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Global expression profiling applied to plant development

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Plant development is controlled by both endogenous genetic programs and responses to exogenous signals. Microarray experiments are being used to identify the genes involved in these developmental processes. Most of the analyses conducted to date have been conducted on whole organs. Although these studies have provided valuable information, they are limited by the composite nature of plant organs that consist of multiple cell types. Technical advances that have made it possible to study global patterns of gene expression in individual cell types promise to increase greatly the information revealed by microarray experiments.

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Abbreviations
AP3 *APETALA3*
cop *constitutively photomorphogenic*
LCM laser-capture microdissection
PI *PISTILLATA*
wri1 *wrinkled1*

Introduction

Several high-throughput techniques (reviewed in [1–4]) are available to examine the expression patterns of thousands of genes in parallel. Depending upon the technology used, expression can be studied at the level of mRNAs, proteins or metabolites. Although it is impossible at present to analyze all of the proteins or all of the metabolites found within a sample, microarray technology makes it possible to analyze 'all' of the mRNA species within a sample. Microarray technology [2] has been widely adopted by the plant biology research community, and so this review focuses on the role of transcriptomic analyses in plant developmental biology.

Plant development is typically analyzed using one of the following experimental designs. First, global patterns of gene expression at a defined developmental stage can be compared between two different genotypes, typically a mutant and the corresponding wildtype. Second, gene expression patterns can be analyzed at different developmental stages or in different organs or tissues from a common genotype. Finally, environmental factors that influence development can be studied by analyzing plants that have and have not been exposed to exogenous stimuli.

Profiling gene expression during development

Plant development is controlled by both endogenous genetic programs and by responses to exogenous signals. The following section briefly summarizes the most recent (post-2001) microarray data from investigations of vegetative and generative development and from studies of the influence of certain abiotic signals on development. Plant responses to biotic signals, such as pathogen attack, have not been included in this review [5]. The findings of microarray analyses of plant development reported before 2002 have been summarized by Aharoni and Vorst [2].

Endogenous genetic programs

Vegetative phase

Various aspects of vegetative development, beginning after pollination with embryogenesis and ending with the onset of flowering, have been profiled using cDNA microarrays. Although the steady-state levels of many genes vary dramatically during the development of maize embryos, genes that encode enzymes that are involved in fatty-acid biosynthesis, the tricarboxylic acid (TCA) cycle and glycolysis are coordinately regulated [6]. Seed filling, which provides nutrients for the growing embryo and the storage of reserve materials for use by the germinating seedling, has been studied using the *Arabidopsis* mutant *wrinkled1* (*wri1*). The seed-oil content of this mutant is just 20% of that of wildtype seeds. Most of the differentially expressed genes in *wri1* are involved in central lipid and carbohydrate metabolism [7]. Analyses of seed filling in rice defined 269 genes involved in different pathways that are coordinately upregulated during grain filling [8]. Together, these studies in maize, *Arabidopsis* and rice [6–8] revealed key components of the regulatory machinery that is involved in embryo development and grain filling. They might, therefore, provide data that are

needed to improve grain quality; for example, by altering the partitioning of photoassimilates between the oil and starch biosynthetic pathways.

During the final stages of embryogenesis and seed filling, fruit maturation and ripening help to ensure the propagation of a species. Genes involved in several processes, including vascular development, oxidative stress and auxin response, that had not previously been associated with fruit ripening are induced during the ripening of strawberry (*Fragaria* × *ananassa*) [9]. During the transdifferentiation of zinnia (*Zinnia elegans* L.) photosynthetic mesophyll cells into xylem cells, the rapid repression of photosynthesis-related genes was followed by the induction of protein-synthesis related genes, auxin-induced genes, genes that are involved in particular morphogenetic events and, finally, genes that are involved in apoptosis [10•]. These findings provide insights into the molecular mechanisms that are involved in both plant transdifferentiation and wood formation. An interesting outcome of these two studies [9,10•] was the parallelism in gene expression patterns during the ripening of strawberries and the differentiation of tracheary elements in *Z. elegans*. This may suggest that the development of the vascular system is coupled to fruit maturation [1].

Several aquaporins and transporter transcripts are differentially expressed during adventitious root formation in poplar (*Populus tremula* × *Populus tremuloides*) [11]. Analyses of these channels and transporters hold great promise for the elucidation of nutrient uptake and assimilation pathways [11].

