The successful existence of all higher organisms is dependent upon their ability to coordinate complex developmental changes and to sense and respond to fluctuations in their surroundings. Responses to developmental and environmental cues occur by stimulus–response coupling: a stimulus is perceived by the cell, a signal is generated and transmitted (signal transduction), and a biochemical change is instigated (the response). This process most often requires the recognition of the stimulus by a receptor and the subsequent use of chemical second messengers and/or effector proteins to transmit a signal that will then trigger the appropriate response. Key properties of signal transduction are speed, sensitivity (achieved by amplification), and specificity, all of which are controlled by a network of positively and negatively acting elements. Whereas the positively acting intermediates are responsible for ensuring a response that is quantitatively appropriate, correctly timed, and highly coordinated with other activities of the cell. Specifically, negative control can (1) allow changes in sensitivity to a particular stimulus, permitting a signaling pathway to work over a broad dynamic range of stimulus intensities; (2) terminate a response when it is completed, even if the stimulus may remain (sometimes referred to as desensitization or adaptation); and (3) allow a signaling pathway to be reused.

In animal cells, the signal transduction events that couple a variety of stimuli with their responses have been well characterized, as have the mechanisms for their negative control. Plants, however, are challenged in different ways from animals because their developmental programs are generally more flexible and because they are unable to escape at will from unfavorable environmental situations. These differences indicate the increased adaptability of plants over animals and lead us to speculate about the nature and control of the sensing systems plants employ to regulate their development and behavior. Have these different constraints necessitated the evolution of mechanisms altogether different from those found in animals? To some extent the answer appears to be yes; both genetic and biochemical approaches have now yielded sufficient information for us to realize that, whereas the basic mechanisms may often be conserved (e.g., the use of the same second messengers and similar protein phosphorylation/dephosphorylation reactions), there are often very real differences in the components of the signaling machinery and in their regulation. In some cases, plant signaling appears more analogous to bacterial signaling systems (Hughes, 1994), whereas in others it may be altogether novel.

Studies of signal transduction pathways are facilitated by some knowledge about the specificity of the stimulus, the biochemical nature of the receptor, and the specificity of the responses. Specificity of the response for the stimulus is particularly important for genetic studies because in many cases it is desirable to generate mutants that are constitutive for a response in the absence of the stimulus, for example, the cop and der mutants, which are constitutive for light responses in darkness, and the ctr mutants, which display constitutive ethylene responses in the absence of exogenous ethylene (see below). In these cases, to be sure that the mutant is blocked in the signal transduction pathway of interest, it is important to be able to show that all of the phenotypes of the mutant are consistent with all or at least a subset of the normally observed phenotype of wild-type plants exposed to the stimulus. This is particularly difficult to demonstrate in plants because many responses can be mediated by different stimuli. For example, the effects of light on plant morphology (photomorphogenesis) and gene expression can be recapitulated to a large extent by cytokinin, and the response to pests and pathogens may involve the actions of (at least) ethylene, jasmonic acid, salicylic acid, systemin, and electrical signals.

Furthermore, genetic screens for constitutive mutants are largely dependent on the assumption that there are negative regulators that normally repress signal transduction in the absence of the stimulus and that the stimulus acts (at least at this step) by overcoming the repression. For some kinds of signal transduction pathways, for example, in which the negative regulator is one step in a linear pathway, loss of function mutations in such a negative regulator would produce a constitutive phenotype in the absence of any stimulus. More frequently, however, individual steps in signal transduction (at
least those upstream of transcription factors) are controlled by counteracting positive and negative regulators. In such cases, loss of function mutations in a negative regulator would not result in a constitutive phenotype unless the signal transduction pathway had a basal level flux passing through it in the absence of the stimulus. This flux could be a result of a basal level activity of the signaling intermediates. For example, although a kinase may be highly stimulated in the presence of inducer, it may also have a low constitutive activity in its absence, as may the "complementary" phosphatase that counteracts the kinase activity. In the case of second messenger pathways, the flux is known as a "current" and relates to a nearly equal hydrolysis and synthesis of the second messenger (e.g., cGMP) in the absence of inducer. Stimulation of the pathway then disturbs this cycle to result in a net production of the active signal transducing molecule (see Koch, 1992).

Whereas genetic approaches can yield information about the role of particular molecules during plant growth, biochemical methods can allow the activities of signal transducing intermediates to be identified. However, as with genetic approaches, biochemical studies of plant signal transduction can be hampered by insufficient knowledge of stimulus-response specificity. Consequently, although several signal transducing molecules (both second messengers and proteins) have been identified biochemically, their roles in specific responses are often obscure.

Notwithstanding the limitations of genetic and biochemical approaches, intensive research is now yielding information about several signal transduction pathways in plants, and some consistent features have emerged. In this review, we attempt to highlight these themes by discussing recently published work on signaling mediated by phytochrome, ethylene, abscisic acid (ABA), and auxin. Particular emphasis is given to the negative control of these pathways, because it is one recurring theme that has emerged. We have illustrated a variety of ideas by presenting models of signal transduction that are consistent with the currently available experimental data. These schemes are intended to be thought provoking and may not necessarily be correct. This review is by no means exhaustive, and the reader is referred to the original articles as well as to other recent reviews on various other aspects of plant signaling involved in these and other responses (Trewavas and Gilroy, 1991; Estelle, 1992; Bush, 1993; Deng, 1994; Jones, 1994; Lamb, 1994).

