

Signal Perception and Transduction: The Origin of the Phenotype

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SETTING THE SCENE

Changes in Scientific Perception

Signal transduction in plant cells has come of age. Twenty years ago the subject did not exist, and the word “transduction” would not have been recognized as having meaning for plant science. Now, however, most plant scientists can be regarded as working on some aspect of signal transduction.

Several milestones have marked the route of this change. In particular, the detection of protein kinases in plants (Trewavas, 1976), the isolation of calmodulin from plant cells (Anderson and Cormier, 1978), and the detection of Ca^{2+} -dependent protein kinases (CDPKs; Hetherington and Trewavas, 1982) all helped, with the latter finding indicating that transduction pathways are branched (Figure 1). Moreover, the critical role of calcium as a second messenger was reviewed as early as 1985 (Hepler and Wayne, 1985). Cytosolic calcium ($[\text{Ca}^{2+}]_c$) and protein kinases still dominate signal transduction investigations, although we are certain that there are additional second messengers (e.g., cGMP, cAMP, and cADP-R) and many more than the 70 protein kinases so far described.

Transduction studies were formerly separated into fast responses, which do not appear to require changes in gene expression (e.g., stomatal closure), and slow responses, which clearly do. We now know that this distinction was misleading and is in fact incorrect—the same transduction network controls both fast and slow responses. When a cell perceives a signal, ion flux and gene expression changes are inextricably linked phenomena that merely occur at different rates. This change in perception was triggered in large part by Neuhaus et al. (1993) and Bowler et al. (1994), who showed that $[\text{Ca}^{2+}]_c$ and cGMP could apparently regulate chloroplast and anthocyanin formation.

“This We Know; All Things Are Connected”

Signal transduction systems often are inappropriately portrayed as simple linear chains of cause and effect, in part because effective conceptualization of what goes on when a cell perceives a signal is impoverished by our inability to put together the experimental information in any meaningful way. What most plant scientists have assumed for years (but have never really formalized) is that every cell contains not one but two information systems. The familiar genetic system, gene→RNA→protein→phenotype, is only truly applicable to the very limited number of phenotypic characters thought to be specified by single genes. By contrast, the epigenetic system involves interacting genes and gene products (i.e., epistasis, polygenic, and pleiotropic effects) that are integrated with networks that transduce a host of internal and external signals.

This integration creates an enormously complex but flexible, unstable regulatory network interposed between the unitary genetic elements and the phenotype. Many hundreds of interlinked proteins are involved, and the behavior of this network could best be regarded as a prime example of chaos theory in action. A map can certainly be constructed of the network and its possible cross-links. However, although we can measure (and thus predict) the flux through parts of the network under precisely defined conditions, these predictions break down as soon as environmental conditions change, even by small amounts (Trewavas, 1986). This is because the characteristics of such networks result from the connections between the constituents, not from their absolute quantitative values (Capra, 1996). We expect that informational and functional redundancy will be found to be common properties of the plant cell signal transduction network.

Topological models can be helpful in conceptualizing this complex situation. As suggested by Waddington (1957), the epigenetic network can be thought of as a surface constructed from hills and valleys. The hills and valleys of this surface are subject to continual changes in position, size, and depth as external and internal signals vary in intensity

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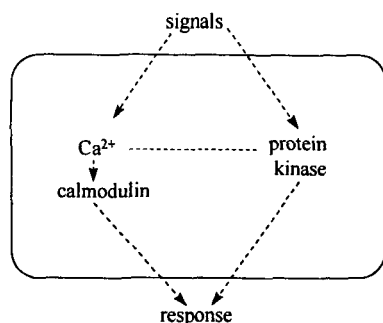


Figure 1. Extent of Understanding of Signal Transduction in Plant Cells circa 1990.

Two transduction pathways had been identified, and they were known to be interrelated, but little else of substance had been established.

and quality from minute to minute. Modifications in a single signal can tweak the surface into a new shape. The signal transduction pathway, visualized as a ball tracking across this landscape, must continually change routes as conditions change so that it ends up at the same place and thus achieves the objective of a similar physiological response. Once a response is initiated, signal perception is altered and the surface topology changes. This is an important modification of Waddington's original proposal, because signal transduction networks were unknown in 1957.

What is it that is sensed by a cell, and what creates the specificity of response to particular signals? The answer is holistic: it is the state of the whole network—the surface in the above analogy. It is the integration of the phosphorylation states and activities of many proteins, including receptors; it is the spatial distributions of $[Ca^{2+}]_c$, kinases, phosphatases, and other crucial proteins; it is the interactions between docking and cross-linking proteins, such as the recently described 14:3:3 proteins (Sehnke and Ferl, 1996) or members of the GTPase superfamily; and it is the stage of development.

Individual plants cannot choose the site in which they grow. Because they are sessile, they must put up with the conditions in which they find themselves and still achieve the biological imperative of reproduction. Consequently, and as is evident from their developmental and metabolic plasticity, plants are very much creatures of their environment. However, they have turned this situation to their advantage; at any one time, plant cells experience a mass of external signals that originate from mechanical, temperature, mineral, light, gaseous, wound, water, physical, and electrical influences. Internally, they are subjected to signals from a stream of growth regulators, peptides, sugars and other metabolites, steroids, wall fragments, turgor-related and wall-imposed mechanical signals from other cells, and further electrical information (Gilroy and Trewavas, 1990). So far as

we know, virtually all plant cells can respond to all of these signals—a tribute to the real complexity built into the signal transduction apparatus.

Stimulus–Response Coupling: The Basic Models

The overall structures of the principal signal transduction pathways used by plant cells have been clarified as a result of the enormous attention they have received over the last 7 years. Signals are first perceived by receptors. Virtually all known receptors are members of families of related proteins and are located in the plasma membrane, nucleus, cytoplasm, or even the cell wall.

A number of well-characterized receptors are protein kinases. In these cases, ligand binding triggers the kinase activity of the receptor, and subsequent signal transduction occurs through a protein kinase cascade. Other occupied receptors activate cytoplasmic or plasma membrane-localized members of the GTPase superfamily, which in turn trigger the synthesis or release of a plethora of second messengers. Either directly or indirectly, many of these second messengers then activate specific protein kinases, which together control the osmotic motors underpinning growth and movement or phosphorylate transcription factors. Although there may be differences in the details, for example, as a result of cross-talking between transduction pathways, these basic models describe the majority of known signal transduction events.

“TODAY WE START WITH THE NAMING OF PARTS”—RECEPTORS

Currently, receptors have been identified for ethylene (Bleecker and Schaller, 1996; see Kende and Zeevaert, 1997, in this issue), red light (i.e., phytochrome; Furuya and Schafer, 1996), and blue light (i.e., cryptochrome; Ahmad and Cashmore, 1996; see Chory, 1997, in this issue). The $[Ca^{2+}]_c$ receptors calmodulin and CDPK (Harper et al., 1991) and many receptor-like protein kinases (Stone and Walker, 1995) have been isolated and sequenced. Although strong candidates for receptors for abscisic acid (ABA; Giraudat, 1995), auxin (Millner, 1995; Bennett et al., 1996), cytokinins (Kakimoto, 1996), and a protein kinase-like receptor for blue light (Short and Briggs, 1994) have been sequenced or investigated in detail, most either lack relevant ligand binding data or exhibit other uncertainties of function. Receptors for inositol 1,4,5-triphosphate (IP_3), cADP-R, fungal elicitors, and Nod factors have been detected but not yet purified (Allen and Sanders, 1994; Nurnberger et al., 1994; Allen et al., 1995; Bono et al., 1995). The identification of these receptors and the elucidation of their properties are clarifying many unknowns in the regulation of plant growth and development.