Generative phase

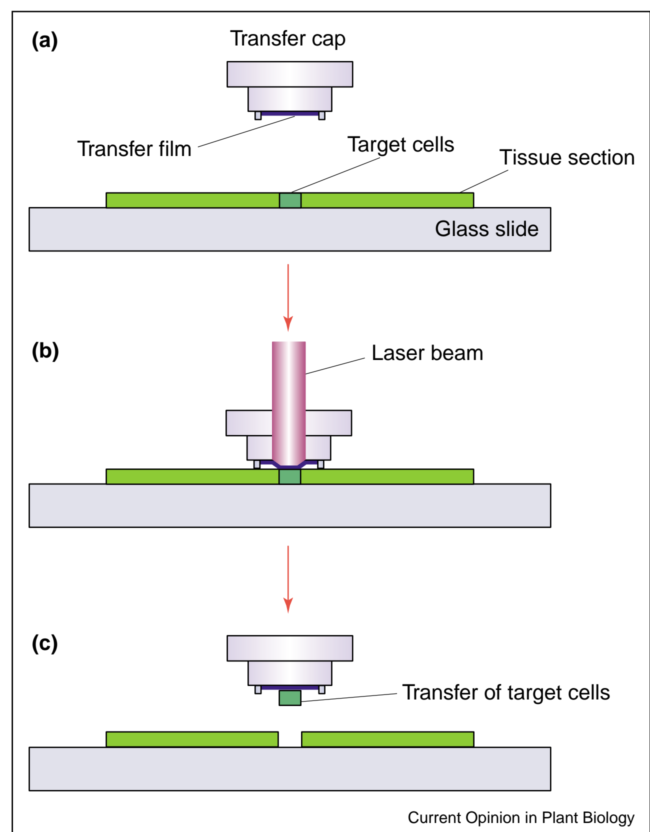
The reproductive phase of plant development starts with the formation of reproductive organs and ends with pollination. In *Arabidopsis*, the floral homeotic genes *APETALA3* (*AP3*) and *PISTILLATA* (*PI*) are crucial for petal and stamen formation. Transcriptome analyses of *AP3* and *PI* suggested that these loci control a small number of genes, most of which are not petal or stamen specific. Because only a few transcription factors were modulated by *ap3* and *pi* mutations, *AP3* and *PI* may act relatively directly to regulate the genes required for petal and stamen morphogenesis [12•]. Microarray analysis of immature and mature anthers and pistils of lotus (*Lotus japonicus*) revealed several clusters of specifically expressed cDNAs, which will be a starting point for a detailed genetic analysis of genes that are involved in the formation of reproductive organs [13].

Environmental signals

In addition to the roles of the endogenous genetic programs involved in plant development, the influence of external cues on plant development has been studied extensively. Analyses of the photomorphogenic mutants *constitutively photomorphogenic* (*cop*), *de-etiolated* (*det*) and *fusca* (*fus*) established that the transcriptomes regulated by these loci are largely, but not completely, overlapping [14]. *cop1* mutants grown in the dark had both microarray profiles and photomorphogenic phenotypes that were similar to those of wildtype plants grown in light [15]. Therefore, COP1 and light regulate the expression of the same set of genes in an antagonistic manner. It is plausible that light regulates genome expression mainly by the inhibition of COP1 activity [15].

Plant development is greatly influenced by hormones. Microarray studies involving the external application of auxin [16], abscisic acid [17,18], gibberellic acid [19], and brassinosteroids [20,21] not only identified cDNAs that are specifically regulated by these hormones but also revealed cross-talk among the different hormone response pathways.