PHYTOCHROME

Phytochrome is the only plant protein that has been unequivocally identified as a receptor (for review, see Quail, 1991; Furuya, 1993). Phytochrome apoproteins are usually encoded by small multigene families—for example, five phytochrome genes (PHYA–PHYE) have been identified in Arabidopsis (Sharrock and Quail, 1989; Clack et al., 1994)—and each phytochrome is thought to have a different physiological role (Whitelam and Harberd, 1994). Holophytochrome is a dimer containing a linear, covalently bound chromophore that mediates light perception. The photoreceptor is synthesized in the red-absorbing Pr form, and absorption of red light alters its conformation to the far-red-absorbing form, Pfr, which is generally considered to be the physiologically active form. Pfr responses can be reversed by far-red light, which converts Pfr back to Pr. Of all the photoreceptors so far characterized, this property, that is, to behave as a molecular light switch, is unique to phytochrome. Its molecular structure is also unique; although we know that it is a receptor, it does not look like any other receptor so far characterized. Most conspicuously, phytochrome is cytoplasmic, and as yet there is no convincing evidence that it ever uses membranes as a platform to mount its biochemical reactions (Quail, 1991).

How the phytochrome molecule transduces the light stimulus is still unknown. Structure–function studies have not answered many of the crucial questions about phytochrome activity, largely because the studies have been confined to overexpression of mutant phytochrome gene sequences in wild-type plants, which also contain the endogenous phytochromes. Although much of the N-terminal region, which contains the chromophore binding domain, is involved in light detection, it appears that the C-terminal region is more likely to be involved in mediating biological activity, that is, signal transduction (Cherry et al., 1993; Boylan et al., 1994). For example, removal of the C-terminal 35 residues from oat PHYA results in a protein that is physicochemically normal in vitro but has no activity in vivo (Cherry et al., 1993). Nonetheless, the first N-terminal 69 residues are probably also important in this regard (Cherry et al., 1992; Stockhaus et al., 1992).

In addition to genetic approaches (see below), phytochrome signal transduction has been studied by a variety of biochemical approaches: (1) using pharmacological agonists and antagonists to interfere with phytochrome-mediated events, (2) studying phosphorylation reactions or examining the effects of antibodies that bind to signal-transducing components of animal cells, and most recently, (3) using a microinjection-based approach to deliver putative signaling intermediates directly into the cells of a phytochrome-deficient tomato mutant. These approaches (reviewed in Millar et al., 1994) have identified heterotrimeric G-proteins as the most upstream component of the phytochrome signaling machinery, although this has not been definitively proven (because the G-protein(s) has not yet been purified and characterized). If true, this would indicate that phytochrome either localizes transiently to membranes or acts through an intermediary transducer to activate this membrane-bound complex. Alternatively, it is possible that the component with which phytochrome interacts does not have all the characteristics of animal heterotrimeric G-proteins, which at any rate are not integral membrane proteins, being localized to membranes via lipid tags (Casey, 1994).

Downstream of G-protein activation, phytochrome utilizes Ca2+ and cGMP (Neuhaus et al., 1993; Bowler et al., 1994a). A role for Ca2+ in phytochrome signaling was first postulated...
10 years ago, and circumstantial evidence has accumulated since then to support this hypothesis (reviewed in Tretyn et al., 1991; Millar et al., 1994). However, it is only recently that a red/far-red–reversible Ca\(^{2+}\) transient was directly observed in plant cells (Shacklock et al., 1992). Subsequently, microinjection of Ca\(^{2+}\) and Ca\(^{2+}\)-activated calmodulin (Ca\(^{2+}\)/CaM) into phytochrome-deficient tomato cells provided direct evidence that these molecules are involved in PHYA signal transduction, specifically in stimulating chloroplast development (Neuhaus et al., 1993). Using the same microinjection methodology, cGMP was found to mediate PHYA-dependent anthocyanin biosynthesis and also to participate, together with Ca\(^{2+}\) and Ca\(^{2+}\)/CaM, in coordinating chloroplast development (Bowler et al., 1994a). The use by the plant of Ca\(^{2+}\) and cGMP to mediate phototransduction has an interesting evolutionary parallel with the photoperception mechanisms found in retinal cells, even though the photoreceptors, phytochrome in the plant and rhodopsin in the retina, are structurally completely unrelated (Koutalos and Yau, 1993).

Figure 1A summarizes our current biochemical knowledge of PHYA signal transduction pathways, based on the results of microinjection experiments in tomato and studies of endogenous gene expression in soybean cells (Neuhaus et al., 1993; Bowler et al., 1994a, 1994b). Most interestingly, the targets of the three different positively acting pathways are divided very clearly along functional lines. The cGMP pathway appears, thus far, to be dedicated to the production of the photoprotective anthocyanin pigments, whereas the Ca\(^{2+}\) and Ca\(^{2+}\)/CaM-dependent pathways control chloroplast development by stimulating genes encoding subunits of the photosynthetic complexes.

Obviously, with these divisions along functional lines, it is very important that the final responses of the different pathways be carefully coordinated to allow, for example, for photoprotectant production before photosynthesis (known as juvenile anthocyanin; Drumm-Herrel, 1984) and to ensure proper stoichiometry of the different photosynthetic complexes. We have begun to understand how coordination is achieved by specifically manipulating signal flow through the different pathways, either by microinjecting large or small amounts of signal intermediates or by using pharmacological agonists and antagonists (Bowler et al., 1994b). Coordination appears to be controlled by negative regulatory mechanisms acting at the level of individual pathways (e.g., desensitization of the cGMP pathway to terminate light-induced expression of the genes encoding anthocyanin biosynthetic enzymes, such as chalcone synthase [CHS]; in continuous light) and by cross-talk mechanisms between the pathways (Bowler et al., 1994b).

