### Title

Signal transduction networks and the biology of plant cells.




### Journal

Biological research, 32(1)

### ISSN

0716-9760



### Authors

Chrispeels, M J
Holuigue, L
Latorre, R
et al.



### Publication Date

1999

Peer reviewed

*Biol Res 32*: 35-60, 1999

# Signal transduction networks and the biology of plant cells*

MAARTEN J CHRISPEELS[1**], LORETO HOLUIGUE[2], RAMON LATORRE[3], SHENG LUAN[4], ARIEL ORELLANA[3**], HUGO PEÑA-CORTES[5], NATASHA V RAIKHEL[6], PAMELA C RONALD[7] & ANTHONY TREWAVAS[8]

[1]Department of Biology, University of California San Diego, La Jolla, CA 92093-0116, USA
[2]Department of Molecular Genetics and Microbiology, Catholic University of Chile, Santiago, Chile
[3]Department of Biology, Faculty of Science, University of Chile, Santiago, Chile
[4]Department of Plant and Microbial Biology, University of California Berkeley, Berkeley, CA 94720, USA
[5]Department of Biology, Faculty of Chemistry and Biology, University of Santiago de Chile, Santiago, Chile
[6]DOE Plant Research Lab, Michigan State University, East Lansing, MI 48824-1312
[7]Plant Pathology Department, University of California Davis, Davis, CA 95616-8680
[8]Institute of Cell and Molecular Biology, University of Edinburgh, Edinburgh, United Kingdom

## ABSTRACT

The development of plant transformation in the mid-1980s and of many new tools for cell biology, molecular genetics, and biochemistry has resulted in enormous progress in plant biology in the past decade. With the completion of the genome sequence of *Arabidopsis thaliana* just around the corner, we can expect even faster progress in the next decade. The interface between cell biology and signal transduction is emerging as a new and important field of research. In the past we thought of cell biology strictly in terms of organelles and their biogenesis and function, and researchers focused on questions such as, how do proteins enter chloroplasts? or, what is the structure of the macromolecules of the cell wall and how are these molecules secreted? Signal transduction dealt primarily with the perception of light (photomorphogenesis) or hormones and with the effect such signals have on enhancing the activity of specific genes. Now we see that the fields of cell biology and signal transduction are merging because signals pass between organelles and a single signal transduction pathway usually involves multiple organelles or cellular structures.

Here are some examples to illustrate this new paradigm. How does abscisic acid (ABA) regulate stomatal closure? This pathway involves not only ABA receptors whose location is not yet known, but cation and anion channels in the plasma membrane, changes in the cytoskeleton, movement of water through water channels in the tonoplast and the plasma membrane, proteins with a farnesyl tail that can be located either in the cytosol or attached to a membrane, and probably unidentified ion channels in the tonoplast. In addition there are highly localized calcium oscillations in the cytoplasm resulting from the release of calcium stored in various compartments. The activities of all these cellular structures need to be coordinated during ABA-induced stomatal closure.

For another example of the interplay between the proteins of signal transduction pathways and cytoplasmic structures, consider how plants mount defense responses against pathogens. Elicitors produced by pathogens bind to receptors on the plant plasma membrane or in the cytosol and eventually activate a large number of genes. This results in the coordination of activities at the plasma membrane (production of reactive oxygen species), in the cytoskeleton, localized calcium oscillations, and the modulation of protein kinases and protein phosphatases whose locations remain to be determined. The movement of transcription factors into the nucleus to activate the defense genes requires their release from cytosolic anchors and passage through the nuclear pore complexes of the nuclear envelope.

This review does not cover all the recent progress in plant signal transduction and cell biology; it is confined to the topics that were discussed at a recent (November 1998) workshop held in Santiago at which lecturers from Chile, the USA and the UK presented recent results from their laboratories.

* This paper contains a summary of research results and relevant background information that was presented at a US-Chile workshop on Plant Cell Biology and Signal Transduction, held in Santiago de Chile November 14-16, 1998. The workshop was organized by Maarten Chrispeels and Ariel Orellana. Support for the workshop was provided by the National Science Foundation (USA), the Fundación Andes, Conicyt, Fundación para Biología Celular, and the University of Chile

The authors are listed alphabetically.

** Corresponding author: Dr. Maarten J. Chrispeels, Department of Biology, University of California San Diego, La Jolla, CA 92093-0116, USA, e-mail: mchrispeels@ucsd.edu, or Dr. Ariel Orellana, Departamento de Biología, Facultad de Ciencias, Las Palmeras 3425 - Casilla 653, Universidad de Chile, Santiago, Chile, e-mail: aorellan@abello.dic.uchile.cl

Received 26.3.99. Accepted 14.4.99

## I. THE EXTRACELLULAR MATRIX: STRUCTURE, BIOGENESIS AND ROLE IN SIGNALING

### *1. The extracellular matrix or cell wall of the plant cell is a multicomponent structure.*

We currently view the cell wall as a three-dimensional network of cellulose microfibrils and cross-linking polysaccharides (hemicellulose) embedded in a gel matrix of acidic polysaccharides rich in galacturonic acid (pectins) and held together by calcium bridges. The two major groups of flowering plants, the monocots and the dicots, differ in their hemicellulosic polymers; monocots have arabinoxylans, and dicots have xyloglucans (see Carpita and Gibeaut, 1993). This network is enmeshed with a variety of structural proteins and glycoproteins and partially cross-linked by aromatic substances (monolignols). Enzymes needed for the biosynthesis or modification of the network (*e.g.*, peroxidase, xyloglucan endotransglycosylase, α-fucosidase, glucanase) or for the modification of metabolites (*e.g.*, invertase, phosphatase, ascorbic acid oxidase) may be ionically or covalently bound to the hemicellulose network or the pectin matrix.

Assembly of the cell wall requires the coordination of the activities of large cellulose synthase complexes on the outer face of the plasma membrane with those of many different glycosyltransferases in the Golgi that assemble the pectin and hemicellulosic polymers. These polymers are transported to the plasma membrane in secretory vesicles, as are the cell wall proteins and glycoproteins, and their cross-linking is catalyzed by cell wall enzymes. In spite of this cross-linking, the walls of young cells remain flexible and can extend through breaking and reforming of cross-links to accommodate the enormous volume changes that characterize the growth of plant cells. In highly differentiated cells such as xylem vessels or fibers, the walls become thick, stiff and incompressible after impregnation with lignin, a polymer that is formed extracellularly by polymerization of secreted monolignol subunits (see Boudet, 1998).

Cell differentiation is accompanied by the synthesis and secretion of different components of the ECM of plant cells, as visualized by the interaction of monoclonal antibodies against cell wall epitopes. This process has recently been studied in Zinnia cells. When exposed to the hormones auxin and cytokinin, Zinnia mesophyll cells transdifferentiate into tracheary elements. This transdifferentiation is accompanied by a new pattern of gene expression and changes in the repertoire of proteins and polysaccharides at the cell surface (Stacey *et al*, 1995).

