



ELSEVIER

Cell signalling and gene regulation Exploring new functions and actions of regulatory molecules

Editorial overview

Jen Sheen and Steven Kay

Current Opinion in Plant Biology 2004, 7:487–490

Available online 10th August 2004

1369-5266/\$ – see front matter
© 2004 Published by Elsevier Ltd.

DOI 10.1016/j.pbi.2004.07.017

Jen Sheen

Department of Molecular Biology, Wellman 11,
Massachusetts General Hospital, Boston,
Massachusetts 02114, USA
e-mail: sheen@molbio.mgh.harvard.edu

Jen's research group is interested in the elucidation of molecular mechanisms underlying plant responses to growth and stress hormones, nutrients, environmental stresses, and pathogens. More information on Jen's work is available at <http://genetics.mgh.harvard.edu/sheenweb/index.html>

Steven Kay

The Scripps Research Institute, Cell Biology -
ICND216, 10550 N. Torrey Pines Road, La Jolla,
California 92037-1000, USA
e-mail: stevek@scripps.edu

Steve's work focuses on the study of the circadian biology of a wide range of organisms, from humans to fruit flies to plants. More information on Steve's work is available at the Scripps Institute website (<http://www.scripps.edu>).

Abbreviations

(A)RR	(<i>Arabidopsis</i>) response regulator gene
CO	CONSTANS
dsRNA	double-stranded RNA
FLC	FLOWERING LOCUS C
miRNA	microRNA
NPR1	NON-EXPRESSOR OF <i>PR</i> GENES1
PR	pathogenesis-related gene
PRR	pseudo response regulator gene
SA	salicylic acid

Plants are creative inventors of large families of regulatory molecules that serve dynamic and diverse roles in complex gene expression programs and signalling networks. The use of integrative genomic, genetic, cellular, biochemical and computational tools has been key to recent advances in our understanding of gene regulation, hormone signalling, immunity, cytokinesis, growth and morphogenesis in multiple plant and cell models.

The completion of plant genome sequences has enabled unprecedented systematic analysis and functional comparison of large gene families encoding transcription factors that are enriched in plants. Ülker and Somssich (pp. 491–498) provide a thorough update on our current knowledge of WRKY transcription factors, which display a broad spectrum of functions in plants. Apart from the identification of *RRS1/AtWRKY52* and *TTG2/AtWRKY44* by traditional genetic screens, the use of a systematic and functional genomic approach has proven to be productive because it circumvents previous limitations. For example, a study of WRKY target genes using microarrays and *in vivo* DNA-binding sites that was based on chromatin immunoprecipitation provided improved resolution in defining WRKY specificity and redundancy at the whole-genome level. The use of virus-induced gene silencing (VIGS) analysis in tobacco and reverse genetics in *Arabidopsis* knockout lines is facilitating the high-throughput analysis of WRKY functions. The success of applying new technologies to determine the functions of a large number of WRKY family members offers new promise of enhancing our knowledge of plant gene regulation and signal transduction.

The identification and extensive analyses of response regulator (*RR*) and related (pseudo response regulator [*PRR*]) genes using a functional genomic approach represent further major advances in our understanding of the unique signalling mechanisms that underlie cytokinin responses and circadian rhythm in plants. Mizuno (pp. 499–505) presents an informative summary of recent progress in work on *Arabidopsis* *RR* (*ARR*) and *PRR* genes. Both gain-of-function and loss-of-function analyses are essential to

determine the functions of *ARR* A-type and *ARR* B-type genes, which display highly redundant functions in cytokinin signalling. Interestingly, recent systematic analyses of the promoter activities of many A-type and B-type *ARR* genes in transgenic *Arabidopsis* indicate characteristic expression profiles for different members. Mizuno raises some excellent questions relating to the physiological roles of RRs, especially in uncharacterized histidine kinase signalling pathways. The value of *Arabidopsis* as a model plant in the functional genomic analysis of evolutionarily conserved signal transduction pathways has been validated by the studies of *RR* and *PRR* genes in maize and rice.

The review by Katagiri (pp. 506–511) examines data from global mRNA expression profiling in *Arabidopsis*. The availability of high-quality, large microarrays for this species has begun to shed light on the massive changes in gene expression that occur during plant responses to pathogen infection. Katagiri reports that plants activate a whole suite of non-specific responses when they detect a pathogen and describes how the major differences between resistant and susceptible responses are quantitative and/or kinetic. In the future, the careful combination of broad-spectrum, quantitative, and sensitive phenotyping methods with other technologies will produce high-resolution quantitative information that will help advance our understanding of the complex signaling networks that regulate plant defense responses.