The negative interactions between the pathways (summarized in Figures 1B and 1C), which we have collectively termed reciprocal control, are presumably important at different times. We believe that the negative regulation of the Ca\(^{2+}\) and Ca\(^{2+}\)/cGMP pathways by high levels of cGMP (Figure 1C) may act during the early stages of light exposure to suppress synthesis of photosynthetic complexes at a time when there are not sufficient photoprotectants, whereas negative regulation

![Figure 1. Summary of Current Biochemical Models of PHYA Signal Transduction.](image-url)
of the cGMP pathway by the Ca\(^{2+}\) pathway (Figure 1B) suppresses the production of anthocyanins when they are no longer required. These hypotheses are generally supported by the timing of expression of genes encoding anthocyanin biosynthetic enzymes and chloroplast components in soybean cells (Bowler et al., 1994b) and in seedlings exposed to red or white light (Drum-Herrel, 1984; Brödenfeldt and Mohr, 1988; Wenng et al., 1990; Ehmann et al., 1991). Furthermore, because we have found that the Ca\(^{2+}\)-cGMP-dependent pathway has a lower concentration requirement (probably by nearly an order of magnitude) for cGMP than does the cGMP-dependent pathway (Bowler et al., 1994b), it would appear that full chloroplast development and synthesis of all the photosynthetic components can proceed in the absence of anthocyanin pigment biosynthesis.

Such a scenario clearly highlights the importance of negative regulation for coordinating phytochrome signal transduction. Although we can only speculate about the precise mechanisms involved, the experimental results so far obtained (Bowler et al., 1994b) indicate that the most obviously apparent forms of negative regulation involve interactions between downstream components of the signaling pathways (Figures 1B and 1C). In addition to these interactions, the sheer number of examples from animal cells would tend to suggest that phytochrome signaling will also be attenuated at the level of the photoreceptor itself. One possibility is that phytochrome is inactivated by phosphorylation, like rhodopsin (Kawamura, 1993) and many other animal receptors. Biochemical data have indicated that phytochrome is phosphorylated in vivo (Hunt and Pratt, 1980). Furthermore, mutation of PHYA N-terminal serine residues to alanine residues results in a molecule that has increased biological activity (Stockhaus et al., 1992), suggesting that one desensitization mechanism for phytochrome signaling may normally be the phosphorylation of these residues. Wong et al. (1986) have purified a polycation-dependent kinase that may be responsible for phosphorylating phytochrome in vivo, and its targets indeed appear to be N-terminal serine residues, at least in vitro (McMichael and Lagarias, 1990). A different phytochrome-associated kinase has also been purified (Grimm et al., 1989). Interestingly, unlike the polycation-stimulated kinase, this kinase has been found to be stimulated by Ca\(^{2+}\) and cGMP (Grimm et al., 1989). This observation suggests that the two signaling molecules that PHYA uses for its responses may also regulate photoreceptor desensitization, providing a mechanism for feedback regulation to control the activity of the photoreceptor.

It may also be significant that the Pfr form of PHYA is known to be very unstable (perhaps because of ubiquitination; Jabben et al., 1989) and is rapidly sequestered into large aggregates in the cytoplasm (Quail, 1991). Controlled removal of physiologically active PHYA from its signaling machinery by sequestration and/or degradation would clearly constitute a form of desensitization, and such mechanisms have now been found in several other signaling systems (see below). Although the other phytochromes are not sequestered, sequestration may well prove to be one mechanism for attenuating PHYA signal transduction.

The importance of negative regulation for light responses is underlined by the types of mutant that have been isolated in screens for mutants with defective photomorphogenesis. In Arabidopsis, two types of mutant have been studied (Chory, 1993): (1) those that have a partially etiolated morphology when grown in the light (e.g., a long hypocotyl), and (2) those that show characteristics of light-grown plants when grown in the dark (e.g., short hypocotyls, open cotyledons, and expression of light-regulated genes). In simple terms, the former class can be thought of as light-insensitive mutants, whereas the latter can be thought of as mutants constitutive for light responses.

The light-insensitive mutants are recessive, indicating that the mutations most likely affect positively acting components of the light signaling machinery. For example, one would expect that some are deficient in photoperception and may contain mutated photoreceptors. This has been borne out by the characterization of the genetic lesions of mutants within this class, denoted hy for long hypocotyl. hy1, hy2, and hy6 mutants are believed to have defects in phytochrome chromophore biosynthesis or attachment (Parks and Quail, 1991; Nagatani et al., 1993); hy3 mutants (now denoted phyB; Quail et al., 1994) have mutations in their PHYB genes (Reed et al., 1993); hy8 mutants (now phyA; Quail et al., 1994) have defective PHYA genes (Dehesh et al., 1993; Nagatani et al., 1993; Whitelam et al., 1993); and hy4 mutants are probably blue-light photoreceptor mutants (Ahmad and Cashmore, 1993). One would also expect some hy mutants to be defective in positively acting light signal transduction intermediates; however, with the possible exceptions of hy5 (Chory, 1992), and fhy1 and fhy2 (Whitelam et al., 1993), such mutants have not been found.

The paucity of mutants that are likely to be defective in positive elements downstream of phytochrome suggests that such mutants are extremely difficult to find in these screens. If signal transduction from each of the phytochromes were to converge in the same pathway, such mutations might even be lethal, because too many processes would be affected. Observations that PHYA and PHYB do have some overlapping functions (Reed et al., 1994) would argue that at least some signaling pathways are indeed shared. However, the fhy1 and fhy3 mutants do appear to affect PHYA responses specifically (Whitelam et al., 1993; Johnson et al., 1994). Whether the cGMP and Ca\(^{2+}\) pathways that have been defined biochemically (Figure 1) are used specifically by PHYA or are shared by different phytochromes is an interesting question. Although these pathways have been linked to PHYA (Neuhaus et al., 1993; Bowler et al., 1994a), it is clearly possible that they may also be used by the other phytochromes or even by the blue/UVA photoreceptors.

