Weaving the Complex Web of Signal Transduction

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"Signal transduction" is the term commonly used to define the diverse array of biochemical mechanisms that regulate cellular physiology. The term "signal transduction" became popular in the early 1980s and now it is considered one of the most intensively studied areas of modern cell biology. This field's fast-paced progress is well illustrated by the results of two PubMed searches (http://www.ncbi.nlm.nih.gov/PubMed/). In 1975, there were no papers retrieved when "signal transduction" was used to query the database. However, during the past year, almost 10,000 papers were published in the "signal transduction" field. The amount of data being generated in the cell signaling field is so immense that the journal *Science* has created a World Wide Web-based signal transduction knowledge environment (http://www.stke.org/)!

Although animal and fungal model systems have had the largest impact on understanding the biochemical mechanisms of signal transduction, the analysis of signaling pathways in plants has come far since the pioneering studies which identified phytochrome as the first receptor in plants (4). Phytochrome was the only receptor known in plants until the early 1990s when a number of candidate receptors were identified. Among the first to be cloned were the S-receptor-like kinases (18), the cryptochromes (1), and the His kinase candidate for the ethylene receptor (6). Shortly after, a number of candidate receptors were identified, including a variety of disease resistance receptors (8) and receptor Ser-Thr kinases controlling plant development (12). With the completion of the sequence of the reference plant Arabidopsis, it is now clear that plants devote about 10% of their approximately 25,000 genes to receptors and other signaling components, such as protein kinases and phosphatases and transcription factors. Still, very little is known about growth factors or other ligands for these receptors, and there is scant information on the biological role of second messengers or scaffolding proteins in plants. In this brief perspective, we will review physiological and genetic evidence for the existence of complex signaling networks in plants, focusing on light and hormone signal transduction cross talk. We will then discuss the complexities in plant cell signaling revealed by the completion of the genome sequence of Arabidopsis.

COMPLEX INTERACTIONS BETWEEN PLANT PHOTORECEPTOR SIGNALING PATHWAYS

Because they are both sessile and photosynthetic, plants have evolved multiple photoreceptor systems for perceiving the quality, quantity, duration, and direction of light in their surrounding environment. Due to the work of many laboratories, using a variety of approaches, the molecular identities of at least three classes of photoreceptors are known: the red/far-red-absorbing phytochromes, the blue-UV-A-absorbing cryptochromes, and the blue-absorbing phototropins. The mechanisms by which these photoreceptors signal is addressed in the excellent review by Briggs and Olney (5). Here, we simply want to discuss the genetic and physiological evidence for complex interactions between the phytochromes and cryptochromes that suggest that light control of development—one of the most intensively studied signaling systems in plants—is the result of information processing by a complex signaling network.

Elegant genetic studies in Arabidopsis have shown that phytochrome signaling involves a complex web of interactions (13). The phytochromes sometimes act independently of one another, but in certain growth conditions and times of development, they may also act redundantly or antagonistically. The genetic complexity of phytochrome signaling has been underscored by biochemical studies in which diverse proteins have been found that interact directly with various domains of phytochromes. Three of these proteins—PIF3, a nuclear-localized basic helix-loop-helix transcription factor; PKS1, a novel cytoplasmic protein that is phosphorylated by phytochrome; and nucleoside diphosphate kinase 2, whose activity is regulated by phytochrome—are the best characterized (16). These three proteins are not structurally or functionally related and appear to interact with different domains of phytochromes. Thus, these proteins do not share a common mechanism of communication with phytochrome.

Genetic analysis has also shown that there is a complex signaling network, not only between phytochromes, but also between phytochromes and the cryptochromes. These studies demonstrate a complex web of interactions within and between the two

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classes of photoreceptors, including redundancy, antagonism, and effector/modulator relationships. Although the mechanisms of integration of red and blue light signals are not clear, one recent study points to a direct interaction of phytochrome and cryptochromes, in which both cry1 and cry2 were shown to be phosphorylated by phytochrome in vitro, and in vivo, cry1 phosphorylation was red-light dependent (2). A novel photoreceptor with homology to both phytochrome and phototropin has recently been isolated from the fern *Adiantum capillus-veneris* (14). In this case, the co-action between blue and red light in phototropism may be through a single photoreceptor.

