been indicated by studies in other systems. In conifers, lignins are supplied as β-glucosides from the adjacent parenchyma cells to the secondary walls of differentiating xylem (Savidge, 1989). Likewise, a Gly-rich protein from *Phaseolus vulgaris* is present in the cell walls of primary xylem elements, but it is synthesized in the xylem parenchyma and then exported to the walls of neighboring protoxylem vessels (Ryser and Keller, 1992).

Interestingly, cell polarity does appear to be cell autonomous and is maintained even without cues from neighboring cells. By scanning electron microscopy, the hole that forms at one end of TEs in planta also forms at only one end of the isolated in vitro TEs (Im et al., 2000). Shinohara et al. (2000) identified an epitope in the cell wall hemicellulose fraction that is distributed in a polarized way in immature tracheids both in planta and in the *Zinnia* cell system. Within the plant, the establishment of polarity may reflect the polar distributions of receptors on the surface of cells (Galweiler et al., 1998), but the maintenance of cell polarity in the culture system may imply some memory mechanism by which the initial polarity of the cell within the leaf has been imprinted.

Our knowledge of molecular signals that mediate cell fate decisions in plants is sadly limited. The long-distance delivery of proteins and mRNAs found within phloem sap that may direct and coordinate developmental events (Lucas and Wolf, 1999) could be the plant equivalent of the endocrine system of animal cells. Paracrine signals are secreted molecules that must be delivered to a few local cells—they must be rapidly taken up by neighboring target cells, destroyed by extracellular enzymes, or immobilized by the extracellular matrix. An example in plants is the secretion of the CLAVATA 3 protein from cells of the outer layer of the shoot apical meristem. This ligand binds to the CLAVATA 1 receptor protein on target cells in an adjacent, more central region of the meristem, probably stimulating their differentiation (Haecker and Laux, 2001). Autocrine signaling involves secretion of signals that act back on the orig-

inal cell and on identical neighbor cells, leading to the coordinate regulation of cellular behavior. Such mechanisms are essential in the maintenance and refinement of the differentiated state of cells. Over 10% of the genes annotated in the Arabidopsis genome sequence have to do with cell communication and signal transduction, and although the *Zinnia* system is not a model genetic system, it should prove to be a useful new paradigm for identifying those genes and proteins involved in intercellular communication and for defining the repertoire of genes required for a plant cell to adopt progressively a vascular cell fate.

## ACKNOWLEDGMENTS

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