The "constitutive" mutants, called det (for deetiolated) and cop (for constitutive photomorphogenic), were isolated in
screens for seedlings showing light-grown morphologies (e.g., short hypocotyls and open cotyledons) in darkness (discussed in Chory, 1993; Deng, 1994). The recessive nature of these mutants suggests that the mutations lie within negative regulatory molecules. Epistasis tests using the hy mutants have indicated that the gene products function downstream of both the phytochromes and the blue/UVA photoreceptors (Chory, 1993; Deng, 1994; but see Millar et al., 1994). It is difficult, however, to conclude that they are light perception and phototransduction mutants because their phenotypes are not restricted to those we would expect if photoreceptor signaling was specifically deregulated (discussed in Millar et al., 1994). The problem lies in the complexity of the photomorphogenic developmental program, because photomorphogenic traits are influenced not only by light but also by (at least) cytokinin and ABA (Millar et al., 1994). For example, it has been shown that det1 can be phenocopied in wild-type seedlings by addition of cytokinin (Chory et al., 1991, 1994). Furthermore, the fact that six of the 11 constitutive mutants characterized to date (det1, cop1, cop8, cop9, cop10, and cop11) are allelic to previously isolated seedling lethal mutants known as fusca (fus) mutants (Castle and Meinke, 1994; McNellis et al., 1994; Misera et al., 1994; Wei et al., 1994a) also indicates that the mutant phenotypes are more pleiotropic than was originally suspected.

With the realization that morphological screens do not allow the specific isolation of mutants with altered phytochrome responses (except for the elegant utilization of responses to constant red or far-red light irradiation to discriminate between different phytochromes; Parks and Quail, 1993), other screens have been developed in an attempt to improve specificity. These screens have been based on the aberrant expression of a light-regulated hygromycin resistance gene (Susek et al., 1993; Li et al., 1994). Although the mutants isolated have not yet been characterized to determine whether they are defective specifically in the response to phytochrome, the type of screens used (i.e., designed to isolate mutants constitutive for a response in the absence of the normal stimulus) and the fact that all but one of the mutations are recessive indicate that these mutants are blocked in some aspect of negative regulation.

Although genetic screens for constitutive mutants select predominantly for mutants with defects in negative regulators, the number of mutants now isolated indicates that negative regulation may be a common mechanism for controlling plant photomorphogenesis and is perhaps as important as regulation by positive elements. Even though the cop and det mutants do not appear to have defects specific to phytochrome signaling, their phenotypes imply that the mutations lie within negatively acting global regulators for plant development that are able to receive signals from multiple stimuli and integrate them into a morphological (i.e., developmental) response. Because many of the cop and det mutants are allelic to seedling lethal mutants, it has been suggested that this class of molecule is critically important for controlling certain aspects of plant development (Castle and Meinke, 1994). It is clearly of great importance, therefore, to understand how such molecules work.

We now have knowledge of the structure of four of these negative regulators: FUS6 (COP11), COP9 (FUS7), DET1 (FUS2), and COP1 (FUS1). FUS6 (Castle and Meinke, 1994) and COP9 (Wei et al., 1994b) have entirely novel sequences but are probably cytoplasmically localized. COP9 exists as a large complex whose formation or stability requires COP8 and COP11, because it is absent in cop8 and cop11 mutants (Wei et al., 1994b). This is some of the first evidence for molecular interactions among different COP/DET/FUS gene products and indicates that at least COP8, COP9, and COP11 may act together to control a particular biochemical process. The sequence of DET1 is also novel, although the protein is nuclear (Pepper et al., 1994). Unlike these novel proteins, COP1 contains a combination of recognizable structural motifs: a zinc-finger domain at the N terminus (similar to a newly characterized zinc binding domain called a RING finger; Boddy et al., 1994), a putative coiled-coil region, and a domain at the C terminus with multiple WD-40 repeats homologous to the β subunits of heterotrimeric G-proteins (Deng et al., 1992; McNellis et al., 1994).

In spite of an extensive analysis of the COP1 gene in an allelic series of cop1 mutants, no domain has clearly emerged as being more important than the others (McNellis et al., 1994). However, the homology of COP1 (particularly the Gβ-homologous domain) with the Drosophila TAF118 protein, a subunit of the TFIID complex that is required for RNA polymerase II transcription initiation, may suggest a role for COP1 in repressing general transcription (McNellis et al., 1994), which would be consistent with its expected function as a negatively acting global regulator of plant responses. If so, COP1 would be expected to be a nuclear protein. The nuclear localization of DET1 may hint that its function is also to repress transcription. Although DET1 does not appear to bind DNA directly (Pepper et al., 1994), its repressor activity may be manifested at a much more general level, for example, by remodeling the nucleosomes to organize repressive regions of chromatin (Cooper et al., 1994; Imbalzano et al., 1994; Kwon et al., 1994). COP1 may have a similar function, because Gβ-homologous domains are present within at least one such global repressor, yeast TUP1 (Deng et al., 1992; Cooper et al., 1994). Biochemical experiments are clearly required to examine this possibility.

The current lack of overlap between the biochemical (Figure 1) and the genetic (Chory, 1992; Ang and Deng, 1994) models of phytochrome signaling deserves some comment and is a major challenge to address in the future. Several points are relevant. First, the biochemical pathways have been defined mainly by their positive elements, whereas all the gene products inferred from mutant studies are likely to be repressors (with the possible exceptions of hy5, fhy1, and fhy3). This is simply a result of the different experimental approaches: the microinjection experiments were designed to identify positively
acting signal intermediates, whereas the genetic screens were
designed to yield predominantly negative regulators.