SNF-1 Kinase and Plasma Membrane Potential: Possible Pseudoreceptors for Respiratory Disturbance

Many chemicals, such as the metabolic inhibitors azide and cyanide, and solvents, such as ether and ethanol, can apparently induce quite specific changes in plant development, including the breakage of seed or bud dormancy, abscission, or the regeneration of roots (Trewavas, 1992). These puzzling experimental data can be explained by invoking the activities of two unusual "receptors."

Azide and cyanide rapidly cause respiratory disturbances through their inhibition of oxidative phosphorylation. Such disturbances may be detected by sucrose nonfermenting-1 (SNF-1) kinases, the activities of which are regulated by the cellular AMP/ATP ratio (Barker et al., 1996). Organic solvents are likely to increase plasma membrane permeability. Such increases would affect plasma membrane potential and could induce the opening of voltage-gated Ca^{2+} channels. Thus, plasma membrane potential may act as a pseudoreceptor that is capable of initiating Ca^{2+} -mediated signal transduction, which, like that triggered by SNF-1 kinases, is likely to circumvent specific endogenous signals that normally control development.

Sensing Signal Direction

To maximize the capture of basic resources, plants have evolved elaborate mechanisms capable of detecting the direction of incoming physical, chemical, and light signals. The vectorial sensing of these signals requires that their receptors be fixed in the cell, and they are commonly assumed to reside on the plasma membrane. The host of signals that are believed to enter the cell from the wall may also be detected first in the plasma membrane, and the demonstrated plasma membrane localization of the ethylene receptor, a number of receptor kinases, calmodulin, and CDPK conform to this contention.

However, not all receptors are localized in the plasma membrane. For example, cryptochrome, which was cloned after the identification of mutants exhibiting blue light-insensitive hypocotyl growth, is a soluble protein. This fact raises obvious difficulties in understanding how it can play a role in detecting the blue light vector. Clearly, there are other blue light receptors that may be responsible for sensing the direction of blue light. These include a plasma membrane-localized, blue light-activated protein kinase (Short and Briggs, 1994) and the receptor involved in the blue light regulation of phototropism (reviewed in Chory, 1997, in this issue).

The interpretation of phytochrome function is subject to similar, apparently unresolved problems. Experimental studies on *Mougeotia* used plane-polarized red light to demonstrate that the form of phytochrome controlling chloroplast rotation is attached to some fixed cellular structure, most likely the plasma membrane. Nevertheless, activated phytochrome can definitely induce $[\text{Ca}^{2+}]_c$ transients (see below)

across the plasma membrane (Shacklock et al., 1992). If phytochrome is not attached to the plasma membrane, how would these transients be induced?

If phytochrome and cryptochrome play a role in sensing light direction, then some form of fixed attachment in the cell is necessary. However, this does not have to be the plasma membrane; attachment to the cytoskeleton close to, or on, the inner surface of the plasma membrane would work equally well. Thus, phytochrome and cryptochrome could act as sensors of the light vector, but on cell fractionation studies they would appear to be soluble. We discuss this possibility in our concluding remarks, but yeast dihybrid experiments using phytochrome or cryptochrome as bait may be quite revealing.

If the above hypothesis is incorrect, then we must assume that tiny but significant amounts of phytochrome and cryptochrome are directly attached to the plasma membrane. If this assumption is true, most of the cellular phytochrome and cryptochrome could then be described as excess, at least with respect to directional sensing. However, this excess could be used to regulate the sensitivity of the cells to light signals (Trewavas, 1991). A further possibility is that phytochrome and cryptochrome become attached to the plasma membrane only after they have absorbed photons. Such a scenario would explain the delays of one or a few minutes between the application of a red or a blue light signal and the initiation of appropriate changes in $[\text{Ca}^{2+}]_c$ or membrane potential.

Receptors Can Amplify Weak Signals

Plant cells can initiate new developmental programs in response to extremely weak signals. In these instances, the physiological response (e.g., growth or movement) must result from substantial metabolic amplification of the original signal, which almost always possesses insufficient energy to actually elicit a response.

Amplification may commence with the receptor itself. Receptor kinases, the ethylene receptor, phytochrome, and others are thought to dimerize upon binding their ligands. Dimerization of receptors with kinaselike cytoplasmic domains may result in intermolecular phosphorylation and the activation of signal transduction pathways. Moreover, the phosphorylated receptor kinase dimer may remain catalytically active in the absence of the ligand. Several possibilities then follow for signal amplification. For example, intermolecular phosphorylation may trigger changes in receptor conformation that could be sensed through direct interaction with downstream signal transduction components until the active state is lost by dephosphorylation. Alternatively, the two phosphorylated receptor monomers may separate and phosphorylate other inactive receptor monomers or other downstream proteins. Phosphorylation of proteins initiating protein kinase cascades would result in further amplification of the signal.

Although this model may be accurate for many receptors, it does not satisfactorily explain phytochrome signal transduction. Although at one time phytochrome was thought to possess protein kinase activity, this is no longer the case. Instead, a close association between a distinct protein kinase and phytochrome, which breaks down during purification, has been observed. Does this phytochrome/protein kinase combination form part of a larger transduction complex? Most plant species express a family of phytochromes (e.g., *Arabidopsis* has five: phytochromes A through E; see Chory, 1997, in this issue), and dimerization of these in various combinations may provide a set of multifaceted light receptors that collectively are capable of detecting and initiating responses to the many different light situations experienced by plants during their development.

Amplification may also help to account for thresholds in plant and cell developmental responses. For example, the cooperative binding of Ca^{2+} at the four Ca^{2+} binding sites of calmodulin ensures that as little as a threefold increase in $[\text{Ca}^{2+}]_c$ can result in the full activation of cellular calmodulin. In this way, signals that are transduced through changes in $[\text{Ca}^{2+}]_c$ may exhibit very narrow dose-response relations. Although thresholds have indeed been observed in single guard cells induced to close by the internal photolysis of loaded caged Ca^{2+} (Gilroy et al., 1990), the dose-response relations of populations of guard cells to ABA suggest anything but a sensitive regulation (Trewavas, 1992; see below). However, it is essential in this case to measure the dose-response relations of a single guard cell, and this difficult task has yet to be performed.

Can Two-Component Systems Explain Activated Cellular States?

The deduced amino acid sequence of the ethylene receptor (ETR1) suggests that some plant receptors may be related to proteins comprising bacterial two-component systems (Bleecker and Schaller, 1996; see Kende and Zeevaert, 1997, in this issue). In these bacterial systems, autophosphorylation of a receptor histidine kinase is followed by transfer of the phosphate moiety to a conserved Asp residue in a transcriptional response regulator. Despite the sequence similarity, ethylene signaling is clearly distinct from the bacterial model because ETR1 probably passes the signal not to a transcription factor but to CTR-1, a protein kinase of the Raf family that initiates mitogen-activating protein kinase (MAPK) cascades. Although many two-component systems are known in bacteria, in higher organisms they appear to have been superseded by protein kinases that phosphorylate Ser, Thr, and Tyr. Nevertheless, the putative cytokinin receptor also has sequence similarity to bacterial receptor/response-regulating proteins, as do osmotic sensors in yeast.