Dugas and Bartel (pp. 512–520) summarize the current state of knowledge concerning the biogenesis and function of microRNAs (miRNA) in plants. This relatively unstudied means of controlling gene expression has, in recent years, become better appreciated for its role in the regulation of several developmental processes in plants. Unlike the small interfering RNAs (siRNA) responsible for post-transcriptional gene silencing, which are derived from their target mRNAs, miRNAs are encoded by their own transcriptional units. These short RNAs are cleaved from imperfectly paired hairpin precursors by Dicer-like ribonucleases to produce double-stranded RNAs (dsRNAs) of around 21 nucleotides in length, a single strand of which is the mature miRNA that is selected for silencing by the RNA-induced silencing complex (RISC). In contrast to the system in animals, in which binding of an miRNA/RISC complex to the 3'UTR of its target message attenuates translation, miRNA species in plants usually direct degradation of the mRNAs with which they share complementarity. miRNAs are characterized by being highly conserved, a feature that has been exploited to identify miRNAs through comparison of the *Arabidopsis* and rice genomes. To date, at least 92 validated miRNAs have been identified in *Arabidopsis*, encompassing a total of 22 miRNA families. Several recent studies have demonstrated that specific miRNAs contribute to important steps in a variety of developmental process,

including the establishment of leaf polarity, flower development, and the promotion of flowering. Although the mechanism is not yet fully understood, early work has made it clear that miRNAs constitute an important means of negative regulatory control over mRNA levels.

This review by Bender (pp. 521–526) presents an overview of the current knowledge regarding the mechanisms involved in transcriptional regulation by DNA methylation in plants. Different regulatory modes are important for regulating transcriptional activity in eukaryotes and DNA methylation is an important mechanism for gene silencing. In plants, this DNA modification appears to be directed by dsRNA species that target methylation to specific sequences. This process is often associated with the production of high levels of dsRNA that occur, for example, in RNA virus infections. There is also a correlation between RNA-mediated DNA methylation and RNA interference (RNAi). Thus, DNA methylation constitutes a primary line of defense against harmful DNAs such as transposons and retroviruses. Different DNA methyltransferases have been identified in *Arabidopsis*. In addition to the mammalian-related activity that maintains methylation in a CG context, Bender describes two plant-specific DNA methyltransferases that are implicated in the methylation of asymmetric cytosines (CNG). Although much progress has been made in this area, we do not yet fully understand how DNA methylation is targeted to exogenous DNA but not to the normal host genes. In addition, there are genes (such as rRNA) that have methylated cytosines and can produce high levels of transcripts. Recent studies on rRNA transcription have provided an explanation for this apparent paradox.

Despite extensive forward-genetic screens, no mutants of small GTPases have been found in plants. The review by Yang and colleagues (pp. 527–536) illustrates the power of reverse genetics for generating new breakthroughs in our understanding of the versatile functions of small GTPases (ROPs/RACs) in plant signalling pathways. It is now clear that ROPs/RACs act as master switches in the transmission of diverse extracellular and intracellular signals. Both conserved and novel modes for the regulation and action of ROPs/RACs are emerging in plants. Newly defined ROP/RAC functions include ABA, auxin, defense, hypoxia, and oxidative signalling, as well as roles in pollen-tube growth and the formation of interlocking jigsaw-puzzle-shaped pavement cells in the leaf epidermis. The association of ROPs/RACs with members of the large family of plant receptor-like serine/threonine kinases (RLKs) suggests the exciting possibility of novel mechanisms that underlie ROP/RAC regulation and signalling.

Guard cells have long been a fascinating model system in which to study channel regulation and dynamic signalling

networks. Assmann and colleagues (pp. 537–546) offer a lucid and updated account of new findings on the functions of channels and transporters, as well as on signalling and regulatory molecules that mediate the control of stomatal closure and opening by ABA, blue light and the extra-cellular matrix. An impressive set of new upstream regulators of calcium, H_2O_2 , and nitric oxide have been discovered and integrated into the multifaceted signalling pathways in guard cells. Recent studies have also revealed a unique aspect of emerging signalling mechanisms that use a heterotrimeric G protein (GPA1) to connect receptors, lipases and channels. The sophisticated combination of genetic, genomic, biochemical and biophysical tools in guard cells will continue to push the frontier of our understanding of complex signalling networks.