Genetic and molecular screens have identified a large number of genes acting downstream of photoreceptors (>50; Fig. 1; Refs. 10, 11, 13). Because different spectral qualities trigger the same developmental responses using different photoreceptors, it is very likely that common late-acting signaling intermediates are used. Mutants in such genes are expected to have the same phenotypes irrespective of light quality. Such loci have been identified. The best-studied class consists of mutants that de-etiolate even in the absence of light (*cop*, *det*, *fus* mutant class). These are pleiotropic, recessive mutations affecting many aspects of plant development, and the proteins encoded are generally considered to be late-acting negative regulators of the light signaling pathways. Early signaling intermediates are expected to have a phenotype only under the specific light conditions activating their photoreceptor. Such mutants have been further classified into genes that affect phyA signaling (defective in far-red light), phyB signaling (defective in red light), or both phyA and phyB signaling (defective in both red and far-red light). These mutations define greater than 20 loci, and the proteins have been found in multiple subcellular compartments (Fig. 1). Thus, in the light signaling networks, there are direct interactions of photoreceptors, as well as cross talk and integration of pathways both early and late in the signaling network.

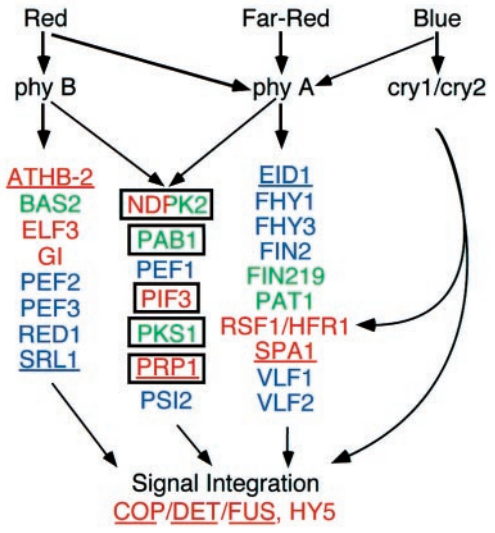

Figure 1. Simplified version of the photoreceptor signaling network controlling seedling development. Cloned genes for nuclear-localized proteins are in red, whereas those that are cytoplasmic are green. Genetically defined genes are indicated in blue. Negative regulators are underlined. Proteins that interact directly with phytochrome are boxed.

CROSS TALK IN PLANT HORMONE SIGNALING

The plant hormone signaling pathways are among a set of core pathways that are used repeatedly in many different developmental contexts. Thus, it is not surprising that plant hormones affect many of the same molecular and physiological processes, such as the control of cell expansion and divisions that define the architecture of vascular plants. Since the classic tissue culture experiments of Skoog and his collaborators (15), it has been known that the ratio of auxin to cytokinin regulates morphogenesis in cultured cells and tissues, and that auxin/cytokinin polarity within a plant defines the architecture of that plant (e.g. the number of lateral branches in the shoot and lateral roots below the ground). The complex interactions in plant hormone signaling are seen in many physiological responses. For instance, gibberellins, auxin, and brassinosteroids have a stimulatory effect on hypocotyl elongation, whereas ethylene, abscisic acid, and cytokinins have inhibitory effects on this process. Cytokinins and auxin both participate in regulating the plant cell cycle. Auxin is known to regulate ethylene biosynthesis. In contrast, cytokinins act antagonistically with brassinosteroids or ethylene to control leaf or fruit senescence, and there are descriptions of abscisic acid acting antagonistically with ethylene and brassinosteroids. Thus, there are numerous physiological examples of synergy, antagonism, and causal relationships among the plant hormone signaling pathways (9).

These interactions between the hormone signaling pathways have been reinforced by the observation that several hormone-resistant mutants are not specific for the hormone pathways for which they were screened. Rather, these mutants exhibit cross-resistance to high levels of several hormones. This was first noted for auxin-resistant mutants (19), but since that report several loci have been identified from different screens. The best example is the identification of *ein2*, not only in ethylene-insensitive screens, but also in screens for abscisic acid and cytokinin signaling mutants (3). Brassinosteroid-insensitive mutants have been reported to be hypersensitive to abscisic acid in root growth assays (7).