Recent biochemical studies of the negative regulatory mech-
ani sms involved in phytochrome signaling (Bowler et al., 1994b)
can, however, allow us to predict the phenotypes of mutants
al tered in specific negative regulatory aspects of phytochrome
signaling, for example, in CHS desensitization or in reciprocal
control. Recessive mutants of this kind might not be expected
to be constitutive for a light response in darkness (as are cop
and det mutants) because at this level in the signal transduction
pathway, there does not appear to be a “dark current” (i.e., turn-
over; see Introduction) of Ca\(^{2+}\) or cGMP (Bowler et al., 1994b).
With respect to their responses in the light (a condition under
which we do believe there is a turnover of second messengers;
Bowler et al., 1994b), phenotypes would be more apparent.
For example, mutant seedlings unable to repress the Ca\(^{2+}\)
and Ca\(^{2+}\)/cGMP-dependent pathways (Figure 1C) might be
expected to display a light fluorescence-dependent bleaching due
to chloroplast development before sufficient photoprotectants
have been synthesized. Because the screens used so far to
isolate photomorphogenic mutants have been based largely
on aberrant phenotypes in darkness rather than aberrant
phenotypes in dynamic responses to light signals, such mu-
nants have not yet been studied.

Currently, the simplest way to reconcile the apparent ab-
scence of a “dark current” with the constitutive phenotypes of
cop and det mutants is if COP and DET act downstream of
the second messengers. For example, they could be general
transcriptional repressors normally inactivated by Ca\(^{2+}\)
and cGMP, among other things. In their absence (i.e., in mutants),
light-responsive and other genes would no longer be repressed
in the dark and constitutive transcription would ensue, even
though Ca\(^{2+}\) and cGMP levels would remain unaltered. Cur-
rent information about the structure of COP1 and DET1 does
indeed suggest that they are somehow involved in regulating
transcription (see above). The possibility that COP and DET
act downstream of Ca\(^{2+}\) and cGMP can be tested by examin-
ing whether or not the pharmacological inhibitors of the
phytochrome pathways (Bowler et al., 1994b) are able to re-
verse the constitutive phenotypes of the mutants in the dark.

A second important difference between the biochemical and
genetic data is that the biochemical pathways have defined
very specific cellular reactions (leading to chloroplast devel-
opment and anthocyanin biosynthesis) and have not even
begun to address the developmental inputs that may affect
the stimulation of these responses. Anthocyanin biosynthe-
sis, for example, is known to be under exquisite photobiological
control during development (Drumm-Herrel, 1984; Frohnmeyer
et al., 1992; Nick et al., 1993). The majority of mutants have,
by contrast, been isolated based on their developmental aber-
ations. Most likely, therefore, the gene products affected in
these mutants are involved in coordinating global develop-
mental switches to a wide range of different signals, including
light, rather than being specific to some aspect of the phyto-
chrome cellular signaling system that has so far been defined
biochemically.

ETHYLENE

Gaseous ethylene is an important growth regulator, influenc-
ing a variety of plant responses that include germination, leaf
abscission, and fruit ripening, as well as responses to environ-
mental stresses such as wounding, chilling, and pathogen
attack. In contrast to phytochrome, biochemical approaches
have contributed little to our current knowledge of ethylene
signal transduction, and recent interest has arisen largely as
a result of genetic approaches that have identified gene
products involved in regulating ethylene responses. These
approaches have exploited a morphological response to eth-
ylene observed in etiolated seedlings known as the “triple
response,” which comprises three distinct changes in seedling
morphology: inhibition of hypocotyl elongation, hypocotyl thick-
ening, and an exaggerated tightening of the apical hook. As
previously described for the photomorphogenesis mutants,
both “insensitive” and “constitutive” mutants have been
screened for and isolated from Arabidopsis (Guzmán and
Ecker, 1990). The success of these morphological screens is
a result of the fact that the simultaneous appearance of these
three responses is highly specific for ethylene; hence, the
mutants isolated are very likely to have defects in the same
signal transduction pathway. This greatly facilitates the inter-
pretation of results from epistasis tests and contrasts with
the mutants so far isolated in screens for photomorphogenic
phenotypes, which may have defects in any one of several
signaling pathways.

For the ethylene-insensitive mutants, three complementa-
tion groups have been reported, designated etr1 (Bleecker et
al., 1988) and ein2 and ein3 (Guzmán and Ecker, 1990; Kieber
et al., 1993). etr1 mutations are dominant and are allelic to the
dominant ein1 mutants isolated by Guzmán and Ecker (1990;
Chang et al., 1993). ein2 and ein3, on the other hand, are reces-
sive mutations. From screens for constitutive triple response
mutants, two mutant classes have been identified that can be
distinguished based on whether or not they overproduce eth-
ylene (Kieber et al., 1993). Ethylene overproducers are not likely
to be mutated in ethylene perception or signal transduction,
whereas those that have normal levels of ethylene are. Of this
latter class, only one complementation group has currently
been reported, designated ctr1. The recessive nature of the
constitutive phenotype indicates that CTR1 is a negative regu-
lator of ethylene responses. Epistatic relationships among
these mutants have been examined, and the results suggest
the order of action as being ETR1, CTR1, and EIN3 (Kieber
et al., 1993).

ETR1 has now been cloned, and the strong similarity of the
C terminus to domains of proteins involved in the two-
component signal-transducing mechanisms of bacteria allows
some speculation about its possible mode of action (Chang
et al., 1993). The two-component pathway of prokaryotes, as
its name suggests, usually contains two proteins (Parkinson,
1993). One is called the “sensor” and contains both an “input”
domain (which senses the stimulus) and a “transmitter.” The
other, called the "response regulator," consists of both a "receiver" module (which receives the signal from the transmitter, via phosphorylation) and an "output" domain, which triggers the response. ETR1 contains a "transmitter"-homologous domain and a "receiver"-homologous domain downstream of an N-terminal region of 313 amino acids whose sequence is novel. It has been postulated that this N-terminal domain may be the ethylene-sensing region, whereas the C terminus may be involved in signal transmission (perhaps like phytochrome). ETR1 may therefore be an ethylene receptor, as is also supported by the fact that etr1 mutants have a lower capacity to bind ethylene compared with wild-type plants (Bleecker et al., 1988). The N-terminal region of ETR1 also contains three potential membrane-spanning domains, indicating that the protein is membrane localized, even though ethylene is able to diffuse through membranes. All the etr1 mutations so far characterized lie within these putative membrane-spanning domains (Chang et al., 1993).