Figure 1



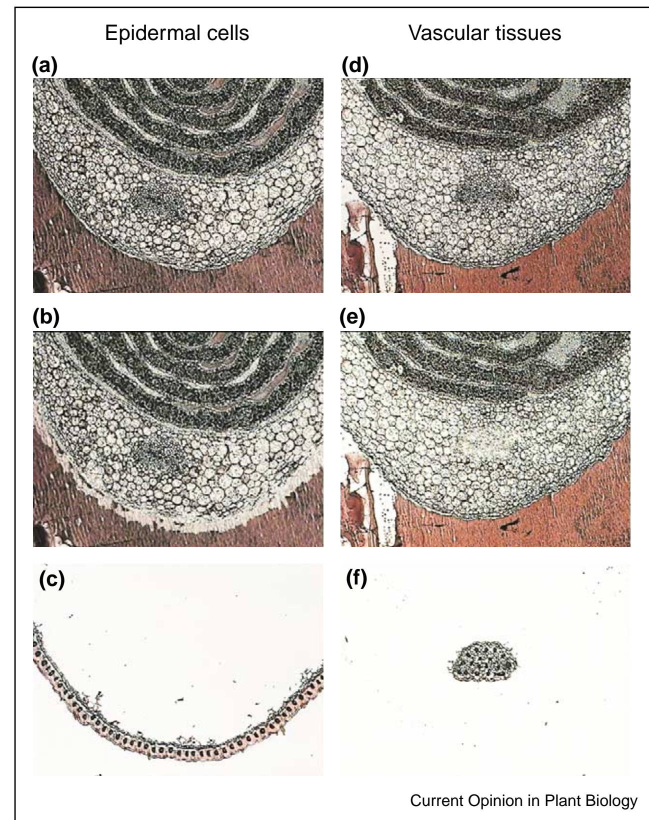
Laser capture microdissection (LCM). **(a)** A transfer cap with a transparent transfer film is placed on a tissue section, which contains the target cells mounted on a glass slide. **(b)** A focused laser beam of short duration (e.g. 1 ms) is targeted on the cells of interest through the transfer cap. The laser beam activates and expands the transfer film, causing it to become locally adhesive and to fuse the target cells. **(c)** The transfer cap is removed from the tissue section. The target cells fused to the transfer film are separated from the rest of the tissue. This schematic describes principles used by the Arcturus LCM; laser-based capture systems that are based on different principles are also available [47,48].

Likewise, various kinds of stresses repeatedly induced similar genes, indicating a central role for these genes in plant protection [22]. Stresses that have been analyzed include salt/osmotic/cold stress [23,24], phosphorus starvation [25,26], drought/salt stress [22], low oxygen in roots [27], high light-stress [28,29], and other stress conditions that induce or repress the expression of transcription factors [30•]. For several stresses, gene expression in the roots and leaves exhibited very distinct reactions [23,25].

Cell-type-specific profiling

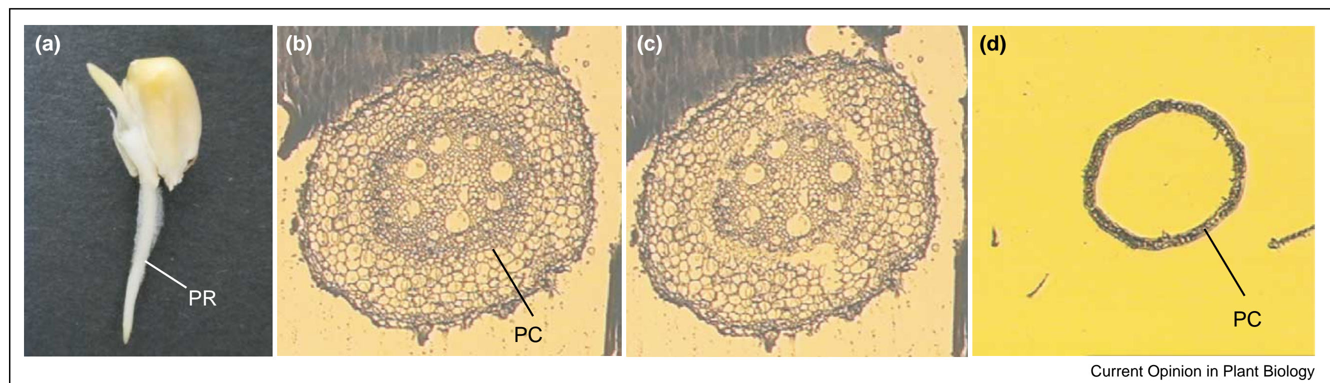
Analyses of global gene expression can reveal much about how genes function and how their gene products interact during development, but to date these analyses have generally been conducted on whole organs, such as leaves or roots. Such analyses are limited by the composite nature of plant organs; plants consist of more than 40 different cell types [10•]. Roots, for example, are made up in the transverse direction of epidermal, cortical, endodermal and pericycle cells plus various cell types in the central cylinder. Each of these cell types expresses a unique transcriptome. Therefore, a transcriptome analysis of a complete root provides average gene expression levels integrated over all cell types. Such an analysis has the potential to mask genes of interest, that is, those that are specifically expressed in a particular cell type. To understand biological processes in individual cells during plant development, methods are needed for the efficient isolation of populations of specific cell types [31]. At least three such approaches have been reported, and some of these strategies are being used to conduct transcriptome analyses that can define cell-type-specific molecular networks.