Experiments on phytochrome and hypoosmotic shock in plant cells suggest that receptor activation may lead to long-

lived excited states. Cultured tobacco cells that are hypoosmotically shocked in the absence of extracellular Ca^{2+} do not exhibit a $[\text{Ca}^{2+}]_c$ transient until Ca^{2+} is returned to the medium (Takahashi et al., 1997). Etiolated cereal leaf segments incubated on distilled water fail to unroll if exposed to a flash of red light but will do so when Ca^{2+} is added to the water. These experiments also show that an activated state is generated by a signaled receptor: by adding Ca^{2+} back at successive times after receptor signaling, Takahashi et al. showed that these "activated" states last no longer than 20 min. Both His and Asp phosphates are chemically unstable with total half-lives of ~ 10 min.

Thus, in these two experimental systems, phosphorylation of His or Asp has a stability similar to that of the activated state produced by signaling, which justifies a search for two-component regulation. Some studies of the phytochrome sequence have implied that His and Asp residues may be phosphorylated (Lagarias et al., 1995).

Do Activated Receptors Move to Adjacent Cells?

During particular stages of development, certain plant cells can impose a developmental program on their immediate neighbors. The clearest examples are found in wound healing, when the cambial ring interrupted by cuts or tissue excision can be regenerated in the callus that is formed on the damaged surface of the wounded stems. The regeneration, which is progressive and often described as contagious, commences from preexisting cambial cells.

Another example occurs during lateral root initiation. A single primordial pericycle cell can, in response to unknown signals, rapidly induce neighboring pericycle cells to form a pseudoprimordium four to five cells in diameter. This primordium forms the basis for a new lateral root meristem, and with the subsequent onset of division of these primordial cells, the characteristic lateral root structure is constructed.

Examples from the animal literature are also informative. The "sevenless" system of *Drosophila* involves a parent cell that imposes a program of development on its neighbor. Receptors on the parent cell plasma membrane directly interact with a plasma membrane-bound protein kinase on the neighboring cell, signaling the new developmental program. However, with a cell wall separating adjacent cells, this possibility seems unlikely in plant cell development. Nevertheless, receptor protein kinases play important signaling roles during plant cell development and cell-to-cell communication (Wilson et al., 1997).

There are two ways in which plant cells may employ these receptor protein kinases. Discrete (but unknown) signals could move from one cell to another through the wall, activating receptor protein kinases in the plasma membrane of the adjacent cell. A more dramatic possibility is that the activated receptor protein kinases themselves move through

the plasmodesmata connecting adjacent cells (see McLean et al., 1997, in this issue).

Although observations of protein movement through plasmodesmata are recent, a specific mechanism has been demonstrated for transport of transcription factors (Gilbertson and Lucas, 1996). These observations have developed from studies on the cell-to-cell spread of invading viruses, some of which suborn a normal cellular mechanism to facilitate their transmission. In these cases, part of the viral RNA genome codes for movement proteins that become localized to plasmodesmata, where they are thought to catalyze the transit of the viral RNA to adjacent cells.

Given these data, the movement of endogenous regulatory mRNAs between cells becomes a real possibility. But plasmodesmata also contain contiguous membrane structures that connect adjacent cells (McLean et al., 1997, in this issue). Direct migration of membrane-bound proteins could then occur through these plasmodesmatal membranes.

Interaction of Receptors with GTPases Commences Signal Interpretation

After they bind their ligand, plasma membrane-localized receptors can interact directly with members of the GTPase superfamily. GTPases with important roles in signal transduction include the G-proteins and members of the Ras family (Bourne et al., 1990; Ma, 1994; Lin et al., 1996). Interaction of the GTPase with the ligand/receptor complex allows it to bind GTP and become activated. The activated GTPase, with bound GTP, is then released from the receptor and can continue to activate numerous downstream enzymes and/or channels until it is inactivated by the hydrolysis of GTP to GDP. The inactive GDP binding GTPase is competent to interact with another receptor and start the signaling process once again.

The involvement of GTPases enormously increases flexibility in signal transduction pathways. In addition to the fact that individual members of the G-protein family can interact with a number of different receptors, the activity of G-proteins can be modulated by associated proteins that catalyze the removal of GDP or the hydrolysis of GTP. The activities of these proteins can in turn be regulated. Current evidence suggests that blue and red light, auxin, gibberellin, fungal elicitor, and stomatal aperture-controlling signals in guard cells are all mediated by GTPases.

GTPases also participate in protein synthesis, secretion, and the cell cycle (see Jacobs, 1997, in this issue). Their involvement in these processes greatly improves the fidelity of molecular interactions. This is because the rate of GTP hydrolysis on the GTPase acts as a timer to test the stability of each GTPase-protein interaction. If a particular interaction between a GTPase and another protein is weak, perhaps because it is nonspecific, the two proteins may separate before GTP is hydrolyzed, precluding the inappropriate activation of further downstream processes.

SECOND MESSENGERS AND CALCIUM

“To Take Arms against a Sea of Troubles”

When plant cells are signaled, the cytoplasmic compartment receives this information via changes in concentration of a plethora of second messengers. Activated G-proteins signal a variety of plasma membrane-bound phospholipases, cyclases, and channels, which in turn regulate second-messenger production. Many second messengers operating in one signaling system or another have been identified by plant scientists. These include phosphatidic acid, IP_3 , cGMP, cADP-R, Ca^{2+} , H^+ , redox reagents such as ascorbic acid or glutathione, hydrogen peroxide, and free radicals. Moreover, diacylglycerol and lysophospholipids, the respective products of phospholipase A and C activation, are likely to remain in the plane of the plasma membrane, where they could further modify membrane protein function.

All of these second messengers could participate in the transduction of any signal. Thus, the precise contribution of each to the control of the overall physiological response must be determined on a system-by-system or environmental-setting-by-environmental-setting basis. Adding to the complexity, some of these second messengers (e.g., Ca^{2+} and lysophospholipids) can activate protein kinases, whereas others (e.g., IP_3 and cADP-R) mobilize $[Ca^{2+}]_c$ via channel opening. Moreover, IP_3 and other phospholipids can bind directly to cytoskeletal components, changing their conformation and function (Irvine and Cullen, 1996).

cAMP and IP_3 : Similar Problems in Identifying a Functional Role

Early analysis of phospholipids in plant cells suggested that the quantities of phosphatidylinositol 4,5-bisphosphate (PIP_2), the precursor for the synthesis of IP_3 , were too low to permit a signaling role. However, subsequent research showed that photolysis of loaded, caged IP_3 in guard cells, protoplasts, and pollen tubes results in IP_3 -dependent mobilization of $[Ca^{2+}]_c$. Measurement of IP_3 levels in each of these cell types indicated that quantities sufficient for signaling are present. Moreover, IP_3 binding proteins have been detected in membrane fractions from several plant sources, and phospholipase C, which hydrolyzes PIP_2 to produce IP_3 and diacylglycerol, has been purified from plant tissues. Thus, it seems likely that the original problem regarding PIP_2 concentration was simply one of dilution; only a small subset of cells may be competent to transduce a specific signal at a particular time, and only those cells will have appropriate levels of signaling intermediates.

