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Signal Transduction in Vascular Plants

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Abstract. We review current evidence for the presence and activity in plants of several paradigmatic components of transmembrane signal transduction systems. Components considered include the second messengers calcium, inositol 1,4,5-trisphosphate, and cyclic AMP; protein kinases and protein phosphatases; and G-proteins. At the current stage of development of the field of plant signal transduction, broad similarities between plant and the more well-studied animal systems are apparent. However, there also exist considerable differences in detail.

Like all organisms, vascular plants face a wide variety of changeable environmental conditions, including light, gravity, wind, temperature, predators, pathogens, and moisture. The genius of plants is that, unlike mesozoa, they are able to cope with variable conditions without resorting to locomotion. Partial solutions to many problems are inherent in the anatomy and physiology of all plants but active responses to changing conditions are necessary as well. Plants must coordinate developmental processes to produce structural preparations for and responses to environmental eventualities, as well as adapt to changing conditions on a more rapid time scale. Active responses to both normal and plastic developmental control take place at the cellular and subcellular levels via recognition of intercellular and intracellular signaling molecules.

Mechanisms by which intracellular signaling molecules exert their effects on cells are generically known as *signal transduction* which implies, by analogy to electronic transducers, the linkage of a number of components that convert information from one form to another. Signal transduction is best understood in animal systems. In one animal paradigm, signal transduction involves perception of the signal (for example, a peptide hormone) at the plasma membrane, leading directly or indirectly to the release of "second messengers" inside the cell. Changes in the intracellular concentration of second messengers affects the biochemical status of the cell, either directly, by affecting enzyme activity, or indirectly, by affecting protein kinase or phosphatase activity. Phosphorylation or dephosphorylation then modulates the activity of cellular enzymes. In this review, we will focus on the events that take place within cells following perception of stimuli in the form of extracellular messengers. Plant hormone receptors, hormone mutants, control of gene expression, and the effects of phytochrome are the subjects of other reviews in this volume and will generally not be discussed here.

Because many aspects of signal transduction in animals are understood in considerable detail, we will begin with a description of signal transduction paradigms derived primarily from research on animals. Research on animal signal transduction is driven by questions about neurotransmission, muscle action, inflammation, immune responses, vision, carcinogenesis, and hormone action, among many others. Although there is little obvious relationship between these phenomena and the activities of plants, it has been reasonably and widely assumed that there is room for similarity in terms of the molecular and biochemical mechanisms that underlie responses to extracellular signals. Arguments for such assumptions include evolutionary relationships as well as the fundamental biochemical similarities between all organisms (Janssens 1988; Palme 1992; Westheimer 1987). Some important issues must be borne in mind in this context, however. First, plants are indeed different from animals in a great many ways. Some differences of rele-

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Table 1. Selected differences between animals and plants of relevance to signal transduction.

Animals	Plants
Most rapid physiological responses: milliseconds	Most rapid physiological responses: seconds or minutes
Many highly specialized cell types	Fewer specialized cell types
Peptide hormones mostly	Peptide hormones rarely
Active circulatory system	Passive circulatory system
Centralized hormone production	Except meristems, relatively decentralized hormone production
Motile	Sessile
Deterministic development; adult architecture fixed	Most cells remain totipotent; adult architecture plastic
No cell wall	Cell wall
Principal energy storage in glycogen/fat	Principal energy storage in sucrose/starch
Nervous system	No nervous system

vance to signal transduction are listed in Table 1. In addition, animal responses depend upon *systems* of biochemical events in which each component depends upon the presence of every other component. Many elements of animal signal transduction chains make no sense out of the context of the system of which they are a part. For example, 3′,5′-cyclic adenosine monophosphate (cAMP) is an extremely important signaling molecule in a variety of evolutionarily divergent groups, and is part of a system that includes receptors, linkages to adenylate cyclase, and cAMP-dependent protein kinase. Because cAMP is generally accepted to have no widespread signaling role in vascular plants (see below), one would not expect to find key elements of that system in plants, except where involved with routine metabolism. It appears, based on analysis of eukaryotic microbes, that animals and plants contained similar signal transduction systems when they diverged in evolution (Janssens 1988). However, there is little other a priori reason, beyond biophysical realities, to expect animal signal transduction systems to have simple parallels with signal transduction systems in plants or other distantly related organisms.

With these issues in mind, we will initially describe in intentionally general terms important components of well-understood (primarily animal) signal transduction systems. The details of the systems themselves will be left to the numerous excellent reviews that also give a greater sense of the degree of complexity possible. After introducing each nonplant paradigm we will consider biochemical and molecular evidence for the activity of each element in plants, and then discuss examples of possible roles, if any, for each element in plant responses to extracellular stimuli.

Second Messengers

Because many intercellular messengers in animal systems are large and/or hydrophilic, a considerable amount of attention has focused on the plasma membrane (PM), which forms a barrier between such messengers and the cellular processes they modulate. Imbedded in the PM are specific receptors that initially interact with signaling molecules and begin the process of signal transduction across the membrane by stimulating the production or release of "second messengers" inside the cell. We will describe the primary second messengers of animal systems and their possible presence in plants, leaving aside the question of the extent to which plant growth regulators may exert their effects at the PM.

Calcium

Two important second messengers in animal systems are calcium and cyclic AMP. Changes in calcium levels are involved in muscle contraction, fertilization, glycogen synthesis and breakdown, and many other processes. Ion channels in the PM and the endoplasmic reticulum (ER) that respond to various stimuli including inositol 1,4,5-trisphosphate (IP_3) mediate the flux of calcium in animal cells, while Ca^{2+}-ATPases maintain a low cytosolic calcium concentration. A primary mechanism by which Ca^{2+} affects enzyme activity involves the binding of Ca^{2+} to calmodulin (CAM), a protein which itself has no enzymatic activity, but which acts by binding to other proteins in response to Ca^{2+}. Important targets of Ca^{2+}-CAM regulation include protein kinases. Calcium signaling pathways also interact with cyclic AMP signaling pathways. This interaction, or "crosstalk," between the systems allows a very complex modulation of responses. Cyclic AMP in plants will be discussed below.

Interest formed in the 1980s regarding the possible involvement in Ca^{2+} in signal transduction in plants (for reviews, see Hepler and Wayne 1985; Gilroy et al. 1987). An important role for Ca^{2+} in plant signaling initially was supported by circumstantial evidence such as the effects of inhibitors and chelators (Hepler and Wayne 1985). With the demonstration of the presence of calmodulin, Ca^{2+}-dependent protein kinases (Roberts and Harmon 1992; see section on kinases), Ca^{2+} channels (Johannes et al. 1991), and elements of an inositol

phosphate system (see next section), the case became much stronger. Additional evidence linking changes in Ca^{2+} levels and signal transduction has come from analysis of Ca^{2+} levels and flux between the cytosol and extra- and intracellular Ca^{2+} stores. This has been made much more quantitative by the use of fluorescent Ca^{2+} indicator dyes and the tools of electrophysiology (Trewavas and Gilroy 1991).

