Minireview

Expression profiling of plant development

Giovanni Mele and Sarah Hake

Address: Plant Gene Expression Center, USDA-ARS, University of California, Berkeley, CA 94710, USA.

Correspondence: Sarah Hake. E-mail: maizesh@nature.berkeley.edu

Published: 29 April 2003

Genome ***Biology*** 2003, **4**:215

The electronic version of this article is the complete one and can be found online at http://genomebiology.com/2003/4/5/215

Abstract

Microarrays have been used to study the response of plants to many signals, including light, hormones and transcription factors. The results in each case can give an overall view of the global response to the signal or identify direct targets of the signal, and can reveal new links between different signaling pathways.

Gene expression is central to the regulatory mechanisms that cells use to respond to internal and external stimuli. Although the final activity of a particular gene is determined by the encoded protein, measurements of RNA levels have proven to be valuable in identifying the molecular changes that occur in cells. The availability of complete genome sequences and large sets of expressed sequence tags (ESTs) has triggered development of efficient methods of RNA detection that allow large-scale analysis of genetic variation and profiling of many genes. These technologies are based on sequence, amplified fragments or hybridization. Microarray technology, on which this article focuses, is hybridization-based and permits large-scale and genome-wide analysis of gene expression from multiple samples. There are two fundamentally different microarray technologies: cDNA microarrays, which consist of a collection of amplified cDNA fragments spotted or printed on a solid surface, and oligonucleotide microarrays, such as the widely-used GeneChips produced by Affymetrix [1], in which gene-specific oligonucleotides are synthesized directly onto a glass surface by photolithography. The most common current application of microarrays is gene-expression analysis, helping to assign new functions to known genes and identify putative functions for unknown genes on the basis of the similarity of their expression profiles to those of known genes. Microarray technology has been widely used to understand the roles of regulators of plant development, such as light, hormones, and transcription factors.

Responses to light

Light is one of the most important environmental signals regulating the growth and developmental programs of plants, and the global response of the genome to light has been studied in *Arabidopsis* [2]. The ability of plants to respond to light is achieved through a network of photoreceptors, which convert the light signal into changes in gene expression. In *Arabidopsis*, two classes of photoreceptors are known: the red/far-red receptors, phytochromes A to E; and the blue-light receptors, CRY1, CRY2 and NPH1. The phytochromes are the best characterized of the photoreceptors [3]. The diverse responses to light depend on interactions between the phytochromes and the basic helix-loop-helix transcription factors such as PIF3 [4,5]. Genome-wide gene-expression profiles of signal transduction in *Arabidopsis* development in response to light [2] suggest that the process involves changes in the expression of up to 30% of the genes in the genome. This massive change is probably the result of activation of a transcriptional cascade [6]. The large number of genes involved in light signal transduction reveals the complexity of the genomic response to one of the most important developmental regulators.

Hormone responses

Brassinosteroids comprise a well-studied class of hormones essential for plant growth and development. Microarray analysis comparing the brassinosteroid-deficient mutant *det2*

with wild-type *Arabidopsis* [7] revealed not only a tight connection between the response to brassinosteroids and the regulation of cell-wall organization but also a possible connection between the responses to brassinosteroids and to light. Although the interaction between the hormone-signaling and the light-signaling pathways has been studied extensively [8,9], the molecular mechanisms that connect the pathways remain unclear. Global expression analysis of the *det2* mutant [7] demonstrated that brassinosteroids down-regulate the helix-loop-helix transcription factor PIF3, which is known to function at the beginning of the light-signaling pathway. PIF3 is localized in the nucleus and interacts with active phytochromes [5,10,11]. In addition, the expression of a large number of early auxin-inducible genes was altered in *det2* mutants, showing that there is a marked overlap between the brassinosteroid and auxin-signaling pathways. In conclusion, the study of *det2* and wild-type responses to signals [7] provides a global view of the effect of brassinosteroids on plants, demonstrating a connection between the brassinosteroid and auxin-signaling pathways and suggesting that brassinosteroids could modulate light signaling through PIF3 to affect plant development.

The hormone auxin has profound effects on plant development; it governs cell division, expansion and differentiation. But the molecular mechanisms underlying these processes are still largely unknown. To gain a more comprehensive understanding of auxin responses, several studies have described the global effects on gene expression induced by auxin [12,13]. The *shy* gene is a member of the auxin-induced Aux/IAA family and has a central role in the auxin-signaling pathway [2]. Studies on wild-type and *shy2* mutant *Arabidopsis* seedlings treated with auxin for 6 hours [12] identified a set of auxin-regulated genes and provided a global picture of the changes in gene expression in the *shy2* mutant.

Other studies on the response to auxin [13] have focused on the early changes in gene expression induced by the hormone. After 15 minutes of auxin treatment, microarray analysis revealed only 30 genes that were differentially expressed compared with untreated plants; among these were a large number of transcription factors of several types, suggesting that auxin signals are mediated by a diverse set of transcriptional regulators [13].