Entirely analogous difficulties have been found with cAMP (Assmann, 1995; Bolwell, 1995). Although the fact that plant cells contain cAMP response elements and proteins that bind to them indicates a capability for response, the quantities of

cAMP that have been detected in plants cells (usually whole tissues) are thought to be too low for a signaling role. Again, the problem may be resolved if cAMP assays were to be performed on the same cells and protoplasts that have been used to resolve the IP₃ question.

Calcium Signaling: The Basic Model

Free [Ca²⁺]_c is maintained at a resting level of ~100 nM, whereas the concentrations of Ca²⁺ in the endoplasmic reticulum (ER), cell wall ([Ca²⁺]_w), and vacuole are 0.1 to 100 mM, many orders of magnitude higher. The membranes that separate the cytosol from these other compartments contain channels that are gated by voltage, stretch, IP₃, cADP-R, or G-proteins. Calcium-mediated signaling takes place when some of these channels open, allowing Ca²⁺ to move down its electrochemical gradient (Ward et al., 1995). A single channel can conduct 10⁶ atoms of Ca²⁺ per second; thus, [Ca²⁺]_c can increase rapidly. Subsequent activation of membrane-bound Ca²⁺-ATPases (or Ca²⁺/H⁺ exchange proteins) in the ER, vacuolar, mitochondrial, and plasma membranes ensures that most [Ca²⁺]_c signals are transients (Poovaiah and Reddy, 1993; Bush, 1995). Mitochondria and chloroplasts not only manipulate [Ca²⁺]_c but also possess their own internal Ca²⁺-signaling systems, which regulate oxidative respiration and the Calvin cycle. Thus, Ca²⁺ orchestrates direct cooperation among the plasma membrane, chloroplasts, mitochondria, the vacuole, and the ER to produce the physiological response.

The diffusion rate of [Ca²⁺]_c in the cytoplasm is several orders of magnitude lower than that of Ca²⁺ in free solution. This important property can confer the capacity for localized [Ca²⁺]_c-mediated signaling. For example, some Ca²⁺-dependent signaling proteins and enzymes are attached to specific regions of the cytoskeleton and can respond only to changes in [Ca²⁺]_c occurring in their immediate vicinity. Such localized changes in [Ca²⁺]_c could arise from the clustering of Ca²⁺ channels.

However, the duration of the Ca²⁺ transient is equally crucial. A short transient results in only limited penetration of the [Ca²⁺]_c into the cytoplasm. A much longer transient ensures greater depth of penetration and a consequent increase in the number of responsive domains that are activated. Activation of spatially separate [Ca²⁺]_c signaling pathways can induce the expression of different genes (Bading et al., 1993). Clearly, then, it is important to monitor the spatial characteristics of the [Ca²⁺]_c signal.

Although a variety of Ca²⁺ binding proteins interpret the [Ca²⁺]_c signal, calmodulin, the sequence of which is highly conserved in all eukaryotes, is the ubiquitous Ca²⁺ receptor. When bound to Ca²⁺, calmodulin activates well over 100 proteins, most of which have not been characterized further. The activities of myosin V, kinesin, Ca²⁺-ATPase, Ca²⁺ channels, glutamate decarboxylase, NAD kinase, and a number of protein kinases are all calmodulin dependent in

plant cells (Baum et al., 1996; Yang et al., 1996). The interactions between calmodulin and these proteins are generally hydrophobic; even minor modifications to the sequence of the hydrophobic patch on calmodulin can disrupt its ability to activate them. By contrast, however, there is little or no sequence conservation among the calmodulin binding sites of the various interacting proteins.

In addition to calmodulin, plants possess an undetermined number of other Ca²⁺ binding proteins. The best characterized is CDPK, although there are sporadic reports of others, including a protein phosphatase (Antonsiewicz et al., 1995; Giraudat, 1995).

The Vacuole and Cell Wall Ca²⁺

A substantial quantity of plant Ca²⁺ is associated with the cell wall. In particular, in the walls of growing cells, Ca²⁺ acts to cross-link pectins, thereby increasing wall rigidity. Indeed, addition of extracellular Ca²⁺ to growing tissues inhibits cell extension, and Ca²⁺ removal by chelation can increase growth rates (see Cosgrove, 1997, in this issue). For the most part, plant cells obtain their supplies of Ca²⁺ through the xylem, but the flow rate through this tissue may vary enormously from minute to minute as availability of water changes. Given the critical role of [Ca²⁺]_w in growing tissues, it seems unlikely that the Ca²⁺ supply would be left to the vagaries of the xylem source.

A more likely source of [Ca²⁺]_w is the vacuole, in which Ca²⁺ concentrations may exceed 100 mM. Some specialized cells, ideoblasts, contain crystals of calcium oxalate in a membrane-surrounded structure that is probably the vacuole. Ideoblasts are found throughout leaf and root tissues, particularly around guard and subsidiary cells. The numbers of ideoblasts (or Ca²⁺-accumulating cells) can be increased by simply supplying a higher concentration of Ca²⁺ to the plant. By contrast, placing plants and tissues in Ca²⁺-free media can result in the complete loss of calcium oxalate within several hours, indicating that this source of Ca²⁺ can be rapidly mobilized. If seedlings or other tissues are placed in solutions of the Ca²⁺ chelator EGTA or the channel blocker La³⁺, [Ca²⁺]_c is greatly increased for several hours, confirming that this mobilization is from internal Ca²⁺ stores.

These observations suggest that the plasma membrane may contain a Ca²⁺ sensor whose functions are to sense and to help regulate [Ca²⁺]_w homeostasis. This proposed sensor may be coupled to a transduction sequence that induces the mobilization of vacuolar Ca²⁺ through the cytoplasm into the wall when required. In highly vacuolated cells, the tonoplast may be in contact with the plasma membrane, which would allow the direct transport of Ca²⁺ to the apoplastic space. When Ca²⁺ is removed from the wall through the application of EGTA or further entry is inhibited by La³⁺, the sensor interprets both changes as indicating a lowering of [Ca²⁺]_w and triggers the mobilization of internal reserves.

There must also be a mechanism for signaling an excess of $[Ca^{2+}]_w$ that would allow unneeded Ca^{2+} to return to the vacuole. It is possible then to envisage that extracellular Ca^{2+} can act as a messenger, a possibility that was originally proposed to explain specific aspects of gravitropism in roots.

Aequorin as a Tool for Measuring Changes in $[Ca^{2+}]_c$

Our knowledge of the signals that trigger increases in $[Ca^{2+}]_c$ levels has increased substantially with the use of plants, such as tobacco, Arabidopsis, and *Physcomitrella*, that have been transformed with the soluble, calcium-sensitive, luminescent protein aequorin (Knight et al., 1991; Trewavas and Knight, 1994; Haley et al., 1995). By using this approach, mechanical signaling (i.e., touch, wind, and turbulence or shaking), hypoosmotic stress, cold and heat shock, oxidative and anaerobic stress, salination, and drought all result in very rapid and transient changes in $[Ca^{2+}]_c$ levels. The duration of these transients seems to be unique for each signal. As described previously, this feature may help to account for the specificity in the physiological responses triggered by changes in $[Ca^{2+}]_c$.

A remarkable degree of independence in the $[Ca^{2+}]_c$ response to particular stimuli has been observed. For example, repetitive exposure to wind rapidly causes total loss of further $[Ca^{2+}]_c$ transients, but the $[Ca^{2+}]_c$ response of these signaled plants to cold shock is completely unaffected. The effect of specific inhibitors on responses to these two signals is also entirely different (Knight et al., 1992). Similar observations have been made using combinations of heat shock, touch, cold, and oxidative stresses.