### *2. The extracellular matrix is a source of signals for development and defense.*

Two types of cell wall macromolecules appear to generate signals for plant cells: the arabinogalactan proteins and the hemicelluloses. Arabinogalactan proteins (AGPs) are extracellular or plasma membrane-anchored proteins that are typically more than 90% carbohydrate. The polypeptide backbone has a domain that is rich in Ala, Ser, Thr, and Hyp, and the carbohydrate moiety consists of short arabinosides attached to Hyp and arabinogalactans O-linked to Hyp or Ser. The arabinogalactan sidechains have 30 to 150 sugar residues, and most have a pure ß(1→3)-D galactan backbone with terminal L-arabinofuranosyl residues on short sidechains (Du *et al*, 1996). AGPs have been shown to promote the differentiation of single, densely cytoplasmic carrot cells into somatic embryos. The AGPs are not produced by the cells that differentiate into embryos, but need to be present in the culture medium or produced by neighboring cells (McCabe *et al*, 1997).

Completely different signals, small oligosaccharides of 5-15 sugar residues, are involved in defense responses and in the loosening of the hemicellulosic network that permits plant cells to grow in size as a result of their internal osmotic pressure. Oligosaccharides involved in defense reactions result from the breakdown of polysaccharides by plant or fungal enzymes. These oligosaccharides bind to receptors

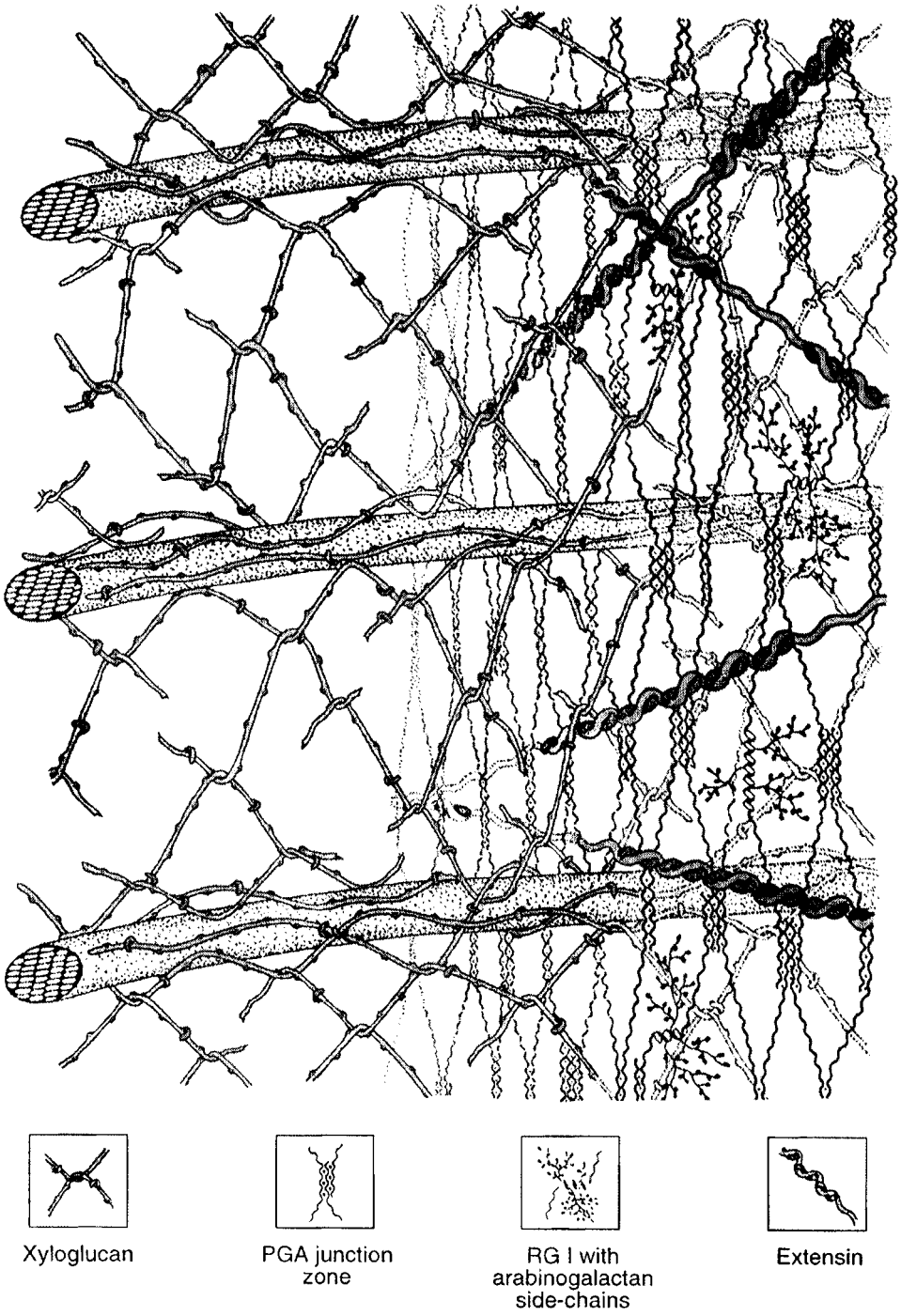


*Figure 1.* Model of a type I cell wall in the process of expanding. A model of possible alterations in structure that first permit microfibril separation and then lock them into place. Microfibrils of a single layer align parallel in a helical arrangement around elongating cells. Xyloglucans can separate from the microfibrils under the action of expansins and be cleaved by glycosidases or endo-transglycosylases. When displacement caused by turgor pressure within the cell is complete, extensin molecules, inserted radially, interlock the separated microfibrils to arrest further stretching. Additional proteins may also be inserted to cross-link extensin, forming a heteropeptide network. Formation of intramolecular covalent bonds among the individual wall proteins signals the end of elongation. From Carpita and Gibeaut, 1993, reprinted by permission of the publisher, Blackwell Science.

on the plasma membrane and this signal is transduced to the nucleus, where a battery of plant defense genes are induced (see below) (Hahn, 1996). Oligosaccharides involved in growth are the result of the action of endoglucanases on the cell wall xyloglucans. They are broken down by exoglucanases and may provide substrates for xyloglucan endo-transglycosidases that are thought to modify the xyloglucan network.

### *3. A gene that encodes a subunit of cellulose synthase has at last been identified.*

Cellulose is a linear polymer made of ß-1,4 linked glucose units. In the primary cell wall the degree of polymerization reaches over 2,000. Fifty to 80 of these linear polymers lie in parallel and bind tightly to each other through hydrogen bonds, creating a microfibril. Electron microscope studies of freeze-fractured membranes provided the first suggestion that a rosette-shaped protein complex located in the plasma membrane may be involved in cellulose biosynthesis. Purification of the protein(s) involved in the synthesis of cellulose proved to be difficult and was unsuccessful at least for plants. However cellulose synthetase was purified successfully from extracts of *Acetobacter xylinum,* a bacterium that synthesizes cellulose. This allowed the cloning of the gene that encodes the catalytic subunit of cellulose synthase. Use of this gene as a probe to search for homologous genes led to the identification of putative cellulose synthases in plants. Analyzing the Acetobacter sequence and the structure of the gene and hydrophobic cluster analysis allowed the identification of a signature sequence (D...D...D...QxxRW) that is present in a group of processive ß-glycosyltransferases, which could be present in cellulose synthases from other organisms as well (Saxena *et al,* 1995). This information helped Delmer's group to identify two ESTs from cotton, obtained by random sequencing of a cottonhair cDNA library, as the first two potential genes encoding cellulose synthase from higher plants (Pear *et al,* 1996). Although direct evidence that these two genes indeed encode cellulose synthase was not provided, all the circumstantial evidence strongly supported this conclusion.