Studies of transcription factors

Microarrays have also been used to identify genes specific to reproductive organs and to discover new genes involved in floral development [14,15]. Endo and colleagues [15] identified groups of genes with similar behavior during anther and pistil development in *Lotus japonicus*. Although most of the genes detected were known to be involved in floral development, some potentially novel floral-development genes were also identified. In addition, it was possible to identify genes involved in the early and late events of anther and pistil development from their patterns of expression. Genetic and molecular studies of floral homeotic mutants, in which one type of floral organ is transformed into another, have led to the identification of several genes that specify floral organ identity. These genes act in a combinatorial manner in overlapping domains to regulate the specification of organ identity in the flower. The analysis of changes in gene expression induced by two *Arabidopsis* genes encoding homeotic transcription factors involved in floral development, *APETALA3* (*AP3*) and *PISTILLATA* (*PI*), confirmed the complexity of the processes of stamen and petal formation [15]. Only a small number of transcription factors were recovered in this experiment, suggesting that *AP3* and *PI* act quite directly in regulating the basic cellular functions required for morphogenesis.

Our group has applied microarray technology to understanding the changes in gene expression induced by transcription factors that are expressed in apical meristems, the multipotent tissues that give rise to shoots, leaves and other plant organs. Members of the KNOX family of transcription factors have important roles in plant development; they affect meristem maintenance and cell differentiation [16]. Our oligonucleotide microarray study comparing wild-type *Arabidopsis* plants with those lacking the *BREVIPEDECELLUS* (*BP*) gene - one of the seven *Arabidopsis* KNOX genes - revealed changes in gene expression of a relatively small number of genes, a large proportion of which are involved in the lignin biosynthetic pathway (G.M., N. Ori, Y. Sato and S.H., unpublished work). The possible link between *BP* and lignin synthesis was confirmed by the discovery of premature lignin deposition in *bp* mutants and decreased lignin in plants overexpressing transgenic *BP*. This study demonstrates how microarray analysis of gene-expression profiles permits the identification of a transcription factor's influence on metabolic pathways.

In conclusion, microarray technology provides a powerful tool for functional genomics and is being widely used to shed light on global changes in gene expression. By clustering genes according to their expression profile, it is possible to assign functions to genes with unknown function and at the same time to assign new functions to known genes. As increasing numbers of microarray experiments are completed, the collections of data retrieved from different analyses will contribute to the resolution of the complex relationships between the numerous signaling pathways that operate in plant growth and development.

References

1. **Affymetrix** [http://www.affymetrix.com]
2. Ma L, Li J, Qu L, Hager J, Chen Z, Zhao H, Deng XW: **Light control of *Arabidopsis* development entails coordinated regulation of genome expression and cellular pathways.** *Plant Cell* 2001, **13**:2589-2607.
3. Quail PH: **Photosensory perception and signalling in plant cells: new paradigms?** *Curr Opin Cell Biol* 2002, **14**:180-188.

4. Ni M, Tepperman JM, Quail PH: **PIF3, a phytochrome-interacting factor necessary for normal photoinduced signal transduction, is a novel basic helix-loop-helix protein.** *Cell* 1998, **95:**657–667.
5. Ni M, Tepperman JM, Quail PH: **Binding of phytochrome B to its nuclear signalling partner PIF3 is reversibly induced by light.** *Nature* 1999, **400:**781-784.
6. Tepperman JM, Zhu T, Chang HS, Wang X, Quail PH: **Multiple transcription-factor genes are early targets of phytochrome A signaling.** *Proc Natl Acad Sci USA* 2001, **98:**9437-9442.
7. Goda H, Shimada Y, Asami T, Fujioka S, Yoshida S: **Microarray analysis of brassinosteroid-regulated genes in *Arabidopsis*.** *Plant Physiol* 2002, **130:**1319-1334.
8. Fankhauser C, Chory J: **Light control of plant development.** *Annu Rev Cell Dev Biol* 1997, **13:**203-229.
9. Kraepiel Y, Miginiac E: **Photomorphogenesis and phytohormones.** *Plant Cell Environ* 1997, **20:**807-812.
10. Fairchild CD, Schumaker MA, Quail PH: **HFR1 encodes an atypical bHLH protein that acts in phytochrome A signal transduction.** *Genes Dev* 2000, **14:**2377-2391.
11. Zhu Y, Tepperman JM, Fairchild CD, Quail PH: **Phytochrome B binds with greater apparent affinity than phytochrome A to the basic helix-loop-helix factor PIF3 in a reaction requiring the PAS domain of PIF3.** *Proc Natl Acad Sci USA* 2000, **97:**13419-13424.
12. Tian Q, Uhlir NJ, Reed JW: ***Arabidopsis* SHY2/IAA3 inhibits auxin-regulated gene expression.** *Plant Cell* 2002, **14:**301-319.
13. Sawa S, Ohgishi M, Goda H, Higuchi K, Shimada Y, Yoshida S, Koshiba T: **The HAT2 gene, a member of the HD-Zip gene family, isolated as an auxin inducible gene by DNA microarray screening, affects auxin response in *Arabidopsis*.** *Plant J* 2002, **32:**1011-1022.
14. Endo M, Matsubara H, Kokubun T, Masuko H, Takahata Y, Tsuchiya T, Fukuda H, Demura T, Watanabe M: **The advantages of cDNA microarray as an effective tool for identification of reproductive organ-specific genes in a model legume, *Lotus japonicus*.** *FEBS Lett* 2002, **514:**229-237.
15. Zik M, Irish VF: **Global identification of target genes regulated by *apetala3* and *pistillata* floral homeotic gene action.** *Plant Cell* 2003, **15:**207-222.
16. Reiser L, Sanchez-Baracaldo P, Hake S: **Knots in the family tree: evolutionary relationships and functions of *knox* homeobox genes.** *Plant Mol Biol* 2000, **42:**151-166.