The full potential of aequorin as a tool to understand how changes in $[Ca^{2+}]_c$ can effect signal transduction is only beginning to be realized. Transformed plants in which aequorin has been targeted to nuclei, chloroplasts, rough ER, and the tonoplast membrane have been constructed. Experiments on these plants confirm that specific combinations of organelles respond to each specific $[Ca^{2+}]_c$ signal. Hence, these signals have been termed calcium signatures.

The aequorin procedure also enables measurements of $[Ca^{2+}]_c$ to be made continuously for many weeks. In both white and red light, circadian oscillations of $[Ca^{2+}]_c$, which disappear in darkness, have been detected. By contrast, circadian oscillations in chloroplast Ca^{2+} are initiated by the onset of darkness (Johnson et al., 1995). The differences between $[Ca^{2+}]_c$ oscillations in these two compartments may form the basis of a possible mechanism for photoperiodic timing (see Kreps and Kay, 1997, in this issue).

Experiments Measuring $[Ca^{2+}]_c$ in Single Cells Threaten a Paradigm Shift

The photolysis of loaded caged Ca^{2+} and caged IP_3 in guard cells caused stomatal closure in each case in which $[Ca^{2+}]_c$

exceeded a threshold value of 500 nM (Gilroy et al., 1990). Thus, guard cells contain the necessary signal transduction sequence that leads from changes in $[Ca^{2+}]_c$ to the regulation of turgor and therefore underpins the stomatal aperture-controlling mechanism (Ward et al., 1995). However, reports from different laboratories (e.g., Gilroy et al., 1991; McAinsh et al., 1992) indicated that treatment of guard cells with ABA concentrations that were sufficient to cause closure in every case initiated $[Ca^{2+}]_c$ elevations only in a limited number of guard cells, with positive responses varying from 30 to 80% of those examined. Later experiments indicated that this range could be extended to anywhere from 0 to 100%, depending on plant growth temperatures (Allan et al., 1994). The percentage of individual guard cells in which $[Ca^{2+}]_c$ exceeded 500 nM was even lower. Furthermore, the kinetic changes in $[Ca^{2+}]_c$ in the cells that did respond were unique to that cell (Trewavas, 1991). How is this information to be interpreted? An appropriate explanation requires the application of the holistic systems perspective we described in the introductory section.

There is inherent "noise" during the complex process of development. Moreover, guard cells do not develop synchronously in leaves, either as a result of the timing of their initiation or as a result of differing environmental conditions. For these two reasons, each mature guard cell contains its own unique complement of signal-transducing proteins. As discussed above, cellular processes result from the integration of all of these signaling proteins into a complex network, with the cell coordinating the activities of these interacting pathways to achieve a state best described as optimum stability. Thus, the topological surface we described earlier can be conceived as folding into an epigenetic transduction landscape of hills and valleys in which overall strain is minimized. The epigenetic landscape in each guard cell will be different because each contains its own unique complement of proteins. Consequently, the transduction track followed by each cell as it navigates this landscape will be different from that of any other guard cell, even though the end point, stomatal closure, is identical.

In some guard cells, this transduction track may not require changes in $[Ca^{2+}]_c$ because prior folding of the landscape obviates the need to navigate this region to effect stomatal closure. However, the absence of $[Ca^{2+}]_c$ changes does not mean that Ca^{2+} -dependent proteins and processes are not used. Ca^{2+} -dependent proteins are an integral part of the epigenetic landscape. If they are removed by inhibitors, then the landscape will refold into a different configuration—one that prevents signal transduction and stomatal closure altogether.

Experimentally, the landscape can also be forced into a new topology by manipulating $[Ca^{2+}]_c$ —one merely has to provide sufficient perturbation to trigger stomatal closure. When plants are grown under different environmental conditions, the complement of guard cell proteins will be specifically altered and their epigenetic landscapes will change, as will all of their signal transduction tracks. Thus, cells that

under one set of environmental conditions exhibit no ABA-induced $[Ca^{2+}]_c$ changes may do so under a different set of conditions, and the number of positively responding guard cells could increase.

Clearly, this holistic perspective gives rise to a radical conclusion: it is the cell that determines the kinetics of $[Ca^{2+}]_c$ appropriate for closure, not the $[Ca^{2+}]_c$ kinetics that dictates closure to the cell.

Other experimental data confirm the conclusions drawn from studies of guard cells. For example, only 75% of etiolated wheat leaf protoplasts exposed to red light exhibited $[Ca^{2+}]_c$ elevations, but all increased their volume by 20 to 30% (Shacklock et al., 1992), and each protoplast exhibited a unique calcium signature (Fallon et al., 1993). Photolysis of loaded caged Ca^{2+} and caged IP_3 increased the volume in all protoplasts measured. The relevance of $[Ca^{2+}]_c$ to cell volume changes was also demonstrated by identifying specific proteins that were phosphorylated after photolysis of caged Ca^{2+} and observing that their phosphorylation state was altered after treatment with red light (Fallon et al., 1993).

Moreover, Neuhaus et al. (1993) and Bowler et al. (1994) microinjected Ca^{2+} and cGMP into epidermal and subepidermal cells of tomato hypocotyls and observed the formation of chloroplasts and anthocyanin in <5% of the cells they examined. These experiments demonstrate that individual cells are sensitive to changes in these second messengers, but the low percentage of cells that actually respond suggests that the appropriate growth and experimental conditions for high numbers of responsive cells have not been identified.

Oscillations, Waves, and Other Novel Aspects of $[Ca^{2+}]_c$ Signaling

In pollen tubes, growth is tightly associated with a tip-focused gradient of $[Ca^{2+}]_c$ that drops from 2 mM to 200 nM over the first 10 to 20 μm from the tip (Pierson et al., 1996). The gradient results from a clustering of Ca^{2+} channels at the tip of the pollen tube, with localization of Ca^{2+} -ATPase pumps in the shaft of the tube (Malhó et al., 1995). Dissipation of this gradient, either by raising (Franklin-Tong et al., 1993; Malhó et al., 1994) or lowering (Pierson et al., 1994) $[Ca^{2+}]_c$, results in the inhibition of tube growth.

When the pollen tubes recover and resume growth, they do so in a random direction. Recovery is concurrent with the formation of a tip gradient of Ca^{2+} channel activity. This activity induces a highly localized hot spot of $[Ca^{2+}]_c$ that predicts the new apex and the new direction of growth. Thus, the growth of pollen tubes is strongly polarized and is underpinned by the spatial separation of channels and pumps.

However, the gradient can also be modified either by the localized photolysis of caged Ca^{2+} on one side of the apical dome (Figure 2A) or by the application of ionophores or channel blockers via a microelectrode applied extracellularly near the tip (Malhó and Trewavas, 1996). Different clusters

of Ca^{2+} channels in the pollen tube tip may separately regulate orientation and growth. In these cases and others, the tube bends toward the zone of higher $[Ca^{2+}]_c$, which seems to be maintained by Ca^{2+} -induced Ca^{2+} release (CICR) while the tube is reorienting. A proposed model for this mechanism is depicted in Figures 2B to 2D.