The identification of a gene that encodes a cellulose synthase of higher plants came from studies with *Arabidopsis thaliana.* Arioli *et al* (1998) identified after extensive screening an Arabidopsis mutant that has impaired cellulose synthesis. The phenotype of this mutant, radial swelling (rsw) of the roots mimics the response of wild type roots to cellulose synthesis inhibitors such as dichlorobenzonitrile. Genetic mapping allowed the identification of the locus RSW1 and the further cloning of the gene. Analysis of the RSW1 amino acid sequence showed that it contains the D...D...D...QxxRW motif and is homologous to the cel-A genes from cotton. The deficiency of cellulose in the RSW mutant could be complemented by transformation with the RSW1 cDNA.

The sequences encoded by these cellulose synthase genes have been used to search for homologous genes through out plant databases, and a number of different genes potentially involved in cellulose biosynthesis have been identified. Thus, a vast field of research to understand the molecu-

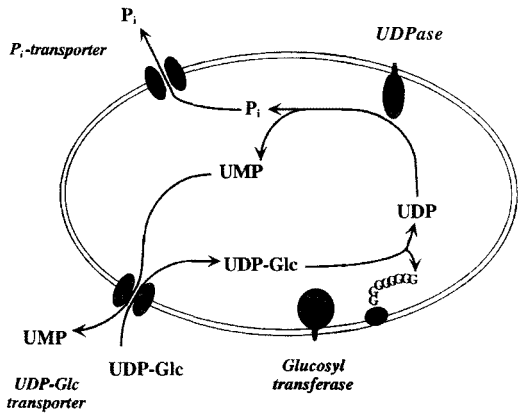


*Figure 2.* Proteins needed for the biosynthesis of a glucan in the Golgi. The cycle begins with the uptake of UDP-Glc by a transporter that also permits uridine monophosphate to leave. UDP-Glc is the substrate of a membrane-anchored glucosyltransferase that adds a glucose (G) to a growing chain, and produces UDP, which is cleaved by UDPase to produce $P_i$ and UMP. Whether the glucan is anchored to a protein (as shown) is not known.

lar basis of cellulose biosynthesis has just emerged.

*4. The biosynthesis of hemicellulosic polysaccharides and the carbohydrate moieties of proteoglycans and glycoproteins occurs in the Golgi apparatus.*

In contrast to cellulose biosynthesis, which takes place in the plasma membrane, hemicelluloses, pectin, and the glycosidic chains of proteoglycans are synthesized intracellularly in the Golgi apparatus (Driouch *et al*, 1993). The formation of polysaccharides in the lumen of the Golgi requires the following enzymes and transporters: (1) glycosyltransferases that use sugar nucleotide diphosphate as substrate and cause the polysaccharide chain to grow; (2) UDPases that degrade nucleotide diphosphate produced by the glycosyltransferases; (3) a transporter that removes inorganic phosphate; (4) a transporter that exchanges sugar nucleotide diphosphate for nucleotide monophosphate (see Fig 2). Little is known about the glycosyltransferases involved in this process, and so far the only enzyme that has been purified and cloned is the xyloglucan α-1,2-fucosyltransferase, an enzyme involved in the fucosylation of xyloglucan, one of the most abundant hemicelluloses in dicots (Perrin *et al*, 1999). The sequence of this enzyme predicts a single transmembrane domain close to the amino terminus, resembling the structure of mammalian glycosyltransferases that participate in protein glycosylation in the Golgi.

Topological analysis of glucan synthase I, an enzyme involved in xyloglucan biosynthesis, indicates that its active site is located in the lumen of the Golgi cisternae (Muñoz *et al*, 1996). Unpublished results from Orellana's and Mohnen's groups indicate that the active site of xyloglucan α-1,2-fucosyltransferase and polygalacturonate α-4-galacturonosyltransferase, enzymes involved in xyloglucan (hemicellulose) and homogalacturonan (pectin) biosynthesis respectively, would also be present in the lumen of Golgi cisternae.

The question is then, how do the nucleotide sugars get into the lumen of the Golgi cisternae? Studies using pea Golgi vesicles show that UDP-Glc can be transported into the lumen of Golgi vesicles by a protein mediated process (Muñoz *et al*, 1996). This membrane transporter seems to be specific for UDP-Glc; therefore it seems possible that different transporters would be responsible for the incorporation of the substrates used by the different Golgi glycosyltransferases involved in both polysaccharides and proteoglycans biosynthesis, as well as protein glycosylation.

After the transfer of the sugar into the oligosaccharide, the nucleoside diphosphate (UDP) is quickly transformed into UMP and inorganic phosphate by a Nucleoside Diphosphatase present in the lumen of the Golgi apparatus (Neckelmann and Orellana, 1998). The hydrolysis of UDP eliminates a potential inhibitor for the transferases and also drives the transfer reaction toward the polymerization of sugars. UMP leaves the Golgi cisternae by a process stimulated by UDP-Glc, suggesting that the UDP-Glc-transporter would be a UDP-Glc/UMP antiporter. On the other hand, inorganic phosphate is not accumulated in the cisternae and is transported out of the cisternae.

*5. The ECM-plasma membrane-cytoskeleton continuum plays a role in signal transduction.*

Recent evidence indicates that receptor kinases are important players in signal transduction pathways. Receptor kinases are proteins that have an extracellular domain that receives the signal and a cytoplasmic domain that transmits the signal. Some other plasma membrane anchored proteins also have large extracellular domains. For example, some AGPs are anchored by a GPI anchor. Recently a membrane-anchored glucanase was discovered that plays a major role in regulating cell elongation (Nicol *et al*, 1998). When plant cells are plasmolyzed, the plasma membrane does not pull evenly away from the cell wall, but the two remain in close contact at very specific adhesion points called Hechtian strands. These

Hechtian strands are not found when cells are incubated with short peptides that contain an Arg-Gly-Asp (RGD) motif. Isolated protoplasts of plant cells readily agglutinate when they are incubated with a genetically engineered protein that contains numerous RGD motifs, suggesting that plasma membranes contain RGD receptors. In animal cells, adhesion is mediated by plasma membrane receptors called integrins that interact with extracellular adhesion proteins (*e.g.*, fibronectin and vitronectin) via the tripeptide Arg-Gly-Asp (RGD), a motif that is conserved in adhesive proteins. On the cytoplasmic side of the membrane integrins interact with proteins of the cytoskeleton, thereby establishing the ECM-plasma membrane-cytoskeleton continuum. There is as yet very little evidence that in plants transplasma membrane proteins interact with the cytoskeleton, but we know that a large number of signals induce rapid cytoskeleton reorganization, leading to the suggestion that a similar continuum exists in plant cells.