Salicylic acid (SA) is a central signalling molecule responsible for the coordinated expression of pathogenesis-related (*PR*) genes and the onset of systemic acquired resistance. Dong (pp. 547–552) contributes a timely summary of exciting new developments in the areas of SA perception and signalling, and of cross-talk with pathways mediated by jasmonic acid, another signalling molecule involved in plant immunity. SA-mediated responses appear to involve multiple steps including early oxidative signalling, which helps to establish the reducing conditions that are necessary for a key regulator, the NON-EXPRESSOR OF *PR* GENES1 (NPR1) monomer, to enter the nucleus. Multiple and redundant TGA transcription factors cooperate with the nuclear NPR1 to activate the expression of late *PR* genes. Mutations in the Cyc residues of NPR1 and some TGAs confirm that protein translocation and transcription activation are modulated by cellular redox states. New evidence also supports the concept that a single NPR1 protein has multiple functions in different subcellular locales, which presumably rely on interactions with distinct or overlapping partners. New transcription factors that are involved in NPR1-independent SA regulation of gene expression have also emerged.

Auxin is the most versatile plant hormone and controls a broad spectrum of essential processes in plants. The review by Berleth and colleagues (pp. 553–563) highlights exciting recent advances in understanding the mechanisms of auxin-mediated gene regulation and pattern formation. Microarray studies clearly show that auxin does not just turn on genes, it can also turn genes off. Molecules that are involved in protein degradation remain at the center stage of auxin signalling. A modified model for Aux/IAA and auxin-response factor (ARF) actions is perhaps needed to explain how different members of the same family could have opposite functions in auxin signalling. Classical, chemical and reverse genetics have unraveled new players and novel aspects of auxin signalling, including the involvement of phosphatase, prolyl

isomerase, G β protein, and ROPs/RACs. The integration of cellular auxin signalling with plant growth and development uncovers the crucial roles of auxin in controlling organogenesis, polarity, phyllotaxis and the development of root hairs, roots and vasculature. Auxin perception and early signalling events are likely to be the focus of future research.

Schepens, Duek and Fankhauser (pp. 564–569) discuss recent work that has brought new understanding to light perception in plants, as well as some challenges to well-established paradigms. Plants rely on a bank of photoreceptors, including phototropins, cryptochromes, and phytochromes, to measure the quantity and quality of the light that falls upon them. In *Arabidopsis*, five distinct phytochromes (phyA–E) contribute to the control of many developmental processes, including seed germination, de-etiolation, shade avoidance and floral induction. Recently, significant advances have been made in understanding the external coincidence mechanism responsible for the photoperiodic control of flowering. In particular, FKF1 (Flavin-binding, Kelch repeat, F-box1) has been identified as a novel blue-light photoreceptor that is important for the establishment of the proper circadian expression of the transcription factor CONSTANS (CO). In addition, phyA and phyB, along with the cryptochromes, regulate the abundance of CO by altering the stability of CO protein. Both of these mechanisms influence the CO-dependent induction of downstream genes that are necessary to promote flowering. Interestingly, a fusion of the amino-terminus of phyB with β -glucuronidase and a nuclear-localization signal (NLS) sequence, displayed signaling activity that was comparable to that of full-length phyB. This implies that the accepted view that phytochromes are composed of an amino-terminal sensing domain and a carboxy-terminal output domain is in need of modification. Upon light exposure, attenuation of phyA signaling is accomplished by ubiquitinylation and subsequent degradation of phyA by the proteasome. It is now apparent that the RING-finger protein CONSTITUTIVE PHOTOMORPHOGENIC1 (COP1), which also targets downstream components of light signaling for degradation, is partly responsible for the turnover of phyA following light exposure. Even with these advances, the complexity of the phytochrome signaling networks and the large number of processes that light impinges upon guarantees that much more can be gained by investigating plant responses to light.