Based on the homology of the C terminus of ETR1 with the prokaryotic proteins, it is possible that perception is signaled via phosphorylation reactions. The prokaryotic transmitters are phosphorylated on a histidine residue, and the conservation of this residue in ETR1 suggests that histidine may also be phosphorylated in ETR1. Histidine phosphorylation was once thought to be restricted to prokaryotes, but with the isolation of not only Arabidopsis ETR1, but also yeast SLN1, an osmolarity sensor that also shows homology with the "transmitter" and "receiver" domains of bacterial two-component systems (Ota and Varshavsky, 1993), the interesting possibility now arises that phosphorylation of histidine residues may prove to be an important regulatory mechanism in eukaryotic cells.

CTR1 has also been cloned recently (Kieber et al., 1993). It contains no obvious membrane-spanning domains, but its C terminus has considerable homology with protein kinases, particularly with members of the Raf family of serine/threonine kinases, which activate MAP kinase cascade pathways (Blumer and Johnson, 1994). All five mutations sequenced are in the putative kinase catalytic domain. By using the plant of ETR1 and CTR1 in ethylene signal transduction therefore appears to be an interesting combination of a prokaryotic signaling system with a typical eukaryotic one. A similar system has also been found in yeast for controlling adaptation to osmotic stress (Maeda et al., 1994).

How does ethylene signal transduction work? One possible model, based on current information, is presented in Figure 2. It is reasonable to speculate that ETR1 is an ethylene receptor and that it controls ethylene responses via a phosphorylation cascade that involves CTR1 and EIN3. Because CTR1 appears to be a kinase, it is unlikely that it acts by itself, and the phosphorylation status of its substrate probably is also controlled by a "complementary" protein phosphatase. However, the nature of the phenotype conferred by ctr1 and the fact that the mutations are recessive suggest that CTR1 is a negative regulator of ethylene responses. Furthermore, the fact that the phenotype is manifested in the absence of exogenous ethylene would therefore indicate that there is always a flux through the pathway (perhaps as a result of low constitutive activities of the phosphatases and kinases; see Introduction) and that one role of CTR1 is to attenuate this signal flow in the absence of ethylene by phosphorylation of its target substrate(s). Mutation of CTR1 would then prevent phosphorylation of its target, allowing the basal level signal to pass this regulatory step unhindered, thus leading to the triple response in the absence of ethylene. In this model, dephosphorylation would be the forward reaction, at least for the CTR1-controlled step.

The triple response elicited by exogenous ethylene probably occurs as a result of increased flux through the pathway initiated by a phosphorylation cascade from ethylene-stimulated ETR1. If ETR1 were to interact directly with the CTR1-regulated step in the presence of ethylene, it could overcome CTR1 repression by inactivating CTR1, by activating the phosphatase partner of CTR1, or by both (Figure 2). In either case, the ethylene-insensitive phenotype of etr1 mutants can be most simply explained if the mutations were to result in an inactive protein that can no longer carry out its subsequent interaction(s). Dominance could then be explained if ETR1

![Figure 2. Possible Model for Ethylene Signal Transduction for Controlling the Triple Response.](attachment:image)
functions as a multisubunit complex, because the presence of mutant subunits would disrupt its activity (Chang et al., 1993). Some of the two-component regulators of bacteria are known to function as dimers (Parkinson, 1993).

If ETR1 were to interact with the CTR1-controlled step in the absence of ethylene (e.g., to activate CTR1), other, more complicated schemes could be proposed to account for the phenotypes of the mutants (discussed in Chang et al., 1993). However, with the current absence of any biochemical information about an interaction between ETR1 and CTR1, all models remain highly speculative. First and foremost, kinase activity must be demonstrated for each protein. Subsequently, important questions that can be answered include whether ETR1 works as a multimer, how the activities of ETR1 and CTR1 change in response to ethylene, and how their activities are altered in etr1 and ctr1 mutants.

If there is a basal level flux through the pathway in the absence of ethylene, ectopic expression of CTR1 would be predicted to result in ethylene insensitivity, because the equilibrium of the phosphorylation reaction would have shifted toward the production of the inactive form of the target of CTR1. However, ectopic expression of a modified (i.e., deregulated) CTR1 molecule should be even more effective in causing ethylene insensitivity.

Overexpression of the gene that encodes the protein phosphatase partner of CTR1 (Figure 2) would cause a constitutive triple response unless its activity were also regulated by ethylene, in which case only a deregulated form might show a constitutive triple response. In analogy with these predictions, pharmacological inhibitors of CTR1 activity might cause a triple response, whereas an inhibitor of the phosphatase might prevent it. Some experiments detailing the effects of protein kinase and phosphatase inhibitors on ethylene responses have been reported, although these have been studied with respect to their effects on the ethylene-mediated induction of pathogenesis-related genes (Raz and Fluhr, 1993). The results indicate that protein kinase inhibitors can block the response, whereas protein phosphatase inhibitors can stimulate pathogenesis-related gene induction in the absence of ethylene, the opposite of what we would predict if CTR1 and its phosphatase partner were, respectively, the targets of these inhibitors. In similar experiments, Ca2+ has also been implicated as a second messenger in ethylene-mediated pathogenesis responses (Raz and Fluhr, 1992), although its potential to regulate the phosphorylation cascade involved in the triple response remains to be critically evaluated.