## II. THE SECRETORY SYSTEM PLAYS AN IMPORTANT ROLE IN SORTING PROTEINS FOR DIFFERENT CELLULAR DESTINATIONS

### *1. Vacuolar targeting requires a specific vacuolar sorting signal.*

The secretory system is a series of organelles that includes the endoplasmic reticulum (ER), Golgi apparatus, plasma membrane and vacuole. Soluble proteins containing an N-terminal signal sequence are co-translationally inserted into the ER lumen, and after their synthesis is complete they are transported through the secretory system in transport vesicles that bud from one compartment and fuse with the next. From the ER, proteins are transported through the Golgi cisternae to the trans-Golgi network, where a major sorting event occurs. Proteins are either secreted to the outside of the cell (the default pathway) or they are targeted to the vacuole via a prevacuolar compartment, a route that is believed to be mediated by membrane-bound receptors to which the cargo proteins bind so that they can be properly sorted.

For a soluble protein to reach the vacuole, it must contain a specific vacuolar sorting signal. Three types of vacuolar sorting signals have been identified (Fig 3). Some proteins contain a propeptide, which comprises the sorting signal and which is removed from the protein upon deposition in the vacuole. Several proteins are known to contain a propeptide at their N-terminus, including sweet potato sporamin and barley aleurain (reviewed in Chrispeels and Raikhel, 1992). Deletion and mutagenesis studies have shown that the N-terminal propeptide is required for transport to the vacuole and have defined a conserved motif within the propeptides, which is essential for proper sorting and

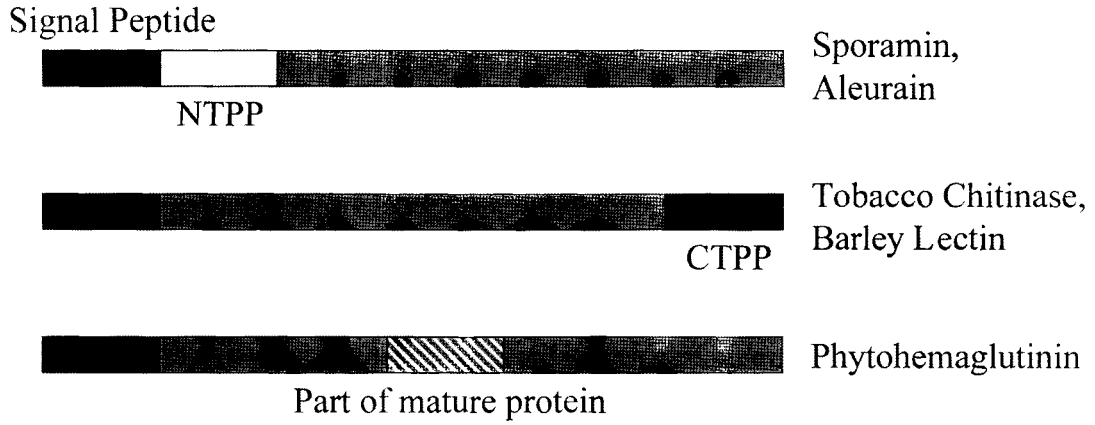


*Figure 3.* Location of vacuolar targeting signals in plant proteins. Vacuolar targeting signals can be propeptides at the C-terminus (CTPP) or the N-terminus (NTPP) of a protein, or be part of the mature protein.

thus is thought to be recognized by the sorting machinery.

The second type of propeptide signal is found at the C-terminus of several vacuolar proteins, including barley lectin and tobacco chitinase (reviewed in Chrispeels and Raikhel, 1992). No common motif can be identified between these sorting signals, and while the propeptides have been shown to be both necessary and sufficient for vacuolar transport, extensive mutagenesis studies have determined that there is no sequence motif that is required for their function (reviewed in Bar-Peled *et al*, 1996). It is still not known whether a receptor protein is present at the trans-Golgi network that recognizes this type of signal.

Finally, a region of the mature protein can also serve as a sorting signal. Bean phytohemagglutinin contains this type of signal (reviewed in Chrispeels and Raikhel, 1992), although the nature of the signal means that it is very difficult to precisely identify the residues responsible for targeting information. Legumin also appears to contain vacuolar sorting information within the mature protein, and the entire legumin α chain is required for efficient sorting (Bar-Peled *et al*, 1996). The mechanism by which these signals work is not known.

*2. Cells have more than one type of vacuole and probably have multiple paths for proteins to reach the vacuole.*

Studies using the fungal metabolite wortmannin, an inhibitor of phospholipid metabolism in tobacco cells, have shown that proteins containing an N-terminal signal are transported to the vacuole by a different mechanism than proteins containing a C-terminal signal (reviewed in Bassham and Raikhel, 1997). In the presence of wortmannin, proteins containing the barley lectin C-terminal propeptide were secreted, whereas those containing the sporamin N-terminal signal were correctly sorted to the vacuole. In addition, membrane proteins are transported by a different pathway compared to soluble proteins (reviewed in Bassham and Raikhel, 1997). Therefore, it appears that multiple pathways to the vacuole exist within a single plant cell. In fact, some specialized cell types have been shown to contain two distinct types of vacuoles, which have their own discrete complement of proteins (Neuhaus and Rogers, 1998). In mature vegetative cells, these vacuoles are thought to fuse to form a single large central vacuole upon which all the vacuolar targeting pathways converge (reviewed in Bassham and Raikhel, 1997).

*3. Vacuolar targeting requires transmembrane cargo receptors that function in traffic between the trans-Golgi network and the prevacuolar compartment.*

Using an affinity column with immobilized peptide corresponding to the aleurain N-terminal propeptide, a protein was isolated from pea clathrin-coated vesicles that is thought to represent a sorting receptor for vacuolar proteins that have this signal (reviewed in Beevers and Raikhel, 1998). This protein (BP-80) is an integral membrane protein that can also bind to other N-terminal signals, but not to the barley lectin C-terminal propeptide. An Arabidopsis homologue of BP-80 (named AtELP) was identified by searching the DNA sequence

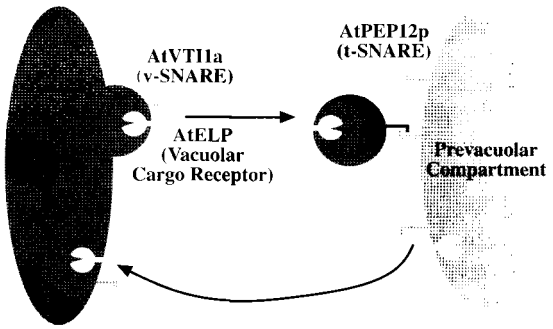


*Figure 4.* Schematic diagram of targeting and fusion of Golgi-derived clathrin-coated vesicles with the prevacuolar compartment. Only three components of the machinery are depicted in this figure: a putative vacuolar sorting receptor, AtELP, a vSNARE, AtVTI1A, and a tSNARE, AtPEP12p. Other members of the machinery that are important for vesicle targeting and target membrane fusion, including soluble and membrane receptors, and proteins that constitute the clathrin coat, are not shown on this diagram.

database using motifs common to sorting receptors from various eukaryotic species (reviewed in Beevers and Raikhel, 1998). Both the pea and Arabidopsis proteins have been localized by electron microscopy to the Golgi apparatus, and in addition AtELP was found in the pre-vacuolar compartment (Sanderfoot *et al*, 1998) (Fig 4). These receptor-like proteins are proposed to bind to their cargos (proteins containing an N-terminal vacuolar sorting signal) at the trans-Golgi network and package them into clathrin-coated vesicles for transport to the prevacuolar compartment.