Simpson (pp. 570–574) reviews the mechanisms of the autonomous pathway in regulating flowering time in *Arabidopsis*. The transition to flowering represents a major commitment during the life cycle of higher plants and is targeted by a number of regulatory pathways, including photoperiod, phytohormones, and an autonomous pathway. The autonomous pathway in *Arabidopsis* functions to

promote flowering, even in the absence of inductive cues such as long days. The key factor operating within the autonomous pathway appears to be the *FLOWERING LOCUS C (FLC)* gene. All of the mutants that affect the autonomous pathway isolated to date alter *FLC* expression and fall into two general categories. They either affect the epigenetic regulation of *FLC* expression (*fld* and *fev*) or the regulation of *FLC* at the RNA level (*fca*, *fpa*, *flk*, and *fy*). The autonomous pathway provides a rheostat to control *FLC* levels in order to control time to flowering. The genes that operate in this pathway do not appear to delineate a series of steps but rather use more global mechanisms to control gene expression, and may therefore provide insights into mechanisms used by other systems.

Leaves are essential to support the life of animals and plants, and have diverse shapes and sizes. Major advances in delineating the regulatory circuits that control leaf morphogenesis are summarized by Tsiantis and colleagues (pp. 575–581). The logical flow from the regulation of leaf initiation to the control of leaf pattern, size and shape serves as an excellent guide to the sequential processes in leaf morphogenesis. The review by Tsiantis and colleagues focuses on the roles of novel transcription factors and the connections between transcription factors the plant hormones auxin, gibberellic acid (GA) and cytokinin. Recent evidence has also suggested that miRNA gradients may convey morphogenetic information. Many *Arabidopsis* transcription factors are likely to be putative targets of miRNA regulation, and so there will certainly be further studies on the novel regulatory mechanisms in gene expression and signalling. Research in model systems will continue to help the elucidation of fundamental mechanisms that lead to the diverse leaf morphology within the plant kingdom.

Arabidopsis has served as an excellent model system in which to study genes that control cell fate in all major root tissues. The review by Birnbaum and Benfey (pp. 582–588) provides a progressively broader overview of the latest findings relating to transcriptional regulation in the root, covering specific cell-fate determination, meristem organization, hormonal signalling, and a global gene-expression map. Studies of root-hair and root development have revealed that cell–cell movement of transcription factors is crucial for cell-fate determination. The nuclear DELLA protein appears to be the convergent point of auxin, GA and ethylene signalling. The application of genomic and computational tools to samples selected by green fluorescent protein (GFP)-based cell

sorting has generated an impressive gene-expression map for roots. The use of laser-capture microdissection and microarrays will provide information on gene expression during the processes of organ differentiation of unprecedented temporal and spatial resolution. The future challenges are to integrate the transcription patterns/profiles with plant hormone signalling and other regulatory pathways.

Pollen tubes and root hairs have been excellent single-cell models for the discovery of novel signalling molecules and for the elucidation of fundamental aspects of signalling mechanisms that are important for tip growth. In their interesting review, Feijó and colleagues (pp. 589–598) introduce novel signalling molecules that guide or repel pollen-tip growth. An amazing array of second messengers and regulators of the actin cytoskeleton have been identified and integrated into the cellular processes that lead to polarized growth. Recent global analysis of gene expression has revealed that pollen tubes are highly enriched for genes that have a role in signal transduction, reflecting the innate requirement for fast and dynamic communication with and to decode signals from the environment. The available genomic information offers an unprecedented opportunity to test new hypotheses using reverse-genetic tools.

Cytokinesis is the final step of cell division. Cytokinesis in plants involves unique temporal and spatial steps that are different from those in yeast and animals. Mayer and Jürgens (pp. 599–604) emphasize new studies that have revealed common mechanistic features that underlie both conventional and unconventional cytokinesis in different plant cell types. Genetic and molecular studies have identified new components and determined the functions of components that are known to be involved in the common mechanism for diverse modes of cytokinesis. A MAPK cascade that is activated by the conserved cytokinesis-specific kinesin plays a crucial role in cytokinesis in tobacco and *Arabidopsis*. Other important players include microtubule-associated proteins, dynamin GTPase, and molecules that are involved in membrane fusion and trafficking. It will be interesting and important to connect these molecules into the mechanistic processes of cytokinesis.

Exciting advances have been achieved in our understanding of plant gene regulation and signal transduction in recent years. We anticipate that the exploration of new functions and actions of plant regulatory molecules will continue and accelerate with the development of new systematic tools and strategies in the near future.