In animals, membrane-bound intracellular stores are one very important source of Ca^{2+}, which is released in response to the binding of inositol 1,4,5-trisphosphate (IP_3) to receptor channels. Ca^{2+} is normally present in the plant cytosol at a concentration of less than 200 nM (Gilroy et al. 1990). Ca^{2+} concentrations in the cell wall ranges around 1 mM; because the surface of the PM is negatively charged, it has been speculated that Ca^{2+} concentrations there will be considerably higher than the average Ca^{2+} concentration in the wall (Drøbak 1992), however this has not been directly measured. The ER, which is the primary source of Ca^{2+} in intracellular signaling in animals, contains in plants approximately 10 μM Ca^{2+}. The highest concentration of Ca^{2+} inside plant cells is found in the vacuole, which contains 0.1–10 mM Ca^{2+} (Evans et al. 1991). Low plant cytosolic Ca^{2+} concentrations are maintained by Ca^{2+}-pumping ATPases and Ca^{2+}/nH^+ antiporters in the PM, ER, and tonoplast (Evans et al. 1991).

One promising area being examined by several laboratories is the role of Ca^{2+} in stomatal control. Stomata close in response to abscisic acid (ABA) when guard cells lose osmoticum (in the form of K^+) and become less turgid (see Tallman 1992 and Kearns and Assmann 1993 for reviews). Schroeder and Hagiwara (1989) used patch-clamp techniques to show that cytosolic Ca^{2+} levels in *Vicia faba* guard cells influence the status of PM K^+ channels. Effects of Ca^{2+} included inhibition of inward-rectifying K^+ channels and activation of voltage-dependent depolarizing anion channels. Using the fluorescent Ca^{2+} indicator dye Fura-2, McAinsh et al. (1990) found that application of extracellular ABA to *Commelina* guard cells triggered an increase in cytosolic-free Ca^{2+}, which was followed by stomatal closure. There is also evidence that stomatal movements are preceded by changes in cytosolic pH (Irving et al. 1992).

The notion that Ca^{2+} was involved in stomatal closure was supported by the experiments of Gilroy et al. (1990) who used a photolabile-caged form of Ca^{2+} to increase intracellular Ca^{2+} levels in guard cells. Release of Ca^{2+} upon photolysis was followed by a narrowing of the stomatal aperture. Release of a similarly caged form of IP_3 caused a rise in intracellular Ca^{2+} and subsequent stomatal narrowing (Gilroy et al. 1990). Blatt et al. (1990) used a similar approach with *Vicia* guard cells and found that release of caged IP_3 inactivates K^+ channels thought to be involved with K^+ uptake and initiates depolarization, which promotes K^+ efflux through another set of channels. Presumably, these effects are mediated by Ca^{2+} (Blatt et al. 1990), supporting the findings of Schroeder and Hagiwara (1989). Schroeder and Hagiwara (1990) suggest that Ca^{2+}-permeable ion channels present in the PM may be indirectly activated by ABA, rather than being ligand-gated ion channels that respond directly to ABA. Hormonal control of ion channel gating has recently been reviewed (Blatt and Thiel 1993).

Although the above experiments seem to indicate that Ca^{2+} is involved in stomatal control, the extent of the role of Ca^{2+} in stomatal control is called into question by other experiments. When guard cells of *Commelina* were treated with ABA in one set of experiments, stomatal closure occurred in 54 of 54 cells tested, but strongly elevated Ca^{2+} levels were observed in only four of 54 cells. Twenty-four cells showed no changes in Ca^{2+} levels (Gilroy et al. 1991). Schroeder and Hagiwara (1990) found effects of ABA on cytoplasmic calcium in only 37% of the cells studied. More work is necessary to sort out these apparent contradictions.

Adding to the confusion are results from barley aleurone. Aleurone responds to gibberellic acid (GA) by producing several hydrolytic enzymes, including α-amylase, which mobilize food reserves stored in the starchy endosperm of the seed. Calcium is required for this effect of GA, which is reversed by ABA (for review, see Jones and Jacobsen 1991). Use of Ca^{2+}-sensitive dyes showed that GA treatment was followed within 3–4 h by a threefold increase in Ca^{2+} in the peripheral cytoplasm of aleurone cells. Subsequent treatment with ABA was followed by reduction of cytoplasmic calcium levels (Gilroy and Jones 1992). This effect of ABA is the opposite of ABA's effect (when an effect is observed) on calcium levels in guard cells (cf. Gilroy et al. 1991).

Inositol 1,4,5-Trisphosphate

In animals, two important second messengers are derived from the membrane itself when receptors activate phospholipase C (PLC), an enzyme which is specific for inositol phospholipids. PLC cleaves the phosphodiester bond of phosphatidylinositol 4,5-bisphosphate (PIP_2) in the inner leaflet of the PM, releasing the products IP_3 and diacylglycerol (DAG). In animal cells, IP_3 is a primary effector of

Ca^{2+} release, interacting with receptors on intracellular Ca^{2+} storage sites to release Ca^{2+} into the cytoplasm (Berridge 1993). Increased intracellular Ca^{2+} leads to changes in the activity of a number of enzymes, some of which were discussed above. The role of DAG is to activate several subspecies of protein kinase C (PKC). PKC is discussed at more length in the section on protein kinases. DAG is also formed in mammalian cells by the action of phospholipase D on phosphatidylcholine and phosphatidylethanolamine, while the action of phospholipase A_2 (PLA_2) on phosphatidylcholine produces free fatty acids and lysophosphatidylcholine (Liscovitch 1992). A primary mechanism by which PLC is activated in animals involves G-proteins, which are themselves activated when receptors in the PM bind their ligands. (See the section on G-proteins for more detail.)

Research on the role of inositol phospholipid-derived molecules in plants has focused on several areas. These include demonstration of the presence of PIP_2 and IP_3 in plant cells, characterization of the enzymes of inositol phospholipid metabolism, and investigations on the effects of IP_3 and DAG in plant cells. Issues related to the role of IP_3 in plants are, of course, closely related to the effects of Ca^{2+}.

Phosphatidylinositol (PI) is about twice as abundant in plant membranes as it is in animal membranes, comprising 8%–15% mol in plant cell membranes. In contrast, the phosphorylated forms of PI, phosphatidylinositol 4-phosphate (PIP) and phosphatidylinositol 4,5-bisphosphate (PIP_2), which are so important in animal signal transduction, are present in much lower amounts in plants than in animals. This, along with technical difficulties and the presence of numerous interfering compounds and metabolic pathways, has hampered research in this area (for reviews see Boss 1989; Einspahr and Thompson 1990; Drøbak 1992, 1993; Coté and Crain 1992). In spite of these difficulties, it is now clear that vascular plants do contain PIP and PIP_2 (Coté et al. 1989; Irvine et al. 1989), as do algae (Einspahr et al. 1988; Irvine et al. 1989).

The enzymes of PI metabolism and IP_3 release, which catalyze the sequential phosphorylation of PI to form PIP and PIP_2, as well as the cleavage of IP_3 from PIP_2, are also present in plant cells. PI-4-hydroxy kinase and PIP-5-hydroxy kinase have been demonstrated to be present mainly in plant PM (Sandelius and Sommarin 1986; Sommarin and Sandelius 1988). In animals, these enzymes are associated with the cytoskeleton as well as with the PM. An association with the cytoskeleton has been recently shown in plants as well (Xu et al. 1992; Tan and Boss 1992). IP_3-specific phosphatases, necessary to help remove second messengers after signaling events, are also present in the cytosol (Martinoia et al. 1993). PLC activity has been reported to be associated with plant PM (Einspahr and Thompson 1990; Tate et al. 1989; Pical et al. 1992; Yotsushima et al. 1993). Thus, most or all of the components needed for the generation of an IP_3 signal are present in plants. Presumably these or similar enzymes are also involved in the metabolism of inositol phospholipids and phosphorylated inositols.