**ABSCISIC ACID**

Among other things, ABA regulates embryo maturation and seed dormancy and inhibits mitotic activity in root meristems. During stress conditions such as water shortage, it also induces stomatal closure (Zeevaart and Creelman, 1988). Mutants defective in these responses have been isolated in screens for seedlings that grow on normally inhibitory concentrations of ABA. Much like the hy mutants, these mutants can therefore be thought of as "insensitives," and they have been named ab1 mutants, for abscisic acid insensitive (Koornneef et al., 1984). These mutants are severely impaired in a wide spectrum of ABA responses, and they show phenotypes including excessive water loss (caused by an inability to regulate stomatal function) and reduced seed dormancy. These phenotypes would therefore suggest that the mutations affect ABA responses specifically. One such mutant, ab1, which is dominant, has recently been cloned (Leung et al., 1994; Meyer et al., 1994). The gene product, AB11, has a novel structure containing a domain highly homologous to the serine/threonine protein phosphatase type 2C, together with an N-terminal Ca2+ binding site. Functionally, therefore, it may be the plant equivalent of calcineurin, the Ca2+-dependent phosphatase present in animal and yeast cells, even though it is structurally very different: calcineurin is a heterodimeric protein composed of a Ca2+- binding regulatory domain and a catalytic subunit that has no homology with protein phosphatase type 2C (Cohen, 1989).

There is very good evidence for the involvement of Ca2+ in ABA responses, at least in controlling stomatal closure (Gilroy et al., 1991). Logically, therefore, the structure of AB11 would indicate that it is a downstream target of ABA-stimulated Ca2+. This can be tested by examining ABA-mediated Ca2+ increases in wild-type and mutant cells and by examining whether responses to artificially increased Ca2+ (e.g., stomatal closure induced by release of caged Ca2+; Gilroy et al., 1990) are affected in the mutant.

Based on current information, models of ABA signaling should incorporate a plasma membrane–localized receptor (Anderson et al., 1994; Gilroy and Jones, 1994), Ca2+, and AB11 and should account for the dominant nature of the ab1 mutation (Koornneef et al., 1984). In simple terms, two functional possibilities exist, dependent upon whether phosphorylation or dephosphorylation is the forward reaction for the AB11-regulated step in ABA responses (Figure 3). If dephosphorylation is the forward reaction, then ABA-mediated increases in cytosolic Ca2+ may activate AB11, which would then dephosphorylate its target (Figure 3A). If phosphorylation is the forward reaction, AB11 would be predicted to function as a negative regulator, constitutively suppressing forward flow through the signal transduction pathway in the absence of ABA (Figure 3B). In the first scenario (Figure 3A), dominance could be explained most simply if AB11 were part of a multisubunit complex, because the mutant subunits would disrupt the activity of the complex. In the latter scheme (Figure 3B), the dominance of the ab1 mutation could be explained if the mutation were a result of a loss of sensitivity to Ca2+, resulting in phosphatase activity that was no longer repressible by Ca2+ and hence causing a permanent switching off of the pathway. Once phosphatase activity of AB11 has been demonstrated,
The ABA receptor is shown to be localized to the plasma membrane and to receive the ABA signal externally (see Anderson et al., 1994; Gilroy and Jones, 1994). In both models, an increase in cytosolic Ca\(^{2+}\) is shown to precede ABU action, and the putative protein kinase partner of ABU is shown as being regulated by Ca\(^{2+}\) in the opposite manner to ABU.

(A) A model in which dephosphorylation, mediated by Ca\(^{2+}\)-activated ABU, leads to activation of the target of ABU and consequently to ABA responses. In this case, Ca\(^{2+}\) would be predicted to repress ABU activity.

(B) An alternative model in which phosphorylation of the ABU target leads to its activation and to subsequent ABA responses. In this case, Ca\(^{2+}\) could be predicted to repress ABU activity.

biochemical experiments should be able to resolve these different possibilities.

AUXIN

Auxin has been implicated in regulating almost every aspect of plant development. Although we now have some understanding of its biosynthesis, biochemical responses, and physiology, molecular studies of its action have proven very difficult (Estelle, 1992). As may also be the case with other hormones, one likely way that auxin responses are regulated is by controlling the amount of free hormone that is available for signal transduction (Szerszen et al., 1994). The conjugation of free hormone to form inactive pools can potentially attenuate signal transduction, whereas its release from these conjugates could activate it. In addition to this type of control, two recent articles have now provided other clues about auxin signaling mechanisms (Leyser et al., 1993; Abel et al., 1994).

The first article details the cloning of the auxin resistance gene AXR1 from Arabidopsis (Leyser et al., 1993). To date, 20 axr1 mutants have been isolated in screens for mutants able to grow on normally inhibitory concentrations of auxin, and all are recessive (Estelle and Somerville, 1987; Lincoln et al., 1980). Other phenotypes of these mutants include decreases in height, in apical dominance, in root gravitropism, in hypocotyl length, and in fertility. Cloning and sequencing of the AXR1 gene revealed significant similarity to the ubiquitin-activating enzyme E1 (Leyser et al., 1993), although there are sufficient important differences between the sequences to make it clear that AXR1 is not an E1 homolog. Nonetheless, the similarities do suggest that the ubiquitination pathway for protein degradation may somehow play an important role in auxin responses. This possibility has also been inferred from an independent series of experiments. Abel et al. (1994) have found that a family of conserved genes induced rapidly and strongly by auxin in excised stem sections encodes nuclear proteins that have very short half-lives. Each of these proteins contains a motif similar to that found in the DNA binding domain of prokaryotic repressor proteins such as Arc, suggesting that they are DNA binding proteins that may regulate auxin responses. Their short half-lives have been proposed to be a result of selective degradation by ubiquitination pathways that possibly involve the AXR1 gene product (Abel et al., 1994), a possibility that can now be tested by examining the half-lives of these proteins in axr1 mutants. Overall, this would provide a mechanism for executing rapid responses to auxin that could be equally rapidly attenuated.