After the formation of vesicles containing vacuolar cargo proteins from the trans-Golgi network, the vesicles have to be directed to the prevacuolar compartment and fuse with that organelle. A number of proteins have been identified from various organisms that are required for the correct targeting, docking and fusion of a transport vesicle with its target membrane. As some components for vesicle targeting and fusion appear to be similar between many different organisms and different stages of the secretory pathway, the basic mechanisms for these processes are probably conserved. Information from the better studied yeast and mammalian systems can therefore be very useful in the study of vesicle trafficking in plants.

### *4. The SNARE hypothesis for vesicle fusion and the discovery of plant homologues that function in this process.*

According to the SNARE hypothesis, vesicle docking requires the interaction of a vesicle SNARE (vSNARE) with a SNARE on the target membrane (tSNARE) (Beevers and Raikhel, 1998). The first vSNARE (syntaxin 1) was isolated from the synaptic plasma membrane of neurons, where it is involved in neurotransmitter release. It has been shown to form a complex with several soluble and integral membrane proteins that are all believed to function in vesicle docking and fusion by mechanism as yet unknown. A number of syntaxin homologues have now been identified in non-nerve cells and in various organisms. They have similar sequences and predicted secondary structures to syntaxin 1, and therefore may function in a similar way in a variety of vesicle fusion events. It has been hypothesized that a different syntaxin isoform may be required for each transport step of the secretory pathway (Rothman and Sollner, 1997).

Typical syntaxin homologues are type II transmembrane proteins. Most of the protein is hydrophilic and faces the cytosol, except for a hydrophobic domain about 20-30 amino acids long at the C-terminus of the protein. The hydrophilic part is thought to interact with other factors involved in vesicle trafficking, whereas the hydrophobic domain is responsible for anchoring the protein in the membrane.

Several syntaxin homologues have now been identified in plants that are also presumably involved in transport through the secretory pathway. By functional complementation of the yeast (*Saccharomyces cerevisiae*) secretory pathway mutant pep12, researchers have isolated a cDNA clone (AtPEP12) from *Arabidopsis thaliana* ecotype Columbia that encodes a protein homologous to yeast Pep12p and other members of the syntaxin family (reviewed in Bassham and Raikhel, 1997). The yeast pep12 mutant is defective in transport of some soluble proteins to the vacuole and is thought to function in transport between the trans-Golgi network and the prevacuolar compartment (PVC). AtPEP12p may therefore play a role in transport to the vacuole in plants. AtPEP12p has been localized by electron microscopy to a PVC in Arabidopsis roots and may act as a receptor for trans-Golgi network-derived transport vesicles en route to the vacuole (Fig 4). By genetic analysis, another Arabidopsis syntaxin homologue, KNOLLE (reviewed in Beevers and Raikhel, 1998), was identified. It is presumably involved in cytokinesis in the embryo, and is located at the forming cell plate during cell division (reviewed in Beevers and Raikhel, 1998). Several syntaxin homologues therefore exist in Arabidopsis, and each of them may be involved in separate steps of the secretory system or function at different developmental stages, or in different tissues. Re-

cently, a vSNARE protein, AtVTI1a, that most likely interacts with AtPEP12 has been identified (H. Zheng and N. Raikhel, unpublished data) (Fig 4).

### III. THE PLASMA MEMBRANE AND THE TONOPLAST CONTAIN CHANNELS AND TRANSPORTERS WITH IMPORTANT SIGNALING FUNCTIONS

*1. Calcium is an important second messenger in many signal transduction networks.*

The concentration of free $Ca^{2+}$ in the cytoplasm is very low (10 to 200 nM) and most of the cellular calcium is sequestered in calcium stores in cytoplasmic organelles or in the cell wall (Fig 5). The movement of $Ca^{2+}$ from the cytoplasm into the stores requires energy in the form of ATP and is carried out by calcium dependent $Ca^{2+}$-ATPases. Movement in the other direction (release into the cytosol) occurs via calcium channels down the electrochemical calcium gradient. Both types of proteins, pumps and channels, are particularly abundant in the plasma membrane and the tonoplast, but also occur in other membranes such as the ER and the mitochondria. In 1957, Hodgkin and Keynes performed a simple but seminal experiment. They injected radioactive calcium and potassium into the cytoplasm of an axon. After several hours they measured the distribution of the two radioactive ions and found that potassium had diffused throughout the cytoplasm, but the radioactive calcium had barely moved from the site of injection. The inescapable conclusion was that calcium is immobile in the cytoplasm. When calcium is artificially elevated in the cytoplasm, the extra calcium will bind to pro-