CICR was first observed in guard cells (Gilroy et al., 1990) and may continue for many minutes after caged Ca^{2+} photolysis. Inositol phosphates may also be involved in CICR and pollen tube reorientation because pollen tubes contain a Ca^{2+} -activated phospholipase C (Franklin-Tong et al., 1996).

The gradient of $[Ca^{2+}]_c$ at the pollen tube tip oscillates quantitatively with a frequency of several minutes (Pierson et al., 1996), and tube growth can exhibit a similar oscillatory variation (Pierson et al., 1995). Oscillations in $[Ca^{2+}]_c$ were first described in coleoptile and guard cells (Felle, 1988; McAinsh et al., 1995) and have now also been observed in root hairs treated with Nod factors, which are rhizobial signaling molecules (Ehrhardt et al., 1996). Oscillations add a new dimension to $[Ca^{2+}]_c$ signaling because the frequencies of the resulting digital signals can be sensed, thereby providing a rich source of complex information to the cell. For example, mitochondria respond to IP_3 -induced $[Ca^{2+}]_c$ oscillations with sustained metabolic activation and discriminate against signals that result in simple amplitude increases in $[Ca^{2+}]_c$ (Hajnóczky et al., 1995).

Oscillations may originate from a continual filling and emptying of intracellular stores of $[Ca^{2+}]_c$. In animal cells, the ER is the primary store and is described as acting like a capacitor, hence the term "capacitative Ca^{2+} signaling" to describe $[Ca^{2+}]_c$ oscillations (Berridge, 1995). The pollen tube tip contains abundant smooth ER, and it is possible that the ER performs an equivalent capacitative function here. However, the vacuole is located toward the rear of the pollen tube and is unlikely to contribute to oscillations at the tip.

Wild plants are subjected to continuous but random environmental variations. The resultant noise is generally thought to be detrimental to the accurate perception of specific signals. However, recent investigations suggest instead that noise may considerably improve the perception of very weak signals. When coupled to an oscillator and a weak signal, noise can enable the stochastic crossing of a threshold above which signaling networks are activated and information flow is permitted (Douglass et al., 1993). This phenomenon, described as stochastic resonance, can be recognized by improved signal detection as noise is increased to an optimal value.

Oscillations of $[Ca^{2+}]_c$ may be coupled to the noise-induced stochastic opening of Ca^{2+} channels. In the presence of weak signals, transduction thresholds may then be periodically approached but not exceeded. Stochastic resonance results when noise increases yet further. More channels will open, thus enabling the critical signaling threshold to be stochastically and frequently crossed. Protein kinase cascades that regulate channel opening may also be used to amplify stochastic resonance. In this way, the perception of very

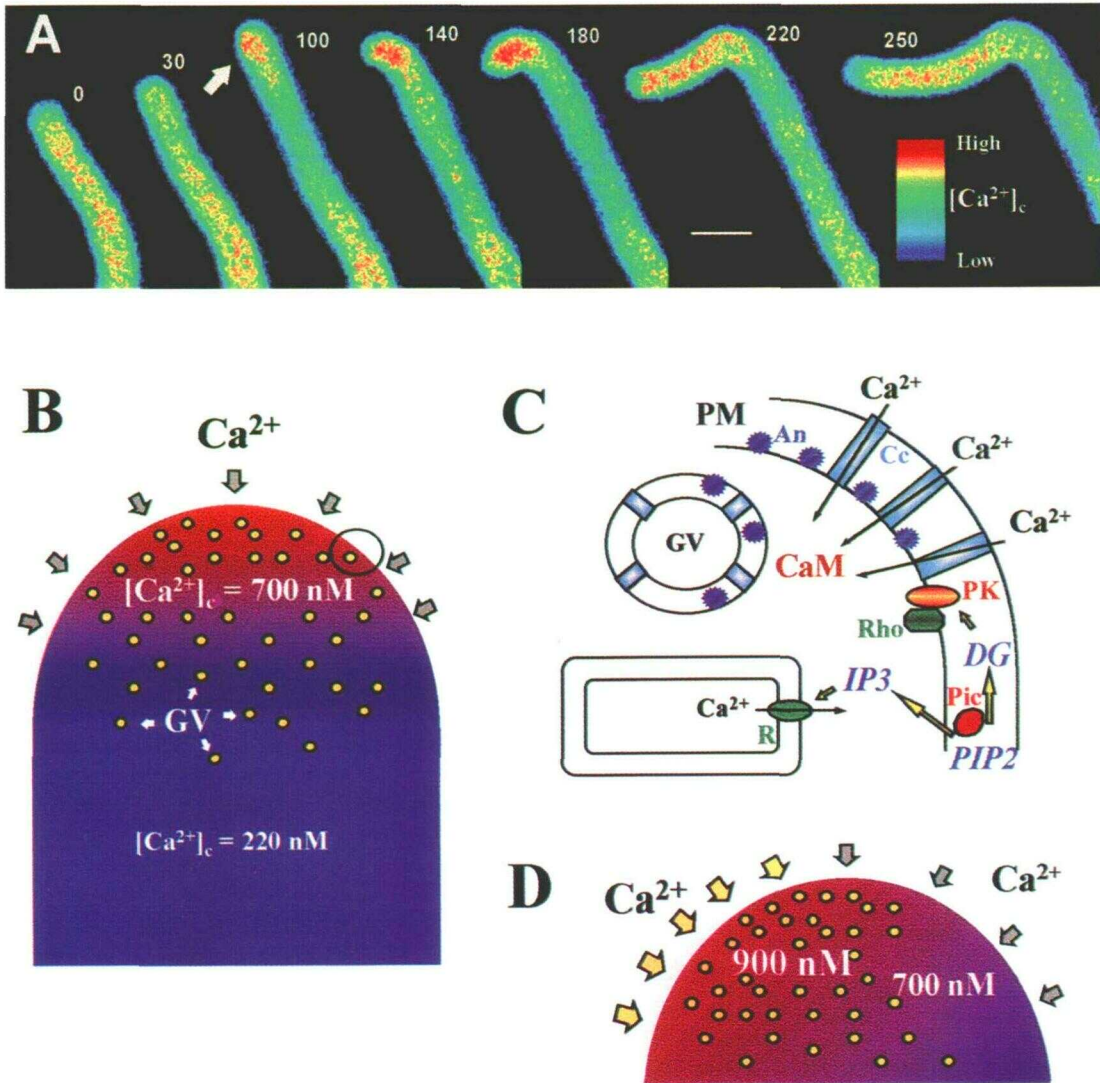


Figure 2. Mechanisms Underlying Pollen Tube Reorientation.

(A) Calcium movement during pollen tube reorientation. Shown is a time-course series of confocal images indicating Ca^{2+} movement after flash photolysis of caged Ca^{2+} in the left hemisphere of the apical zone at ~ 95 sec. The times (in seconds) at which images were taken are shown adjacent to the tip of the growing tube. A pronounced reorientation of the pollen tube growth axis follows the localized elevation in $[\text{Ca}^{2+}]_c$ in the left side of the tube apex. Color images are coded according to the scale shown. Bar = 10 μm .

(B) to (D) Proposed model for pollen tube reorientation.

(B) Apical region of a growing pollen tube. A higher but symmetric influx of Ca^{2+} in the extreme apex generates a tip-focused gradient of $[\text{Ca}^{2+}]_c$, and fusion of Golgi vesicles (GV). As a result, the tube grows straight.