If products of PIP_2 hydrolysis are involved in signaling, then it is expected that changes in concentration of components of the phosphatidylinositol system should be detectable in response to stimuli. Changes in cytoplasmic Ca^{2+} in response to IP_3 should likewise be observable, as should changes in cell function. There have been several reports correlating changes in metabolism of PI cycle components with the application of different stimuli including light, auxin, fusicoccin, cytokinin, gibberellic acid, osmotic shock, and cell wall-degrading enzymes (reviewed in Drøbak 1992). Some of these reports have been subject to criticism on technical grounds (e.g., Boss 1989), and few appear to have been reproduced in other laboratories. This lack of replication is due in part to the complexity of the system as well as the use of a number of different research organisms. For example, at least four laboratories have looked at the effects of auxin using four different organisms, and have found effects on five different parameters, none of which were assayed by any other research group (Table 2 in Drøbak 1992). There have been reports that attempt to directly link release of caged IP_3 inside cells with changes in cell function, but these results are not as straightforward as they might appear. (See earlier section in this article on Ca^{2+}.) In addition, Moreau and Preisig (1993) suggest that treatments with materials such as fungal elicitor and cellulase may have less specific effects on membrane lipids than may be evident from the plant IP_3 literature.

It has been suggested that an IP_3 system is only functional in a limited number of often-stimulated cells, including guard cells, pulvini, and stem epidermal cells (Trewavas and Gilroy 1991). An additional possibility is that heterogeneity of plant tissues and asynchrony of responses act to compound quantitation problems which stem from low concentrations of IP_3 during a response and the small cytoplasmic volume of vacuolated cells (Drøbak 1992). Drøbak (1992) has calculated that typical plant cells might be expected to contain less than 0.2 amol (attomoles, 10^{-18} mol) of IP_3 during a response. The most sensitive assays for IP_3 operate in the sub-pmol range, necessitating the near-synchronous response of more than 10^6 cells for the production of a detectable signal even under opti-

mal conditions. This calculation underscores an unfortunate aspect of the use of guard cells as models for signal transduction research. Although it is possible to obtain relatively large quantities of guard cell protoplasts (Kruse et al. 1989), producing enough material for extensive classical biochemical analysis (i.e., hectogram or kilogram quantities) would be laborious, or more likely, impossible. This difficulty will impede important avenues of research in this area.

Cyclic AMP

As noted above, cyclic AMP and Ca^{2+} are two vital second messengers in animals. Levels of cAMP in animal cells are controlled by both adenylate cyclase, which interacts with a variety of PM-localized receptors and produces cAMP from ATP in response to extracellular stimuli, and phosphodiesterase, which degrades cAMP. As with Ca^{2+}, signaling by cAMP in animals culminates in activation of protein kinases, in this case the cyclic AMP-dependent protein kinases (PKAs).

Cyclic AMP has been demonstrated to function in signal transduction in diverse organisms including bacteria, eukaryotic microbes, and animals. With the discovery of a widespread role for cAMP in these organisms, there developed considerable interest in possible functions for cAMP in plants (Amrhein 1977). However, in spite of concerted efforts spanning many years, no such role for cAMP has yet been found.

The very presence of cAMP in plant tissues remains controversial. Spiteri et al. (1989) examined seeds and other tissues of five species of plants, at least three of which had been previously reported to contain cAMP (Table 1 in Brown and Newton 1981). Spitieri and coworkers used a highly sensitive radioimmunoassay technique, and carefully eliminated possible artifactual origins of cAMP. Levels of cAMP above the detection limit (<0.5 pmol/gFW) were found in only two of 14 tissues tested. cAMP in the two positive samples (imbibed and dry lettuce seeds) was apparently of microbial origin, as cAMP was undetectable in imbibed seeds which had been separated from their external tegument. Cyclic AMP levels in animals are on the order of 100–500 pmol/gFW (Robison et al. 1971; cited in Amrhein 1977). Reports identifying cAMP in plants appear from time to time (e.g., *Lemna,* Gangwani et al. 1991; maize, Janistyn and Ebermann 1992). Such recent reports evidently do not suffer from the technical problems that have plagued the field in the past (Amrhein 1977), but neither do they always include quantitative data. Most reliable accounts make it clear that plants, when they contain cAMP at all, contain much lower amounts than do animal tissues. As with IP_3, it has been suggested that the presence of cAMP in a minority of signaling cells in a plant tissue might be undetectable in a homogenate of the entire tissue (Trewavas and Gilroy 1991).

Although direct, unequivocal demonstration of cAMP in vascular plant tissues is problematic, several papers have suggested that DNA and protein sequence comparison data may indicate a role for cAMP in plants (Trewavas and Gilroy 1991). Working with tobacco, Katagiri et al. (1989) found two DNA-binding proteins, TGA1a and TGA1b, with homology to basic-leucine zipper regions of mammalian nuclear factors including CREB (cyclic AMP response element binding protein) and two others. DNA binding by CREB is thought to be modulated via phosphorylation by cAMP-dependent protein kinase (PKA), protein kinase C, and/or casein kinase II (Gonzalez et al. 1989; Hunter and Karin 1992). Katagiri et al. (1989) suggest TGA1a and TGA1b may be involved in PKA-mediated signaling, although only TGA1b contains a consensus PKA phosphorylation site, KRXXS/T; in animals RRXS/T is most commonly used (Scott 1991). A third CREB-like sequence, VBP1, from *Vicia faba* contains neither PKA phosphorylation site (Ehrlich et al. 1992). Like TGA1a and TGA1b, VBP1 contains homology to the basic-leucine zipper region and can bind DNA. All three plant CREB-like proteins contain phosphorylation sites for casein kinase II (S/TXXE; Kemp and Pearson 1990), and numerous other serine and threonine residues, leaving open the possibility that DNA binding is modulated by kinases other than PKA. Examination of plant CREB-like sequences thus actually appear to provide evidence against the involvement of cAMP in plant signal transduction. Caution should be also used in drawing conclusions from the presence of CRE-binding sites adjacent to plant genes: of the many CRE-binding proteins, CREB is the only one that has been identified as a mediator of cAMP action (for reviews see Karin and Smeal 1992; Hunter and Karin 1992).

Another line of evidence that involves phosphorylation site specificity is the presence in petunia extracts of kinase activity capable of phosphorylating Kemptide, an artificial substrate often used to assay PKA activity (Polya et al. 1991). The petunia Kemptide kinase activity is responsible to bovine PKA regulatory subunit in a cAMP-dependent manner, but endogenous cAMP-responsive regulation was not observed. The lack of endogenous cAMP regulation could be the result of differential degradation of the regulatory subunit, or it could be in-

dicative of the presence of a cAMP-independent kinase with the same phosphorylation target sequence as PKA (Polya et al. 1991). It should be noted that PKA is not the only kinase capable of phosphorylating Kemptide (Kemp and Pearson 1990). Plants have been demonstrated to contain proteins that are responsive to phosphorylation by mammalian PKA, such as maize leaf sucrose-phosphate synthase (Huber and Huber 1991) and phosphoenolpyruvate carboxylase (Terada et al. 1990). Endogenous kinases capable of phosphorylating these plant proteins are apparently cAMP-independent; phosphorylation site specificity for these kinases is unknown.