With the identification of a plethora of hormone mutants, mostly in Arabidopsis, genetic studies have reinforced the notion that there are complex interactions and cross talk in plant hormone signaling pathways. These studies also give us a glimpse of the

challenges for the future. For example, is a "negative regulator" as defined by loss-of-function mutations truly a negative regulator or is the effect due to the loss-of-function of a positive regulator in an antagonistic pathway? These are the questions that we are now poised to answer.

THE COMPLEXITY OF SIGNALING REVEALED BY STRUCTURAL GENOMICS

Given the rapid progress made in cell signaling over the past 25 years, it is appropriate that we end with a snapshot of all the signaling molecules in a plant. With the recent announcement of the completion of the sequence of Arabidopsis, we now have the first glimpse of the complexity and redundancy of signaling components in a higher plant (17). As outlined in Table I, it is now clear that Arabidopsis devotes a significant percentage of its genome to cell signaling (about 10%). For instance, Arabidopsis has more than 850 predicted protein kinases, whereas *Caenorhabditis elegans* has about 400 and Drosophila even less (approximately 250). There are greater than 1,000 predicted transcription factors, often found in large families. Why so many kinases and transcription factors and why so much redundancy within gene families? There are many possible explanations for expansion of gene families within plants that will be addressed over the next years. One to consider in terms of the evolution of signal transduction networks in plants is the large number of environmental signals that need to be integrated with intrinsic developmental programs. These are rapidly changing biotic and abiotic signals that are perceived in different parts of the plant and which must be integrated to give a fine-tuned and appropriate growth response. Thus, it seems fitting that there has been significant expansion of both receptors and transcription factor families that define the input and output layers of cell signaling. Whether these proteins have overlapping or distinct functions is only beginning to be revealed. An even bigger unknown is how the activation of these receptors is integrated to give the final gene expression response, a question which will not be easily answered using forward genetic screens.

Table I. *Representative examples of cell signaling components predicted in the Arabidopsis genome*

Putative Protein	Pfam Domain	Approximate No.
Protein kinases (all types)	PF00069	>860
NBS-LRR receptors	PF00560; PF00931	>100
Leu-rich repeat receptor-like proteins	PF00931	>60
Photoreceptors	Many domains[a]	>10
Protein phosphatases	PF00149; PF00481	~90
ras family GTPases	PF00071	>60
Scaffold proteins (nph3-type)	BLASTp search[b]	27
TPR domain	PF00515	75
Ankyrin repeat domain	PF00023	95
PDZ domain	PF00595	14
PH domain	PF00169	21
GRAS (GAI/RGA/SCR)	BLASTp search[c]	27
Transcription factors (all types)		>1700
Families		
myb-type	PF00249	177
AP2-domain	PF00847	117
Zn-finger C_2H_2 class	PF00096	105
basic-helix-loop-helix	PF00010	83
MADS domain	PF00319	68
Zn-finger CCHC class	PF00098	64
basic Leu zipper	PF00170	59
homeobox domain	PF00046	49
B3 domain	PF02362	35

[a] PF00360, PF00512, PF00989, PF01590, PF00875. [b] BLASTp search against protein NPH3 (Genbank accession no. AAF05914). [c] BLASTp search against proteins SCARECROW (Genbank accession no. AAB06318), GAI (EMBL accession no. CAA75492), and RGA2 (EMBL accession no. CAA72178).

CHALLENGES FOR THE NEXT 25 YEARS

Twenty-five years ago, the concept of signal transduction networks did not exist. Now, we know that many signaling domains are conserved throughout the plant and animal kingdoms and that significant percentages of eukaryotic genomes encode information for receptors, signaling enzymes, and transcription factor targets of these signaling pathways. Signaling molecules are often found in large complexes or anchored to discrete membrane regions that may confer specificity to a signaling pathway. However, understanding signal pathway cross talk will become increasingly important for our understanding of complex signaling networks. Catalogs of protein kinases and phosphatases from sequencing projects and bioinformatics efforts used together with global expression analysis methodologies will allow the deciphering of which signaling networks are present in different cell types. The major challenges will be to develop proteomic methodologies for dissecting global posttranslational modifications in response to discrete stimuli and during particular times in development and to model the protein-protein interactions in vivo. Given that cell signaling networks are four-dimensional and that thousands of molecules might be involved in signaling in a particular cell, the challenges are immense.

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