A proposed role for AXR1 must account for the hormone-insensitive, recessive nature of axr1 mutants. Most simply, there are two formal possibilities: (1) that it degrades a repressor (i.e., that it negatively regulates a negative regulator) or (2) that it is involved in positively regulating auxin responses. The second possibility is difficult to reconcile with a role in protein degradation, although auxin responses may involve the repression of other pathways. AXR1 could achieve this by degrading key positive regulators within these pathways. Further genetic and biochemical characterization is necessary to examine these possibilities. Unfortunately, a major obstacle for assigning an auxin-specific function to AXR1 will be the known pleiotropy of auxin responses.

Although the function of AXR1 in auxin signal transduction is unclear, its identification as a possible participant in ubiquitin-mediated protein degradation is interesting because selective protein degradation is an emerging theme for desensitizing some signal transduction pathways in animal cells. Examples include regulation of cyclins, the human growth regulator p53, Drosophila Ftz, and yeast MATa2 (Gottesman and Maurizi, 1992). Compared with phosphorylation and other reversible modifications, proteolysis is a drastic way to inactivate a protein, but it is especially important for controlling proteins that are required either for only limited periods of time or under specific developmental or metabolic conditions and whose presence may be detrimental under other conditions. For example, cyclin acts to stimulate cell division, but its degradation is an essential regulatory mechanism for allowing the cell to advance to the next phase of the cell cycle.
This brief overview of recent advances in the understanding of plant signal transduction mechanisms serves to illustrate several emerging features: (1) the involvement of novel proteins containing more than one functional domain, (2) the concerted utilization of signaling systems previously thought to be specific for prokaryotes or eukaryotes, (3) the presence of basal level fluxes through signaling pathways in the absence of a stimulus, (4) the central importance of negative regulation, and (5) the significance of crosstalk between pathways.

The mechanism by which hybrid molecules such as COP1, ETR1, and ABI1 receive and transduce signals will be important to understand, because it is possible that they are central players in transmitting crosstalk information, for example, between different hormones. Cross-talk is likely to be of extreme importance in plants because of their developmental plasticity. The importance of crosstalk has indeed been inferred from the phenotypes of many of the mutants isolated in screens designed initially to be specific for one stimulus. Witness, for example, the pleiotropy of the cop and det mutants and the resistance of auxin-resistant mutants to other hormones (Pickett et al., 1990; Wilson et al., 1990). Such mechanisms are likely to be elucidated largely by genetic approaches; in fact, the information already available gives us some clues about possible mechanisms. For example, ABI1 may be regulated by Ca²⁺ signals generated not only from ABA, but also from other hormones or from phytochrome, because all are known to stimulate increases in cytosolic Ca²⁺ (see Bush, 1993, and references therein). Similarly, the COP and DET proteins may be targets for more than one stimulus, thus allowing access by different stimuli to the same signal transduction pathway or at least the same target. In addition, it is possible that different signals activate the same target in different cell types or that the same protein can behave as either an activator or a repressor, depending on the context (Lehming et al., 1994).

The emergence of negative regulation as a central theme in plant signaling may to some extent be a biased interpretation of the true situation because of the type of genetic screens used to date. Nonetheless, there are clearly negative regulatory molecules of extreme importance in plant development, of which the COP, DET, and CTR1 proteins are obvious examples. Recessive mutations in some of the ACD and LSD genes result in plants that spontaneously form necrotic lesions on leaves even in the absence of pathogens (Dietrich et al., 1994; Greenberg et al., 1994); these genes are also likely to encode negative regulators, implying that the defense response to pathogens may be mediated, to some extent, by release of repression mechanisms. The presence of signaling pathways primed to respond but blocked by repressors (such as the ethylene pathway and the defense response pathway) is a conceptually simple as well as an efficient mechanism for permitting crosstalk. Moreover, the lack of rigid developmental constraints during plant development may indicate that such negative regulatory mechanisms will be found to be prevalent in plant signal transduction.

Future work must confirm or identify by biochemical methods the functions of gene products identified in genetic screens. Activities proposed for different proteins—e.g., ETR1, CTR1, and ABI1—must be demonstrated, their regulation understood, and their substrates defined. The development of new methods may also be required to open up a signal transduction pathway biochemically. For example, the extension of microinjection and related methodologies to Arabidopsis should prove to be extremely powerful, as has been recently shown for tomato (Neuhaus et al., 1993; Bowler et al., 1994a).

Combined with biochemical approaches, we believe new genetic screens should be designed to isolate mutants more likely to be affected in specific signaling pathways. Rather than designing screens aimed at isolating mutants that are constitutively switched on or off, it is now both feasible (Millar et al., 1992) and worthwhile to design screens for isolating mutants that are affected in their quantitative responses to different stimulus strengths. By screening for aberrant phenotypes in dynamic responses to a particular stimulus, mutants with defects specific to the stimulus are more likely to be obtained. Furthermore, the nature of the phenotype (e.g., a hypersensitive or an amplitude response) can give an indication of whether the mutation affects the activity of the receptor or downstream signal-transducing steps within the signaling pathway.

In summary, both the genetic and biochemical approaches that have been applied to the study of plant signal transduction are clearly beginning to reveal the details of a variety of signaling networks. The union of these approaches should now be seen as the next major objective, because it will allow both the elucidation of the underlying mechanisms and the identification of the molecules responsible. Ultimately, it should permit a thorough understanding of how plants perceive, respond, and adapt to changing developmental and environmental stimuli.

ACKNOWLEDGMENTS

We would like to thank our colleagues in the laboratory for many stimulating discussions and especially Drs. Diana Horvath, Raphael Mayer, and Diane Shevell for their comments on the manuscript. We are also grateful to Dr. Joe Ecker for sharing his insights into ethylene responses with us. C.B. was supported in New York by a Science and Engineering Research Council (North Atlantic Treaty Organization) postdoctoral fellowship and later by a fellowship from the Norman and Rosita Winston Foundation. Research in New York was supported by National Institutes of Health Grant No. 44640 to N.-H.C.

Received July 15, 1994; accepted September 16, 1994.

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