There are recent reports that suggest the involvement of cAMP in some plant responses, indicating continued interest in this topic. One example is the down-regulation of elicitor-induced phenylalanine ammonia lyase (PAL) in suspension cultures of *Phaseolus vulgaris* (Bolwell 1992). In this work, down-regulation of PAL was observed in response to added forskolin, an activator of adenylate cyclase activity in animal cells. Addition of both elicitor and forskolin led to increased levels of cAMP of two- to sevenfold over the basal level of around 4 pmol/gFW (Bolwell 1992). The suggestion by Morsucci et al. (1991) that cAMP may be involved in stomatal movement in *V. faba* is based on transmission electron microscope cytochemistry and incubation of epidermal peels in cAMP solutions. Concentrations of 0.1–0.5 mM cAMP led to maximum stomatal apertures. However, given the low cAMP levels present in plants, it could be argued that the levels of cAMP used by Morsucci et al. (1991) were not physiological.

Pending further investigations of the possible second messenger regulation of the Kemptide kinase and other aspects of cAMP in vascular plants, it must be stated that even after considerable efforts evidence for the widespread involvement of cAMP in signal transduction is extremely limited, or negative. The presence or role in plants of other cyclic nucleotides, such as cGMP, have received far less attention that cAMP, and their involvement cannot yet be ruled out. There is a need for a comprehensive, authoritative, impartial, and critical review of this subject.

Protein Phosphorylation

It would be difficult to overemphasize the degree to which protein kinases are now known to be involved with the functioning of animal cells (Fischer and Krebs 1989). Indeed, our understanding of the presence and roles of protein phosphorylation in animal cells has grown so vast that the topic is rarely if ever reviewed as a whole. Although this review cannot be comprehensive, published reports on phosphorylation in plants will still fit in a few, if bulging, file folders. Progress toward an understanding of phosphorylation in plants began as it did in animals, with biochemical approaches. Recently molecular techniques have greatly aided the search.

Protein Kinases

Protein kinases are a large and diverse group of phosphoryltransferases which transfer the terminal phosphate from ATP (or, less commonly, GTP) to substrate proteins. Covalent phosphorylation induces conformational changes in the structure of enzymes, modulating their activity (Sprang et al. 1988).

Protein kinase C (PKC) and PKA are the best understood of the many protein kinases which have been described in animals. There are 10 subspecies of PKC, comprising three main groups. The PKC subspecies differ in their response to a number of activators, including DAG, Ca^{2+}, phosphatidylserine, lysophosphatidylcholine, and *cis*-unsaturated fatty acids. Not all PKC subspecies are activated by DAG or Ca^{2+}, but all require phosphatidylserine. PKC is also activated by limited proteolysis which renders the enzyme Ca^{2+}-independent (Nishizuka 1988). PKA is activated by the presence of cAMP. Inactive PKA consists of two catalytic subunits complexed with two cAMP-binding regulatory subunits. The catalytic subunits are activated when the regulatory subunits bind cAMP and dissociate. As with the PKC family, there are multiple forms of PKA, and further complexity is possible because there also exists multiple forms of the regulatory subunit. For example, in humans there are three genes for catalytic subunits and four genes for regulatory subunits (for review see Scott 1991).

Most initial attempts to identify protein phosphorylation activity in plants focused on cAMP-regulated phosphorylation, which has still not been demonstrated in vascular plants. The first biochemical evidence for cAMP-independent protein phosphorylation in plants was the work of Ralph et al. (1972). By 1976 there was sufficient evidence for and interest in phosphorylation in plants to warrant a review of protein modification by phosphorylation (Trewavas 1976). This review provides a snapshot of an early phase of both animal and plant kinase research and is a useful source of perspective. Regulation of phosphorylation in early reports was at the substrate level (i.e., histone-specific, casein-specific, etc.). The first reports of protein kinase

activity modulation at other than the substrate level involved Ca^{2+}-stimulated protein kinase activity in pea shoot membranes (Hetherington and Trewavas 1982) and zucchini hypocotyl membranes (Salimath and Marmé 1983). Soluble Ca^{2+}/calmodulin-activated kinase activity was reported in wheat germ extracts (Davies and Polya 1983), and phosphorylation of specific proteins in corn was shown to be Ca^{2+}-sensitive (Veluthambi and Poovaiah 1984). Reports of Ca^{2+}-stimulated phosphorylation were particularly interesting because of the potential importance of changes in cytoplasmic Ca^{2+} to developmental and metabolic changes in plant cells (Hepler and Wayne 1985). It has now been confirmed that phosphorylation occurs in all parts of plant cells including nuclei, mitochondria, plastids, and microsomal membranes (Ranjeva and Boudet 1987).

Interest in Ca^{2+}-stimulated phosphorylation in soybean (Putnam-Evans et al. 1986) led to the discovery of a Ca^{2+}-activated protein kinase which was calmodulin-independent (Harmon et al. 1987). The soybean protein kinase was purified (Putnam-Evans et al. 1990), and subsequently microsequenced and cloned (Harper et al. 1991). The deduced amino acid sequence for the clone demonstrated that it was a member of a unique class of protein kinases, the Ca^{2+}-dependent protein kinases (CDPK). This type of kinase appears to be unique to plants and protists, and has yet to be demonstrated in an animal species (Roberts 1993; Harper et al. 1993). The CDPKs are characterized by a structure that includes an N-terminal kinase catalytic domain contiguous with a C-terminal calmodulin-like Ca^{2+}-binding domain (Harper et al. 1991). Monoclonal antibodies to soybean CDPK have been produced (Putman-Evans et al. 1990) and have been found to cross-react with proteins from many organisms and subcellular locations including corn, *Mougeotia, Tradescantia,* onion, *Chara,* oat and zucchini PM, and soybean root nodules (Roberts and Harmon 1992). Immunological results must be interpreted with caution because the antibodies recognize an epitope near the nucleotide binding site in a region that is fairly well conserved in all kinases (Alice Harmon, University of Florida, personal communication). Thus, sequence analysis is currently the sine qua non for identification of a kinase as a CDPK-type (Harper et al. 1993). CDPKs have been recently comprehensively reviewed (Roberts and Harmon 1992; Roberts 1993).

In addition to CDPK, which can respond to Ca^{2+} without the agency of calmodulin, a putative Ca^{2+}/calmodulin-dependent protein kinase has been recently cloned from apple (Watillon et al. 1993). The sequence, CB1, was identified by screening an expression library for the ability to bind ^{125}I-labeled calmodulin. CB1 shares significant sequence homology to rat Ca^{2+}/calmodulin protein kinase II in both the kinase catalytic and calmodulin-binding domains (Watillon et al. 1993). Thus, plants may contain at least two different routes by which changes in Ca^{2+} levels may effect changes in phosphorylation.