Figure 2



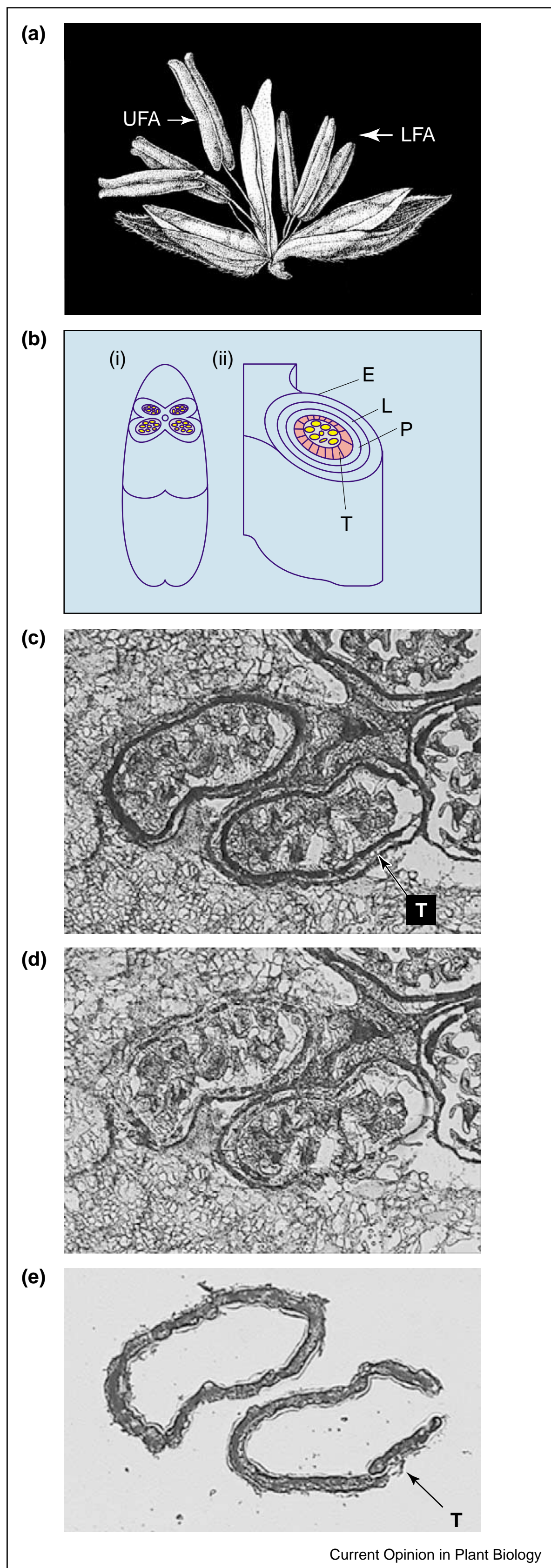
The isolation of epidermal cells and vascular tissues from 6-μm-thick sections of maize coleoptiles using LCM. **(a,d)** Sections before LCM. **(b,e)** Sections after LCM. **(c,f)** Captured cells on the cap. Figure reprinted from Nakazono *et al.* [41••], the material is copyrighted by the American Society of Plant Biologists and is reprinted with permission.

Figure 3



The isolation of pericycle cells from maize roots using LCM. The pericycle (PC) is the outermost cell layer of the central cylinder of the root and gives rise to secondary root initials. **(a)** A maize seedling three days after germination (DAG). Note the root hairs, which indicate the differentiation zone of the primary root (PR). **(b)** Cross-section (8 μm thick) in the differentiation zone of a three DAG primary root before LCM. **(c)** The same cross-section after removal of the pericycle cell layer by LCM. **(d)** The pericycle cell layer captured from the cross-section by LCM. LCM performed and photographs provided by Katrin Woll (University of Tübingen).

Figure 4



The isolation of tapetum from maize anthers using LCM. **(a)** Maize spikelets consist of an upper and a lower floret, each containing three anthers. The anthers in the upper florets (UFAs) mature several days earlier than those in the lower florets (LFAs). **(b)** (i) Each anther consists of four locules. (ii) The anther wall consists of the epidermis (E), the endothelial layer (L), middle layer(s) (P) and the tapetum (T). **(c)** A cross-section (10 μm thick) of two locules from a single early microspore anther before LCM. The tapetum is the innermost cell layer of the anther wall. **(d)** The same cross-section after LCM of the tapetum. **(e)** The tapetum captured from the cross-section shown in (c) by LCM. (a) Drawn by Miwa Kojima (Iowa State University) from Liu *et al.* [49], the material is copyrighted by the American Society of Plant Biologists and is reprinted with permission. (b) Adapted by Tsui-Jung Wen from Horner and Palmer [50] with permission. (c–e) Provided by David Skibbe (Iowa State University).