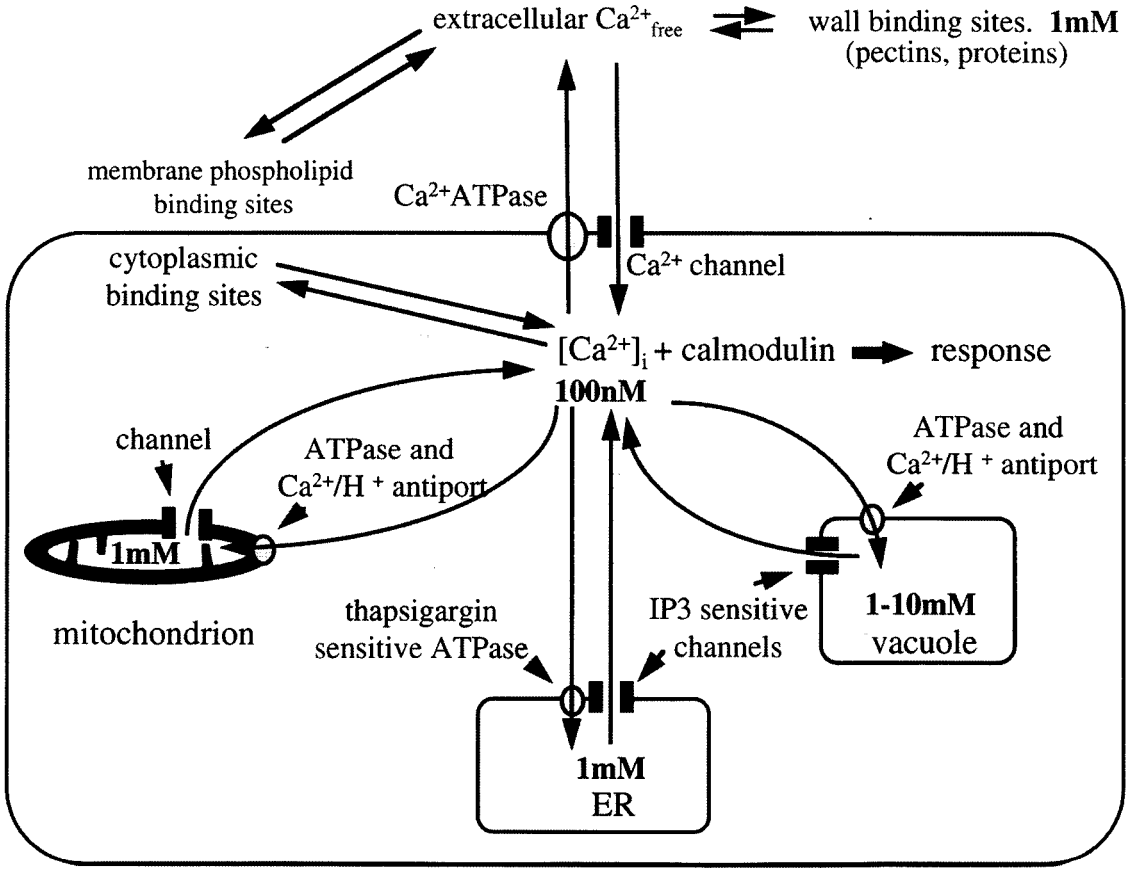


*Figure 5.* Summary of the major calcium relationships in a plant cell as presently understood. The figure indicates the presence of channels and calcium-ATPases and estimates of concentration in the main organelles. The presence of cytoplasmic binding sites inhibits direct calcium diffusion in the cytoplasm and necessitates the production of calcium waves using $IP_3$-calcium sensitive channels in the ER and vacuole.

teins and is sequestered into $Ca^{2+}$ stores. These stores are in the cell wall, the vacuole, the ER and the mitochondria. Calcium channel proteins permit the flow of calcium from the stores back into the cytoplasm. These channels open when cells are signaled in some way, and calcium then enters the cytoplasm down in its electrochemical gradient. Families of calcium channels are known to exist: some are activated by changes in membrane potential, some by membrane stretch and yet others by second messengers. A single channel can transmit $10^6$ atoms of $Ca^{2+}$ per second. Elevation of calcium at the channel mouth can be rapid, and concentration here can reach 100 mM. The channels rapidly close when cytosolic calcium is elevated and calcium is then pumped back into the intracellular stores or into the wall. Transient elevations of calcium, sometimes called spikes, may last anywhere from a few seconds to many minutes, depending on the characteristics of the stimulating signal.

*2. How does calcium make waves, and how do the waves move?*

Calcium itself does not move through the cytoplasm; however, this calcium spike can propagate itself as a wave (Malho *et al*, 1998). This propagation depends on the second messenger, inositol 1,4,5 triphosphate ($IP_3$). This molecule is generated through the action of the plasma membrane bound enzyme, phospholipase C. When phospholipase C is activated it hydrolyzes phosphatidyl inositol diphosphate ($PIP_2$) to yield diacylglycerol and $IP_3$. Unlike calcium, $IP_3$ is freely mobile in the cytoplasm, and $IP_3$-sensitive calcium channels are found in organelles throughout the cytoplasm. When $IP_3$ is present, the calcium channels open and calcium enters the cytoplasm. The role of $IP_3$ in mobilizing calcium was demonstrated in plant cells a decade ago (Alexandre *et al*, 1990; Gilroy *et al*, 1990), and $IP_3$ binding to the channels was discovered more recently (Allen *et al*, 1995). These observations help us understand how calcium makes a wave. The $IP_3$-sensitive calcium channels need both $IP_3$ and calcium to open (Marchant and Taylor, 1997). When $IP_3$ binds to a channel, a calcium binding site is briefly exposed. If calcium is absent, the channel rapidly inactivates. If calcium is present, the calcium binding site is occupied and the channel opens. However, the binding of calcium only serves to delay the eventual IP3-induced channel inactivation. Mobilization of calcium through a single IP3-dependent calcium channel is therefore brief and self-limiting. So how does the wave move? When an IP3-dependent calcium channel opens, the calcium concentration near adjacent channels will increase. The calcium-binding sites of these channels will be occupied and they will briefly open, in turn enabling the opening of others. Calcium is therefore responsible for inducing further calcium release and this release underpins wave movement. The wave is not a forward movement of calcium, but a forward movement of calcium release. IP3-induced inactivation of channels generates direction in calcium wave movement away from the point of origin. Because these channels are located in membranes, the wave moves across the face of the membrane.

*3. Plasma membrane potassium channels play fundamental roles in signal transduction processes in plants.*

Potassium transport processes play a fundamental role in plant cell physiology. Potassium ion movements across the cell membrane are involved in leaf movement, cell elongation, plant growth and development and in the regulation of stomatal aperture (for reviews see Schroeder *et al*, 1994; Cherel *et al*, 1996; Fox and Guerinot, 1998). Potassium is the dominant cation within the vacuole, and accumulation of $K^+$ inside this organelle is essential for generating the turgor pressures that drive cell expansion. Potassium transport in plants is mediated by carriers and channels and is customarily divided into high- and low-affinity $K^+$ uptake (Schroeder *et al*, 1994). High-affinity $K^+$ transport is defined as the $K^+$ influx that occurs when the external $K^+$ concentration is in the range of 1 to 200

mM (*e.g.*, Fox and Guerinot, 1998) and was thought to be mediated exclusively by carriers. Low-affinity $K^+$ uptake is mediated by voltage-dependent inward rectifier $K^+$ channels when the external concentration of $K^+$ is > 0.3 mM. These channels belong to a superfamily of ion channels designated the S4 superfamily, which includes $Na^+$, $Ca^{2+}$, and $K^+$ voltage-dependent channels (Jan and Jan, 1992). In all of these channels, the fourth transmembrane domain (S4) contains several positively charged amino acids in every third position (Fig 6B).

KAT1 and AKT1 (Fig 6A) were the first inward rectifier $K^+$ channels cloned from the plant *Arabidopsis thaliana* (Schroeder *et al*, 1994). The KAT1 channel is expressed in guard cells and the vascular system of the stem and plays a crucial role in stomatal opening (Fox and Guerinot, 1998). AKT1 is present in roots and plays an important role in $K^+$ uptake from soil. Mutants from *Arabidopsis thaliana* in which the AKT1 channel gene was disrupted show reduced $K^+$ uptake and grow poorly on media containing <100 mM $K^+$ (Hirsch *et al*, 1998). This result suggests that inward rectifier channel activity might also mediate high-affinity uptake (Schroeder *et al*, 1994). KST1, a gene-encoding a guard cell $K^+$ channel, was cloned from a *Solanum tuberosum* cDNA library based on its similarity to KAT1. KST1 is also present in flowers, albeit at a low level. Four more inward rectifier $K^+$ channels genes have been identified in *Arabidopsis thaliana* and *Solanum tuberosum*: AKT2 and AKT3 and SKT2 and SKT3. Two outward rectifier $K^+$ channel genes have also been cloned from *Arabidopsis thaliana*: SKOR (Gaymard *et al*, 1998) and KCO1. SKOR is present in root stelar tissues and its role is to release $K^+$ into the xylem sap. KCO1 is expressed in seedlings and at low levels in leaves and flowers. For original references see Fox and Guerinot (1998).