Because of the important functions present in the PM, as well as by analogy to animal signal transduction systems, it has long been assumed that the PM would be the site of some degree of protein kinase activity. This supposition was supported by reports of protein kinase activity associated with PM of pea bud (Hetherington and Trewavas 1984), suspension-cultured ryegrass (Polya et al. 1984), oat root (Schaller and Sussman 1988; Schaller et al. 1992), silver beet leaf (Kulcis and Polya 1988), corn root (Ladror and Zielinski 1989), and zucchini hypocotyls (Verhey et al. 1993). Several of these reports involved Ca^{2+}-stimulated kinase activity (Hetherington and Trewavas 1984; Schaller and Sussman 1988; Schaller et al. 1992; Verhey et al. 1993).

An enzyme presumed to be responsible for at least part of the calcium-stimulated kinase activity in pea PM was purified (Blowers et al. 1985) and found to have several curious characteristics. Its size (18 kDa) was considerably smaller than known protein kinases, it displayed rapid cycling of autophosphorylation, and it could apparently phosphorylate PIP to form PIP_2 (Blowers and Trewavas 1989). A sugarcane enzyme with very similar molecular weight and biochemical characteristics has recently been demonstrated to be a nucleoside diphosphate kinase (Michael Harrington, University of Hawaii, personal communication).

Protein kinases from the PM of other species have been characterized as well. Oat root PM contains a 79 kDa lipid-activated, calcium-stimulated protein kinase that cross-reacts with antibody to soybean CDPK (Schaller et al. 1992). Upon limited digestion with trypsin, the 79 kDa kinase is converted into a smaller, soluble, lipid-independent polypeptide (unlike PKC; see below). There have been several reports of similar soluble and PM-associated protein kinases (Kulcis and Polya 1988; Klimczak and Hind 1990). PM from various organs of zucchini contain a number of calcium-requiring protein kinase subspecies, some of which cross-react with anti-CDPK antibody (Verhey et al. 1993; Verhey and Lomax, unpublished data). This biochemical demonstration of multiple protein kinases with similar characteristics is predicted by molecular data indicating the presence of a gene family (Harper et al. 1991).

Biochemical approaches to identifying protein kinases have the advantage that they identify enzymatically active kinases, but technical difficulties can make the biochemical route arduous.

Analysis of the nucleic acid and amino acid sequences of a great many nonplant protein kinases has made molecular approaches and crucial sequence comparisons possible in plants, since protein kinase sequences contain invariant amino acid residues in each of eleven conserved subdomains (Hanks et al. 1988).

Knowledge of conserved kinase sequences has been used to clone several putative protein kinase cDNAs. For example, an abscisic acid-inducible transcript encoding a putative protein kinase (PKABA1) has been isolated from wheat embryos by screening a library derived from ABA-treated tissue with a probe produced by kinase-specific PCR (Anderberg and Walker-Simmons 1992). PKABA1 transcripts were dramatically induced when plants were subjected to dehydration stress or to low levels of ABA. The phosphorylation state of several proteins in carrot somatic embryos is apparently affected by treatment with abscisic acid (Koontz and Choi 1993). The first plant protein kinase to be cloned using sequence information from a purified protein kinase was soybean CDPK (Harper et al. 1991). CDPK clones have also been obtained from carrot by probing a cDNA library with degenerate oligonucleotides (Suen and Choi 1991) and from *Arabidopsis* by probing a cDNA library with part of the soybean gene for CDPK (Harper et al. 1993).

Several putative protein kinase sequences have been isolated for which the cloning strategy or subsequent complementation experiments gave hints as to possible functions. An elegant approach was used by Feiler and Jacobs (1991) to clone the gene for a pea homologue of the eukaryotic cell cycle regulatory kinase *cdc2*. PCR products generated by use of *cdc2*-specific primers with a pea cDNA library were ligated into sites flanking a deletion derivative of yeast *cdc2* in an *Escherichia coli* expression vector. A monoclonal antibody was used to screen immunoblots for the reconstitution of the deleted region containing the epitope recognized by the antibody. Genomic Southern analysis indicated the *cdc2* sequence was present in a single copy in pea (Feiler and Jacobs 1991). The pea *cdc2* has been used to identify a cDNA clone from alfalfa (*Medicago sativa*), called MsERK1 (Duerr et al. 1993). MsERK1 bears considerable sequence homology to a class of animal kinases known as mitogen-activated protein (MAP) or extracellular signal-regulated kinase (ERK) that participates in control of cell proliferation.

Site-directed mutagenesis suggests the activity of expressed MsERK1 may depend on phosphorylation of a tyrosine residue, a regulatory mechanism similar to that found in *Xenopus,* mouse, and rat cells (Duerr et al. 1993). If confirmed in vivo, this result would represent the first example of the effect of tyrosine phosphorylation on enzymatic activity in plants. There have been other hints that plants contain tyrosine kinases, including APK1, a protein kinase identified among protein kinase-specific PCR products from *Arabidopsis* (Hirayama and Oka 1992). When the APK1 sequence was expressed in *E. coli,* in which phosphotyrosine is not normally found (Letwin et al. 1988), several bacterial proteins were found to cross-react with anti-phosphotyrosine monoclonal antibody. No such proteins were present in uninduced cells, suggesting APK1 was the source of the tyrosine phosphorylation. However, no tyrosine phosphorylation could be observed in vitro using APK1, casein, or enolase as substrates (Hirayama and Oka 1992).

Several plant kinases have been described that have characteristics of putative receptor kinases. In animals, receptor kinases are a class of transmembrane proteins in which the extracellular domain is associated with recognition of and binding to extracellular signaling molecules, while the intracellular domain is generally a tyrosine kinase. The plant kinase ZmPK1, identified by kinase-specific PCR of corn cDNAs, was the first putative protein kinase sequence with a transmembrane sequence to be found in plants (Walker and Zhang 1990). The catalytic domain resembles *raf-1,* a soluble serine/threonine kinase from human, and also human epidermal growth factor receptor, which is a membrane-spanning tyrosine kinase. The extracellular domain of ZmPK1 is strikingly similar to the extracellular domain of S-locus glycoproteins (SLG) found in *Brassica* species. In the Brassicaceae, SLGs are thought to be involved in the expression of self-incompatibility in pistils (Ebert et al. 1989). However, in maize ZmPK1 mRNA is expressed primarily in shoots and roots of seedlings, and to a reduced degree in silks. Southern analysis indicated the presence of a ZmPK gene family (Walker and Zhang 1990).

The suggestion that a protein kinase might be involved with the regulation of self-incompatibility was extended when sequence analysis of SLG clones from *Brassica oleracea* identified regions with kinase catalytic domain homology in polypeptides with membrane-spanning regions (Stein et al. 1991). The S-receptor kinase (SRK) catalytic-like and SLG-like domains were each found to hybridize to similar-sized transcripts from pistil and, to a lesser extent, anther. Unlike ZmPK1, the *Brassica*

sequences were not detected in leaf or shoot tissue (Stein et al. 1991). Similar sequences have been cloned from *Arabidopsis* and *B. napus* (Tobias et al. 1992; Goring and Rothstein 1992). The *A10* allele of an SRK from a self-compatible *B. napus* has recently been shown to encode a message with a 1-bp deletion, causing a frameshift which is predicted to lead to premature termination of translation (Goring et al. 1993). This supports the concept that a functional SRK gene is required for expression of self-incompatibility.