The microcapillary method

Karrer *et al.* [32] developed a method for the isolation of mRNA and the preparation of a cDNA library from the contents of specific living cells (e.g. epidermal cells and guard cells) isolated using a microcapillary. Brandt *et al.* [33•] used the microcapillary method to obtain mesophyll and epidermal cells, and performed cDNA-array hybridizations using RNA extracted from these cells. Brandt *et al.* [34] used the expression of the visible marker green fluorescent protein (GFP) under the control of a cell-type-specific promoter to solve the problem of identifying specific cell types. In transgenic plants, the expression of GFP has been used to identify target non-surface cells [34]. A disadvantage of this technique is that very few cell-type-specific promoters are currently available. In addition, this approach would be difficult to implement in species that are not easily transformed.

Protoplast preparation

A second approach for the collection of uniform cell populations is to use protoplasts. Using flow cytometry, protoplasts derived from cells of interest can be separated according to their sizes and/or chlorophyll contents [35], or by the presence of a visible marker whose expression is specific to the target cells [36]. When this method is used to monitor gene expression in individual cell types, however, the expression patterns of some genes could possibly be changed as a result of the manipulations required to generate protoplasts [37].

Laser-capture microdissection (LCM)

In this approach, cells of interest are physically stuck to a transparent film with the aid of a laser. Laser-capture microdissection (LCM) an attractive approach because it can be used to rapidly and precisely isolate a wide variety of cell types from heterogeneous tissues (Figure 1; [38]). Because these tissues have been quickly fixed or frozen before capture undesirable changes in gene expression that could arise during sample preparation are minimized. Although LCM has been combined with DNA microarrays to study gene expression in mammalian cells (reviewed in [39]), plant cells are less

amenable to LCM because of their rigid cell walls and large vacuoles [31].

Recently, three research groups have succeeded in using LCM to isolate individual plant cell types. Asano *et al.* [40••] constructed a cDNA library from approximately 150 phloem cells from rice leaves that were isolated by LCM, and thereby identified phloem-specific genes. Nakazono *et al.* [41••] reduced the problems presented by rigid cell walls and air spaces between cells by freezing cells, and used LCM in combination with cDNA microarrays to conduct high-resolution analyses of the global gene expression of selected cells (Figure 2). Approximately 1.5% of the genes detected by the cDNA microarrays were preferentially expressed in the epidermal cells or vascular tissues of maize coleoptiles [41••]. Kerk *et al.* [42••] used RT-PCR to examine gene expression patterns in RNA samples from cells obtained from paraffin-embedded tissue sections using LCM. They found that cell structures were generally better preserved in paraffin-embedded sections than in frozen tissue sections.

Only a small amount of RNA (between a few to a hundred nanograms) can be extracted from LCM-isolated cells. Because a few micrograms of mRNA are required for hybridization, it is necessary to amplify the RNA from LCM-isolated cells before conducting microarray analyses. T7 RNA polymerase-based RNA amplification is a highly reproducible method for amplifying RNA from LCM-selected plant cells [40••–42••]. A recent modification of RNA amplification [43] might make it possible to conduct microarray analyses on even fewer cells, facilitating the study of cell types that can be isolated only with great difficulty (e.g. egg cells).

Conclusions

Global expression profiling experiments are beginning to define the genes that are involved in specific developmental programs. DNA microarray analyses, in combination with new technologies such as LCM, are expected to become increasingly important tools for monitoring changes in transcript accumulation in specific types of plant cells in which morphological and/or physiological changes occur during development (e.g. cells of the pericycle and the tapetum [Figures 3 and 4]). Significant challenges for expression profiling remain, however, including the improvement of designs for microarray experiments, the application of rigorous statistical approaches to analyze these experiments, and the development of improved technologies for isolating particular cell types. It would also be desirable to be able to assay not only mRNAs but also proteins and metabolites in captured cells. Fortunately, progress is being made in the application of LCM to proteomics. For example, proteins in LCM-selected mammalian cells have been subjected to two-dimensional polyacrylamide gel electrophoresis and mass spectrometry [44,45]. In addition, ProteinChip™ arrays (CIPHERGEN), in combination with surface enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS), can be used to examine protein profiles in LCM-isolated mammalian cells [44,46].

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