(C) Detail of the circled region in **(B)**. Ca^{2+} enters the cell through Ca^{2+} channels (Cc) located in the plasma membrane (PM). The high levels of $[\text{Ca}^{2+}]_c$ in this region promote the association of annexins (An) with the plasma membrane, which in turn enhances the fusion of vesicles carrying wall construction material and further channels. This process may be regulated by protein kinases (PK) and Rho GTPases (Rho). The activity of kinases is regulated in turn by the phosphoinositide pathway. Perception of a reorienting stimulus increases the Ca^{2+} influx, and the signal is transduced to target molecules, such as calmodulin (CaM), through an IP_3 -dependent Ca^{2+} wave. DG, diacylglycerol; IP_3 , inositol 1,4,5-triphosphate; Pic, phospholipase C; PIP_2 , phosphatidylinositol 4,5-bisphosphate; R, IP_3 receptor.

(D) Detail of reorienting tip. After perception of a reorienting stimulus, Ca^{2+} influx at the tip becomes asymmetric, which leads to localized fusion of vesicles and modification of the pollen tube growth axis. The distribution of open and closed Ca^{2+} channels in the apical plasma membrane must be in a constant dynamic state and is probably regulated by many factors, such as tension, voltage, and intracellular Ca^{2+} stores. The possible role of the cytoskeleton, recently reviewed by Cai et al. (1997), is not depicted in this model.

weak signals in a noisy environment is made possible. Very weak blue light signals associated with guard cell opening and first positive phototropic curvature of coleoptiles and weak chemical signals controlling pollen tube orientation may be sensed with some accuracy when environmental variation, and thus noise input, increases. Indeed, endogenous $[Ca^{2+}]_c$ oscillations may simply be required to improve the recognition of weak signals that otherwise would be destroyed by noise (Gilroy and Trewavas, 1990).

$[Ca^{2+}]_c$ spiking that may represent stochastic resonance was observed by Sedbrook et al. (1996) in *Arabidopsis* roots subjected to anaerobic conditions. Excision of the root caused the spiking to disappear, suggesting that some form of $[Ca^{2+}]_c$ oscillation may be involved in tissue communication.

The importance of $[Ca^{2+}]_c$ oscillations in explaining various aspects of plant development can hardly be underestimated. Integration of the $[Ca^{2+}]_c$ oscillations of individual cells may invoke $[Ca^{2+}]_c$ rhythms of longer frequency that perhaps underpin the rhythmic movements of leaves and petioles. Experiments using channel blockers and calmodulin binding inhibitors support this proposal. Longer signal integration may also result in the circadian variations in $[Ca^{2+}]_c$ that have been described (Johnson et al., 1995). However, the appropriate production of such long-term variations may require the existence of pacemaker cells that oscillate strongly and coerce their neighbors into the same pattern of variation. The regions of intense luminescence (corresponding to high levels of $[Ca^{2+}]_c$) that we have observed at the tips of cotyledons of aequorin-transformed seedlings (N. Wood, H. Page, N. Read, and A.J. Trewavas, unpublished observations) may represent these proposed pacemakers.

There are many patterns in plant development that result from some basic oscillatory property of the tissue in which they originate. Good examples are in phyllotaxis, the periodic production of leaves along a stem, and the successive production of primordia-specifying petals, stamens, and other tissues of the flower (see Kerstetter and Hake, 1997, in this issue). The single-celled alga *Acetabularia* produces whorls of bractlike organs. This developmental phenomenon, which is clearly related to phyllotaxis, is dependent on changes in $[Ca^{2+}]_c$ (Goodwin, 1994).

In animal cells, Ca^{2+} waves are known to regulate meiosis, mitosis, fertilization, and secretion (Bock and Ackrill, 1995), and the documentation of free-moving Ca^{2+} waves in plant cells suggests that Ca^{2+} may regulate these important processes in plants as well. For example, Ca^{2+} waves were induced in pollen tubes either after photolysis of loaded caged IP_3 or by localized photolysis of caged Ca^{2+} in the vicinity of the nucleus and the associated rough ER (Franklin-Tong et al., 1996; Malhó and Trewavas, 1996). These Ca^{2+} waves, which traveled at speeds similar to slow Ca^{2+} waves in other organisms (Jaffe, 1993), were observed to trigger the reorientation of growth in *Agapanthus* pollen tubes and the cessation of tube growth in poppy as they moved toward the tip. The Ca^{2+} wave is clearly carrying information, which may be transduced via an IP_3 -based relay (Figures 2B

to 2D). Hypoosmotic signaling of *Fucus* has also been observed to induce a Ca^{2+} wave (Taylor et al., 1996).

It is possible that Ca^{2+} waves can move between cells to coordinate tissue activities. Indeed, Tucker and Boss (1996) have suggested that intercellular communication in stamen hair cells may be conveyed by IP_3 , and possible examples of tissue movement of $[Ca^{2+}]_c$ waves have been imaged using aequorin-transformed plants (Knight et al., 1993).

"I Am a Thousand Winds That Blow"—Protein Kinases

Post-translational modification of Ser, Thr, and Tyr residues by protein kinases and phosphatases is undoubtedly a major transduction route for many signals. These modifications afford the potential for extensive interaction and cross-talking among transduction pathways (Bourne, 1995). To date, >70 plant protein kinases that regulate many different aspects of metabolism, growth, and division and mediate second-messenger changes have been isolated and characterized (Stone and Walker, 1995). We expect that an order of magnitude more will be identified in the future; in fact, some estimates suggest that 2 to 3% of plant genes may encode protein kinases.

One of the most important groups of protein kinases comprises the MAPK cascade (Hirt, 1997; see Jacobs, 1997, in this issue). This cascade includes three distinct types of kinases: (1) MAPKs, which are Ser/Thr protein kinases activated by dual phosphorylation of Thr and Tyr residues by (2) bifunctional MAPK kinases, which in turn are activated by (3) MAPK kinase kinases, which are members of the Raf family. Representatives of each of these protein kinase families have been identified in plant cells, and the MAPK cascade is now implicated in the transduction of mechanical signals and signals triggered by gibberellin, ABA, ethylene, osmotic stress, wounding, auxin, fungal elicitors, and factors regulating cell proliferation.

The importance of this cascade is that it links initial stimuli to responses at the level of gene expression, usually via transcription factor phosphorylation. However, the stimulus perception end of the MAPK cascade is also of considerable interest. In animal cells, the cascade usually initiates at receptors coupled to GTPases, such as Ras, which, as discussed above, have also been implicated in plant signal transduction. At present, the precise start point of the MAPK cascade and GTPase transduction mechanism in plant cells is unknown. However, a number of disease resistance gene products are protein kinases (Hammond-Kosack and Jones, 1996; Wilson et al., 1997), suggesting that future research activity in this area will be intense. The likely complexity of the network of interactions between the MAPK cascade and other signal transduction pathways is indicated in Figure 3 (see also Pelech, 1996).

A second important class of protein kinases is the CDPK family (Harper et al., 1991). Members of this family are found attached to microfilaments and to the plasma membrane.