A fourth report of a plant protein kinase sequence with a transmembrane region involves a protein with an extracellular domain resembling the leucine-rich repeat motif found as part of the extracytoplasmic domains of a number of mammalian membrane proteins, rather than the S-protein homologous domains of ZmPK1 or the SRK sequences (Chang et al. 1992). Only a few transmembrane serine/threonine kinases have been reported from animal species; the majority phosphorylate only tyrosine (Ullrich and Schlessinger 1990).

Much interest has centered on the question of whether plants contain protein kinase C. There have been reports of protein kinases that cross-react with antibody to bovine brain PKC (Elliott and Kokke 1987), as well as reports of kinases that are activated by Ca^{2+} and fatty acids (Lucantoni and Polya 1987). Protein kinase activity in zucchini microsomes is stimulated by platelet-activating factor, a phospholipid (Scherer et al. 1988). However, no plant kinases have yet been identified that meet the biochemical definition for PKC. PKC activity is phosphatidylserine- and (usually) calcium-dependent. The presence of diacylglycerol dramatically increases the affinity of PKC for Ca^{2+}; limited digestion with trypsin or calcium-dependent protease activates the enzyme and renders it totally independent of Ca^{2+} (Kikkawa and Nishizuka 1986). CDPK from *Arabidopsis* (Harper et al., 1993) and oat (Schaller et al. 1992) has been analyzed for lipid dependence and response to proteolysis. The *Arabidopsis* kinase is stimulated by lysophosphatidylcholine and phosphatidylinositol; phosphatidylcholine and phosphatidylserine had no effect. Calcium and lipid stimulated the enzyme synergistically (Harper et al. 1993). CDPK from oat is similarly activated by calcium and phospholipid. The presence of lipid has no effect on the affinity of the oat enzyme for calcium, and limited proteolysis converts the enzyme into a form that is lipid-independent but still calcium-dependent (Schaller et al. 1992). Thus, CDPK from both *Arabidopsis* and oat have biochemical properties that are dissimilar from those of PKC.

Protein Phosphatases

Much less research has been devoted to protein phosphatases than to protein kinases. This belies their importance, as the phosphorylation state of proteins reflects a balance between kinase and phosphatase activity, and dephosphorylation as well as phosphorylation affects enzyme activity. For example, spinach sucrose phosphate synthase is activated by dephosphorylation (Huber and Huber 1991). Four main classes (or types) of protein phosphatases have been identified in mammalian cells (for review, see Cohen 1989). Phosphatase classes are differentiated on the basis of the effects of inhibitors and ions, and at least three of the classes have been found in plants. Type 1 phosphatases have been identified in pea and carrot (MacKintosh et al. 1991), *B. oleracea* (Rundle and Nasrallah 1992), and maize (Smith and Walker 1991). The *Brassica* and maize enzymes have been cloned (Rundle and Nasrallah 1992; Smith and Walker 1991). Phosphatase types 2A and 2C are present in carrot, pea, and wheat; type 2A is also present in *B. napus* (MacKintosh et al. 1991).

Phosphatase types 1 and 2A are very sensitive to the inhibitor okadiac acid, and the effects of this and other inhibitors have been exploited in efforts to link the activity of phosphatases to various cellular processes. For example, okadaic acid causes mitotic arrest in tobacco (Zhang et al. 1992). Application of okadiac acid and the phosphatase inhibitors microcystin-LR and microcystin-RR at specific points during the course of mitosis affects metaphase transit times as well as the pattern of sister chromatid separation in *Tradescantia* (Wolniak and Larsen 1992). A cDNA clone of an *Arabidopsis* type 1 phosphatase has been reported to be able to rescue the *Schizosaccharomyces pombe* mutant *dis2*-11, which fails to complete chromosome disjunction under nonpermissive conditions (Nitschke et al. 1992).

Role in Plants

Whereas considerable molecular and biochemical phenomenology is being collected, relatively few processes or enzymes are known to be controlled by phosphorylation in plants. Changes in protein kinase activity or phosphorylation have recently been correlated with a number of extracellular signals, including syringomycin (Bidwai and Takemoto 1987), blue light (Short and Briggs 1990), oligouronides (Farmer et al. 1991), cutin (Bajar et al. 1991), fungal elicitor (Schwacke and Hager 1992), and the elicitor α-aminobutyric acid (Raz and Fluhr 1993). Examples of processes or enzymes that may

be modulated by phosphorylation or dephosphorylation include self-incompatibility (Goring et al. 1993), initiation of mitosis (Li and Roux 1992, Duerr et al. 1993), isoprenoid biosynthesis (MacKintosh et al. 1992), cytoplasmic streaming (Tominga et al. 1987; McCurdy and Harmon 1992), sucrose phosphate synthase activity (Huber and Huber 1991), MsERK1 activity (Duerr et al. 1993), and phosphoenolpyruvate carboxylase activity (Bakrim et al. 1992).

In addition, plant growth regulators have been demonstrated to affect protein kinases, and phosphorylation in general. Transcription of one putative kinase sequence is stimulated by ABA (Anderberg and Walker-Simmons 1992), and phosphorylation of several proteins is stimulated by ABA (Koontz and Choi 1993). Treatment with ethylene stimulates phosphorylation in tobacco leaves (Raz and Fluhr 1993). Phosphorylation in oat coleoptiles is affected by auxin (Veluthambi and Poovaiah 1986).

It appears that kinases in plants, as in animals, are fundamentally involved with the regulation of cellular function. However, it is also becoming clear that there are important differences: there is no conclusive evidence to indicate that plants contain either PKA or PKC, which act together as well as independently to modulate many vital cellular processes in animals. In addition, while transmembrane kinases are present, they are thus far exclusively serine-/threonine-type kinases in plants. On the other hand, plants contain CDPK, a class of kinase that may not be present in animals. In the case of phosphatases, it appears that plants are similar to animals in that plants contain most or all of the major types of protein phosphatases.

G-Proteins

Guanine nucleotide-binding regulatory proteins (G-proteins) carry out a wide variety of regulatory roles, and their presence has been documented in organisms ranging from *E. coli* to mammals (for reviews see Simon et al. 1991; Hepler and Gilman 1992). G-proteins themselves are a diverse group, but all share the ability to undergo conformational changes in response to the binding of GTP or GDP which then affects the ability of G-proteins to interact with other proteins.

Small G-Proteins

One class of G-proteins, consisting of a single polypeptide chain, comprises the so-called *small G-proteins*. Small G-proteins are involved in functions including intracellular vesicle trafficking and secretion, cell growth and differentiation, protein synthesis, and cytoskeleton organization. One example is the well-known oncogene *ras*, which encodes a small G-protein (for review see Hall 1990, 1992). Genes with homology to *ras* have been found in *Arabidopsis* (Anai et al. 1991), and *Zea mays* contains genes with homology to the *ras*-related *ypt* gene family (Palme et al. 1992). The function(s) of these genes in plants has not yet been established.