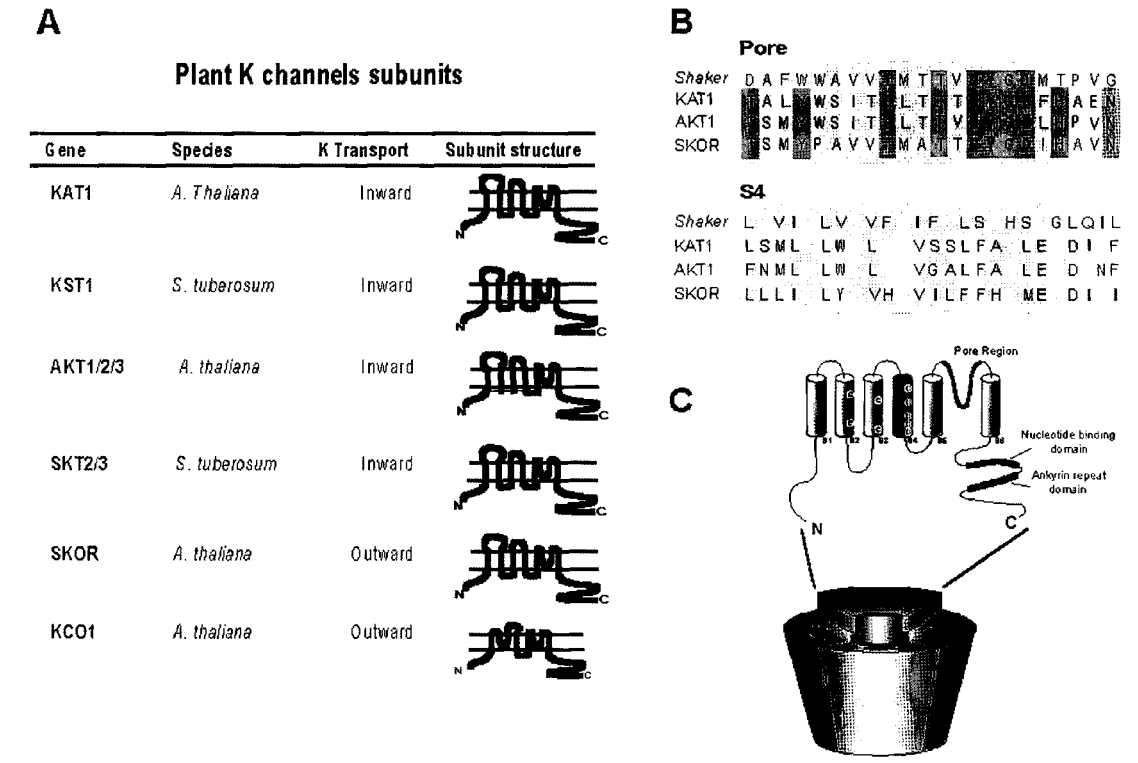


*Figure 6* A. Cloned plant potassium channels and their subunit structure. B. Primary sequence alignment of the pore region (top) and S4 segment (bottom) for three plant channels in comparison to Shaker. Potassium channel fingerprint GYGD is highlighted. Conserved positively-charged residues in the S4 segment are indicated in white by the amino acid number. C. Proposed transmembrane structure of one potassium channel subunit indicating membrane spanning segments S1 through S6 and the pore region (top), and tetrameric subunit assembly to form one channel (bottom).

*4. Inward and outward rectifying potassium channels of plants and animals have the same basic structure.*

As can be appreciated in Figure 6, inward rectifiers and SKOR channels share the same subunit structure consisting of six transmembrane domains (S1-S6) and a pore domain (P) linking segments S5 and S6. The S4 domain contains conserved, regularly-spaced, positively-charged amino acid residues (Fig 6B). Many different $K^+$ channel genes in metazoans have this same basic structure, and *Shaker*, the first $K^+$ channel gene cloned from the fly *Drosophila melanogaster* (Tempel *et al*, 1987), is a classic example. In *Shaker* $K^+$ channels, the S4 domain forms part of the voltage sensor and the P region is part of the ion conductive pore (Papazian and Bezanilla, 1997; Doyle *et al*, 1998). The pore region of $K^+$ channels is extremely well conserved (Fig 6B). The *Shaker* $K^+$ channel is a tetrameric structure formed by four identical subunits (Fig 6C), and KAT1 and AKT1 are also likely to form tetramers (Cherel *et al*, 1996). Additionally, a putative cyclic nucleotide-binding domain is present in the carboxyl terminus in both KAT1 and AKT1. In AKT1 an ankyrin domain is present downstream of the cyclic nucleotide-binding domain (Fig 6C). It is worth noting here that KAT1 has a great similarity to the *ether a-go-go* (*eag*) *Drosophila* channel. The *eag* channel forms part of a large class of $K^+$ channels containing a cyclical nucleotide binding domain and is activated by hyperpolarizing voltages (*e.g.*, Trudeau *et al*, 1995).

*5. Aquaporins, or water channel proteins, occur in the plasma membrane and the vacuolar membrane.*

How does water flow through a biological membrane? Does it simply diffuse through the lipid bilayer, or does it pass more or less unimpeded through specialized pores? Recent discoveries show that water-flow through membranes is facilitated by aquaporins (AQPs), integral membrane proteins that can increase the water permeability of a membrane 10-20 fold (Preston *et al*, 1992; Maurel *et al*, 1993). Aquaporins are not pumps, but simply facilitate the passage of water through the membrane when the osmotic or hydrostatic pressure is higher on one side than on the other. Aquaporins belong to the MIP family of membrane proteins, which also includes glycerol channels and possibly some ion channels. These 26-28 kDa proteins have six membrane-spanning domains and the signature sequence NPAVT.

The activity of water channel proteins is most conveniently assayed by expressing them in Xenopus oocytes and then measuring the swelling rate when the oocytes are shifted to a hypotonic condition. AQPs form tetramers in the membrane, but the monomer is probably the unit that transports water. A molecular model suggests that two cytoplasmic loops are half-buried in the lipid membrane to form the aqueous pore.

Both the abundance and the activity of aquaporins can be regulated. Some aquaporins are expressed in a tissue-specific or organ-specific pattern. This is true both in mammals and in plants, where aquaporins have been most studied. In *Arabidopsis* there are at least 23 different cDNAs that fall into two large groups on a cladogram, TIPs (tonoplast integral proteins) and PIPs (plasma membrane integral proteins). In addition, there is a small group of more distantly related aquaporins (Weig *et al*, 1997). It is important to note that all of these genes have not yet been shown to encode active aquaporins. Indeed, some of the proteins appear not to be active as aquaporins when their mRNAs are introduced in Xenopus oocytes. This could mean that they do not encode aquaporins or that the polypeptides made in the oocytes do not behave as active aquaporins. Perhaps they do not arrive at the plasma membrane, or they need to be post-translationally modified or they need to interact with other proteins not present in oocytes. In any case, we cannot rule out the idea that the these members of the MIP family have completely different functions and may be involved in the transport of other substances.