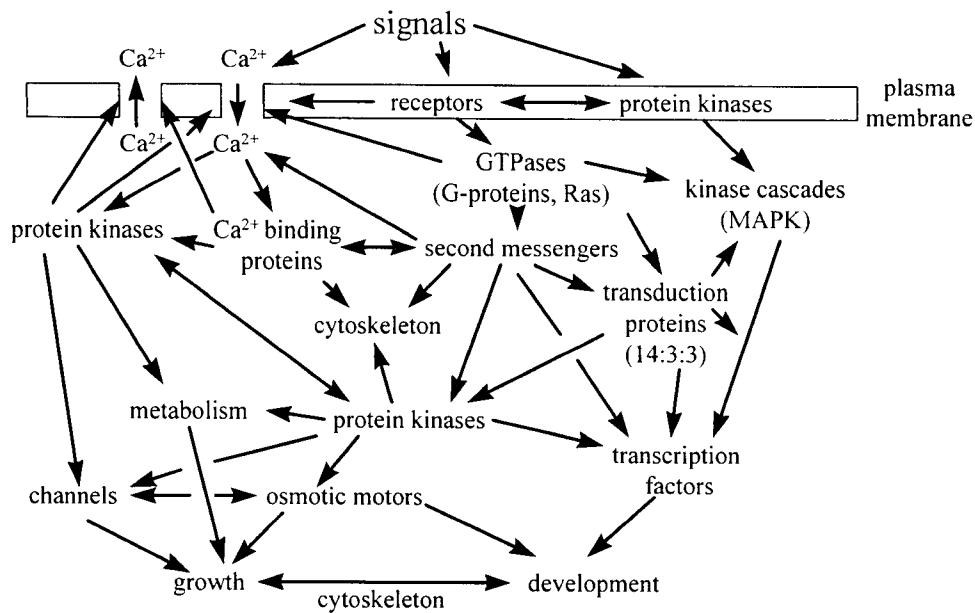


Figure 3. Present Understanding of the Signal Transduction Network in Plant Cells.

The figure indicates that signals can alter membrane potential, occupy and activate plasma membrane receptors, or modify plasma membrane protein kinases. For the sake of clarity, some important details, including soluble receptors, second-messenger synthesis, transduction proteins, and kinase cascades, have been omitted. Nevertheless, the figure indicates the extent of interactions that collectively constitute the signal transduction network.

CDPKs have a domain that is clearly related to calmodulin, and therefore, they do not require calmodulin for activation. The active site of CDPKs is normally bound to an autoinhibitory region that is removed when the kinase binds Ca^{2+} .

Several protein phosphatases have been detected in plant cells, and protein phosphatase inhibitors can have striking effects on growth and development, possibly through the inhibition of protein kinase cascades (Giraudat, 1995; Stone and Walker, 1995).

"IF YOU CAN DREAM AND NOT MAKE DREAMS YOUR MASTER"—THE FUTURE

Mechanical tension is one readily identifiable characteristic that may underpin the origin of plant form as organs are initiated at meristems. Growth occurs in meristems by cell division and expansion (see Clark, 1997; Cosgrove, 1997; Kerstetter and Hake, 1997; Schiefelbein et al., 1997, in this issue). During development, local stresses are generated because division, expansion, wall growth, and turgor changes do not occur synchronously. However, tissues are constructed from cells joined through their walls. Thus,

these stresses will eventually be propagated throughout the organ (Selker et al., 1992). Nodal points, representing small regions of high or low tension, will be generated. These could be used to specify the genesis of specific cells, tissues, or organs (Trewavas and Knight, 1994).

The outer cell walls of epidermal cells are thicker than their inner cell walls. The resulting differences in epidermal cell wall extensibility could act to increase the stress inside tissues by constraining growth (see Cosgrove, 1997, in this issue). Indeed, the tensions generated by epidermal cell walls can account for many of the fundamental shapes that are observed in plant form. For example, a less extensible epidermal outer wall around a whole organ would generate a sphere (Trewavas and Knight, 1994).

To understand how these physical stimuli trigger morphogenetic responses (i.e., changes in organ or plant shape), the corresponding signal transduction mechanisms need to be investigated further. More specifically, the sensitivity of plants to mechanical stimuli should be measured, and mutants affected in the perception and transduction of these signals should be isolated and characterized (Braam and Davis, 1990). There is already evidence that growing seedlings, in which growth-induced mechanical signaling is probably continuous, express exquisite sensitivity to even

slight mechanical stimulation (Knight et al., 1991). How is this sensitivity achieved?

Changes in $[Ca^{2+}]_c$ during mechanical signaling may be required to continually disrupt and respecify cytoskeletal structures. It has been proposed that the transduction of mechanical signals may operate through integrin-like proteins that are embedded in the wall, traverse the plasma membrane, and join to microfilaments through vinculin-like and talin-like proteins (Ingber, 1991). Although this is an attractive model, there is little evidence for the presence of true plant analogs of these animal proteins. A group of membrane-associated methyl guanylate kinase proteins, which may be important in connecting environmental influences to intracellular signaling pathways and the cytoskeleton, have recently been identified in animals (Anderson, 1996); it will be interesting to test whether similar proteins may also be present in plants.

We cannot emphasize too strongly that much of the specificity in signaling derives from the restricted spatial distribution of cellular signaling components. Uncontrolled activation, which would result from second messengers navigating throughout the forest of kinases and phosphatases, would wreak havoc on the cell. Indeed, mechanisms that anchor kinases and phosphatases to the cytoskeleton through particular protein subunits are being uncovered rapidly (Faux and Scott, 1996). Are these the first indications that signal transduction takes place in large protein complexes, entities that may be termed "transducons"?

What would make up a transducon and how would it function? We speculate that transducons could consist of an aggregate of receptors, channels, bound calmodulin, protein kinases and phosphatases, and Ca^{2+} -ATPases; they may be specifically positioned within a cell; they may operate as on/off switches in the metabolic circuitry—a form of hard wiring of the cell that ensures the localized specificity of a response. Transducons could be coupled together in feedforward or feedback loops by such mobile components as second messengers, mobile (receptor) protein kinases in membranes (Braun and Walker, 1996), substrate proteins, or 14:3:3 proteins (Sehnke and Ferl, 1996). Such interactions would help to improve the rate and accuracy of information transfer and processing. Transducons could also act as integration and information storage facilities for the cell, characterizing its history and providing a chemical memory of past experience (Bray, 1995). Using this analogy, the phenotype of a plant represents the indissoluble linkage between genes, which can be thought of as a kind of read only memory (ROM), and the signaling networks, which are the biological equivalent of random access memory (RAM). The keys are punched by environmental and developmental cues.

The 14:3:3 proteins are a recently identified group of proteins whose function seems to be to join together other transduction proteins. 14:3:3 proteins exist as a family of at least 10 different members, one of which, the so-called fusococcin receptor, controls plasma membrane ATPase activity through a specific Ca^{2+} -dependent and fatty acid-depend-

ent protein kinase (Van der Hoeven et al., 1996). In addition, 14:3:3 proteins inhibit nitrate reductase activity, thus directly linking nitrate metabolism to signaling pathways, as was proposed many years ago (Trewavas, 1983). In animal cells, 14:3:3 proteins activate Raf, other protein kinases, and phosphatases; new functions for plant 14:3:3 proteins should be identified soon.

Finally, it seems likely that those who investigate the details of tissue-specific gene expression, the production of form, metabolism, and cell growth and division will increasingly find that signaling constituents play important roles in the process. Continuous signaling is an integral component in the establishment and maintenance of cellular identity; we can no longer meaningfully separate it from gene expression, protein synthesis, or any other elements or constituents that in former times were thought in some way to be more fundamental to development.

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