Heterotrimeric G-Proteins

The second class of G-proteins includes the heterotrimeric G-proteins, which is the group most associated with transmembrane signal transduction (Simon et al. 1991; Hepler and Gilman 1992). Heterotrimeric G-proteins (more commonly simply called *G-proteins*) consist of three subunits, α (39–46 kDa), β (37 kDa), and γ (8 kDa). The β and γ subunits are present as a tightly associated complex that interacts with α as a unit. G-proteins are attached to the inner leaflet of the PM, via prenylation of γ and myristoylation of some species of α. The unstimulated G-protein complex includes a molecule of GDP, tightly bound to α. The presence of GDP maintains the affinity of α for $\beta\gamma$; when the complex makes contact with a hormone receptor that has a bound ligand, the rate of dissociation of GDP is increased. GDP is then exchanged for GTP, and α loses its affinity for $\beta\gamma$. The α subunit then interacts with an effector such as PLC, ion channels, or adenylate cyclase. There is growing evidence, at least in some cases, that $\beta\gamma$ interacts with the same or different effector as well. The α subunit possesses a constitutive GTPase activity. Once GTP has been cleaved to GDP the subunits reassociate, returning the system to the unstimulated state (Hepler and Gilman 1992). The rate of GTP hydrolysis thus determines the lifetime of the interaction between α subunits and their effectors.

Biochemical examination of multiple G-proteins, along with molecular cloning, has led to the identification of many different G-protein α subunits (Gα). All share the biochemical characteristics of GDP binding and GTP binding and hydrolysis. More than 30 genes for Gα have been cloned from animals (Simon et al. 1991). Analysis of the cDNA clones suggests that 20% of the amino acids are invariantly conserved, and that Gα fall into four main classes (Gα_s, Gα_i, Gα_q, and Gα_{12}). Each class consists of two or more subclasses, all of which differ in terms of various biochemical properties, including rate of GTP hydrolysis, nucleotide binding affinities, and susceptibility to ADP-ribosylation

by pertussis toxin (PTX) and cholera toxin (CTX). PTX dissociates susceptible G-proteins from their receptors, blocking signal transduction, while CTX activates G-proteins by inhibiting GTPase activity. By treating intact cells with PTX or CTX and examining the effect, evaluation of G-protein function and correlation with G-protein types and subtype is possible (Hepler and Gilman 1992). Different Gα also display different tissue distribution and receptor associations (Simon et al. 1991).

Over 100 G-protein-linked receptors have been described in mammals alone. With a single possible exception (Nishimoto et al. 1993), all G-protein-linked receptors described to date contain seven transmembrane domains. Distinct subtypes of receptors that respond differently to the same ligand have been identified. Because receptor subclasses can be coupled to different second messenger pathways and to ion channels, or, alternatively, multiple receptor subtypes can activate a single effector, very complicated signaling networks are possible (Simon et al. 1991). G-protein-linked receptors have not yet been identified in plants.

Initial clues to the presence of G-proteins in vascular plants came in the form of evidence of binding of [^{35}S] GTP-γS, a nonhydrolyzable analogue of GTP, to solubilized microsomal proteins from pea epicotyls (Hasunuma et al. 1987). A somewhat more thorough report of [^{35}S] GTP-γS binding to microsomal proteins from etiolated zucchini hypocotyls also provided the first immunological evidence of plant polypeptides resembling G-proteins (Jacobs et al. 1988). Two bands, at 50 and 33 kDa, were seen on Western blots probed with antibody to $G\alpha_s$. Kinetic analysis of binding showed an overall K_d of 300 nM; Scatchard analysis suggested the presence of more than one type of binding site (Jacobs et al. 1988).

Proteins with characteristics of animal Gα, such as [^{35}S] GTP-γS binding and antigenic similarity, were reported in a number of plants including *V. faba, Arabidopsis thaliana,* and *Commelina communis* by Blum et al. (1988). Antigenic similarity was based on cross-reactivity using antibody raised to the highly conserved nucleotide binding region of mammalian Gα subunits ($G\alpha_{common}$). Immunostainable plant proteins were described at 31–38 kDa, slightly smaller than the range of sizes of mammalian Gα (39–52 kDa; Stryer and Bourne 1986). Using a different approach to identifying GTP-binding proteins by molecular weight, Zbell et al. (1990) employed [^{35}S] GTP-γS overlays on nitrocellulose blots, and reported binding proteins with molecular weights of 24 and 28 kDa in soybean PM. Similar experiments with zucchini PM yielded a binding protein at 30 kDa (Perdue and Lomax 1992). The zucchini and soybean PM GTP-binding proteins are considerably smaller than the size range for known Gα subunits. Parallel experiments with membrane proteins from rat revealed a single [^{35}S] GTP-γS binding protein, at 28 kDa, indicating that Gα does not bind [^{35}S] GTP-γS under these conditions (Zbell et al. 1990). The soybean and zucchini [^{35}S] GTP-γS binding proteins are similar in size to small G-proteins (Hall 1990).

Conservation of Gα sequences allows the application of molecular approaches to questions about G-proteins in plants. Using PCR, a gene for a Gα homologue was cloned from *Arabidopsis* (Ma et al. 1990). This sequence was subsequently used to clone cDNA sequences for a related Gα from tomato (TGA1; Ma et al. 1991). The predicted sequences of the tomato and *Arabidopsis* proteins are very similar to one another, with 85% identical and 93% similar (identical plus conservative replacements; Ma et al. 1991). TGA1 is 27% identical and 51% similar to rat Gαs and 34% identical and 59% similar to the Gα of bovine transducin. The tomato sequence encodes a 44.9 kDa protein that includes all of the conserved regions characteristic of known Gα, as well as consensus myristoylation and cholera toxin ADP-ribosylation sites. Southern analysis of tomato and *Arabidopsis* genomic DNA indicate that the plant Gα sequences are each single-copy genes. Stringency of the tomato Southern experiment was such that sequences of >60% similarity were detectable. Known Gα sequences that are less than 60% homologous are considered to be in different classes, leaving open the possibility that tomato contains genes encoding members of other Gα classes (Ma et al. 1991). Indeed, lower stringency probing of *Arabidopsis* Southerns indicated other Gα sequences were present (Ma et al. 1990).

Role in Plants

As it became clear that plant cells contain GTP-binding proteins, workers began to try to link GTP-binding with application of extracellular stimuli or activation of effectors. Thus, Zaina et al. (1990, 1991) reported preliminary evidence of an effect by auxin on GTP-binding and on GTPase activity. Other laboratories have detected changes in phospholipase C and phospholipase A_2 activity in response to auxin (Ettlinger and Lehle, 1988; Zbell et al. 1989; André and Scherer 1991). In animals, PLC and PLA_2 activity are often controlled via G-proteins (Simon et al. 1991).

Ion channels are one class of effectors controlled by G-proteins in animals. Regulation of plant ion channels has recently been reviewed (Blatt and

Thiel,1993). Using patch-clamp experiments, Fairley-Grenot and Assmann (1991) provided evidence for G-protein regulation of potassium channels in guard cells of *V. faba*. Perfusion of the cytosol with 100–500 μM GDPβS, which binds tightly to Gα and locks G-proteins in the inactive GDP-bound state, resulted in an increase in inward-directed K^+ channel current. Treatment with GTP-γS, which locks G-proteins in the active GTP-bound state, had the opposite effect in that current was reduced. Thus, the results indicate that activation of a G-protein results in a decrease of inward K^+ current. Interestingly, both CTX and PTX decrease K^+ current (Fairley-Grenot and Assmann 1991). This is contrary to the expectation that CTX would decrease current (by activating a G-protein) and PTX would have either no effect or increase current, depending upon the nature of the upstream activation of the G-protein, that is, the type of receptor binding involved, and whether the Gα involved had a target site for PTX (Simon et al. 1991). The authors suggest the possibility of a PTX-sensitive G-protein with a function opposing that of the CTX-sensitive G-protein (Fairley-Grenot and Assmann 1991). Lee et al. (1993) have recently reported that pretreatment of epidermal peels of *C. communis* with PTX, or microinjection with GTP-γS into guard cells promotes stomatal opening. Further work will be necessary to reconcile this result with those of Fairley-Grenot and Assmann (1991).

There have been at least two reports of changes in G-protein behavior in response to extracellular stimuli. Working with PM from etiolated pea epicotyls, Warpeha et al. (1991) found that low-fluence blue light induces a GTPase activity 1–2 min after irradiation. A 40 kDa polypeptide present in the PM was recognized by antiserum directed against the Gα subunit of transducin, a vertebrate G-protein that couples the action of a photon of light on rhodopsin to the activation of cyclic GMP phosphodiesterase. CTX and PTX both ADP-ribosylated a protein with the same molecular weight as the immunostained pea protein. Interestingly, PTX had no effect on the 40 kDa protein in the presence of blue light and GTP, while CTX was effective only in the presence of blue light and GTP. Finally, using a nonhydrolyzable UV-crosslinkable analogue of GTP, a 40 kDa protein was found to bind GTP only in the presence of blue light (Warpeha et al. 1991). Given the characteristic structure of animal G-protein-linked receptors that recognize chemical signals, it will be interesting to elucidate the nature of the receptor that links blue light with a G-protein. It will also be interesting to see whether there is a role for cGMP in the blue-light response.

A second approach to the role of GTP-binding proteins in responding to extracellular stimuli involved the use of soybean suspension culture cells (Legendre et al. 1992). The soybean cells contained a 45 kDa protein that cross-reacted with antibody raised against the $G\alpha_{common}$ sequence. The antigen-binding fragment (F_{ab}) of the anti-$G\alpha_{common}$ antibody was delivered into the cell via receptor-mediated endocytosis, a process facilitated by biotinylation of the protein. When this was followed by treatment with the fungal elicitor polygalacturonic acid (PGA), cells responded by producing up to ten-fold more H_2O_2 than did controls involving heat-treated F_{ab} or biotinylated BSA. The elicitor response could also be triggered by treatment of the cells (in the absence of PGA) with mastoparan, a 14-residue oligopeptide from wasp venom. In mammalian cells, mastoparan activates G-proteins by mimicking the G-protein-recognition region of the activated receptor proteins. These results suggest that at least two G-proteins may be involved in the oxidative response to PGA: because introduction of F_{ab} into the cell, with the presumed neutralization of the 45 kDa G-protein, prolongs and intensifies the response to PGA, the authors suggest that this G-protein is involved in turning off H_2O_2 production. On the other hand, mastoparan, which directly activates G-proteins, may affect a G-protein involved in the initiation of the response to elicitor, since it activates the oxidative burst in the absence of elicitor (Legendre et al. 1992).

Compared to the situation with plant protein kinases, relatively little is known about G-proteins in plants. Still, even at this early stage in the development of this field, several cellular events have been linked with G-proteins. These include perception of blue light (Warpeha et al. 1991), response to auxin (Zaina et al. 1990), response to elicitor (Legendre et al. 1992), regulation of ion channels (Fairley-Grenot and Assmann 1991; Li and Assmann 1993), and stomatal opening (Lee et al. 1993). There is every reason to believe that the coming months will bring a rapid increase in our understanding the roles of G-proteins in plants.

Conclusion

The rate at which progress is being made toward understanding signal transduction in plants is increasing rapidly. However, it does not seem likely to match even early rates of progress on animal signal transduction. This is only partly because of the relatively small number of researchers arrayed against the problem or because of the difficulties of working with vacuolated cells surrounded by cell walls. Progress in animal systems has proceeded at

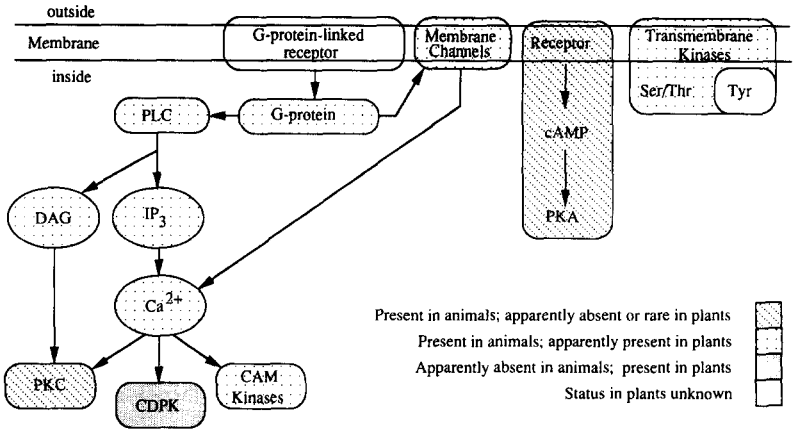

Fig. 1. A simplified, schematic diagram representing current knowledge of similarities and differences between plant and animal transplasma membrane signal transduction components. Abbreviations as per text.

a rate directly proportional to the availability of the experimental tissue. Thus, some of the earliest work on animal signal transduction utilized rabbit skeletal muscle (e.g., Krebs and Fischer 1956) and liver (e.g., Sutherland and Cori 1956). Insights gained from abundant tissues were subsequently helpful in beginning to sort out signaling in other, more precious tissues such as rod outer segments from bovine retina and hair cells from bullfrog inner ear. It would have been difficult to proceed in the opposite direction, yet that is what appears to be necessary in plant signal transduction: most experimental tissues are relatively difficult to obtain in pure form. Comparisons with animal systems, which were initially expected to substitute for abundant tissues, have certainly been helpful, but it is now clear that there are important differences between plant and animal signal transduction (Fig. 1), just as there are differences between plant and animal physiology (Table 1). In spite of the current lack of a rabbit skeletal muscle equivalent in the plant world, these are exciting times to be involved with research on plant signal transduction.

If considered at the lowest level of resolution, then it is clear that plants contain, in some form, the major components of signal transduction systems found in many organisms ranging from bacteria to mammals. For example, phosphorylation–dephosphorylation is known to control the activities of a number of plant enzymes, as well as heterotrimeric small G-proteins are present, and plant cells appear to possess the machinery for generation of several different second messengers. Looked at more closely, however, certain differences become striking: at this time, plants do not appear to contain a signaling pathway based on cyclic AMP; nor do they appear to contain an enzyme regulated precisely like PKC; plants do contain CDPK, an enzyme that may not be utilized by animals. It is much too early to draw conclusions based on these observations, but the observations do have important consequences. At this stage of the development of our understanding of plant signal transduction, we have not yet identified the variety in transduction systems that would lead to a robust network of signaling pathways, finely tuned by cross-talk between the pathways. Such cross-talk is a crucial part of well-understood animal models. If cross-talk is part of the mechanism by which a handful of plant growth regulators are able to have diverse effects in different tissues, then it is to be expected that one or more fundamental signaling pathways are yet to be discovered.

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