Aquaporin expression can be altered by hormones or environmental signals, such as light or drought. Phosphorylation may regulate the activity of AQP as has been shown for *Arabidopsis* α-TIP and spinach PM28A. When spinach plants experience water deficit, the plasma membrane aquaporin PM28A is less phosphorylated than when the leaves are turgid and this inhibition of phosphorylation may decrease the activity of the water channel. When PM28A is expressed in *Xenopus* oocytes, its water channel activity is inhibited by agents that prevent phosphorylation (Johansson *et al*, 1998).

*6. Aquaporins have multiple roles in cellular and whole plant physiology.*

What is the role of aquaporins in cellular physiology or in the whole plant? Determining the role of aquaporins is likely to take many years of research, but some hypotheses are beginning to emerge as a result of studying the expression patterns of aquaporins. Expression has so far been studied mostly at the mRNA level and not at the protein level, simply because it is much easier to make gene-specific probes than to make protein-specific antibodies when dealing with a family of homologous proteins. Analysis of the expression pattern of the active aquaporin γ-TIP from *Arabidopsis* and ZmTIP1 from *Zea mays* shows that expression is highest in meristematic and expanding cells, young root epidermis, the endodermis, the parenchyma cells that surround functional xylem vessels, and in the pedicel (Barrieu *et al*, 1998; Chaumont *et al*, 1998). What does this tell us about the possible functions of aquaporins? The high expression in the meristem and expanding cells may be related to the biogenesis of new vacuoles and the rapid expansion of vacuolar volume that accompanies cell enlargement. TIPs may be needed to sustain the rapid water influx into the vacuole that sustains cell growth in size. The high expression in the epidermis, endodermis and pedicel suggests that TIPs may be present at high levels in cells that can undergo sudden fluxes in ions or metabolites. If ions and metabolites suddenly enter the cytoplasm, then the cell has to adjust its osmotic pressure, and the most readily available source of water is in the vacuole (Fig 7). However, it is only readily available if the tonoplast is truly permeable to water. High expression in the parenchyma cells that surround the active xylem vessels could indicate an important role in transcellular water flow. Water flows radially in and out of vessels, and this flow will be less impeded if the tonoplast is permeable to water. In this case the water permeability of the tissue would reside in and be regulated by the plasma membrane. Whereas these are all interesting hypotheses, they need to be supported by evidence and such evidence will probably have to come from mutants that lack specific aquaporins. A recent report indicates that a

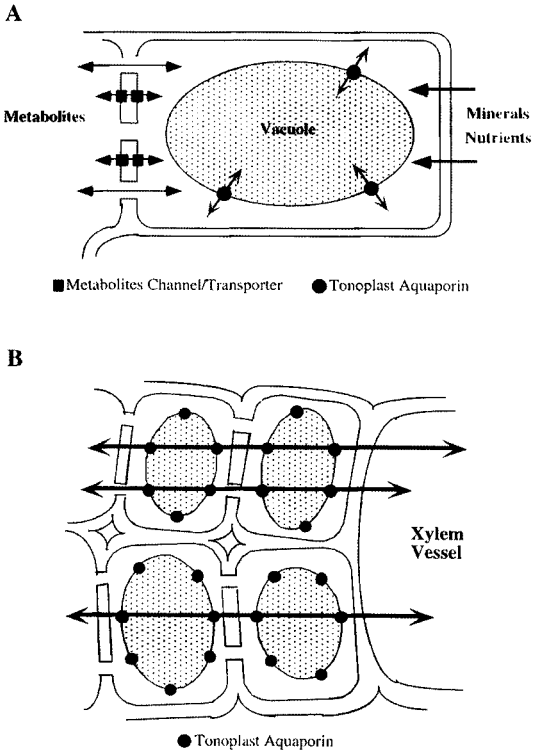


*Figure 7.* Schematic representation of two roles for tonoplast aquaporins. A. Tonoplast aquaporins are needed for cytoplasmic osmotic equilibration in cells that can experience rapid fluxes of metabolites or mineral nutrients. B. Tonoplast aquaporins permit rapid transcellular flow and increase the effective cross-section of the cytoplasm for symplastic flow. From Barrieu *et al*, 1998. Reprinted with permission of the publisher.

PIP antisense mutant of *Arabidopsis* has an unusually large root system as if the plant were trying to compensate for slower water loss from the shoot.

## IV. SIGNAL TRANSDUCTION NETWORKS IN ABIOTIC STRESSES

### *1. Plants have both rapid and slow responses to water deficit.*

An important difference between plants and animals is that plants cannot move around at will. This leads to a big difference when it comes to the way they interact with their environment. During evolution, animals developed mechanisms to respond to the environment by their behavior. Plants, on the other hand, developed mechanisms to respond to their environment developmentally. For plant biologists, it has always been important to understand how plants respond to their environment, especially the stressful environments, because this is critical for agriculture. Perhaps the most common problem for farmers worldwide is water stress, which can result from drought, salinity, or cold temperature. Plants respond to water stress by both rapid and slow pathways. The **rapid pathway** involves the regulation of ion channels that close the stomatal pores in the leaves by reducing guard cell turgor. Potassium fluxes through potassium channels (see below) play an important role in this process. The **slow response** involves cascades of gene expression that shape the developmental program of the plant.

Ion channel regulation in guard cells provides a nice model for studying the early steps in the water stress pathway. Studies have shown that water stress elicits at least two early pathways; one involves production of a plant hormone abscisic acid (ABA), and the other is ABA-independent (Fig 8). A number of reports suggest that the ABA-dependent pathway further branches out to contain calcium-dependent and calcium-independent pathways, both of which regulate the activity of several ion channels in the plasma membrane and tonoplast (Assmann, 1993; Ward *et al*, 1995). Calcium-dependent pathways inactivate the inward $K^+$ channel, and calcium-independent pathways activate the outward $K^+$ channel. Both pathways may be involved in the activation of outward anion channels. The combination of these effects causes a net efflux of ions and decline in guard cell turgor, a prelude to stomatal closure.

An ABA-independent pathway directly utilizes the osmotic stress as a signal that regulates ion channels through a volume or osmo-sensing mechanism (Liu and Luan, 1998). If a guard cell is in a swelling state during stomatal opening, the inward $K^+$ channel is activated, while the outward $K^+$ channel is inactivated. This leads to increase in the turgor pressure and accelerates the opening process. If a guard cell is in a "shrinking state" during hyperosmotic shock caused by drought or darkness, the inward $K^+$ channel will be inactivated, and the outward $K^+$ channel will be activated. This leads to further efflux of $K^+$ and speeds up the closure process. The volume, or osmo-sensing mechanism, provides a way for both positive feedback of stomatal movements and for response to water stress.

In the slow response, a water stress signal is perceived at the plasma membrane and transduced all the way to the nucleus where

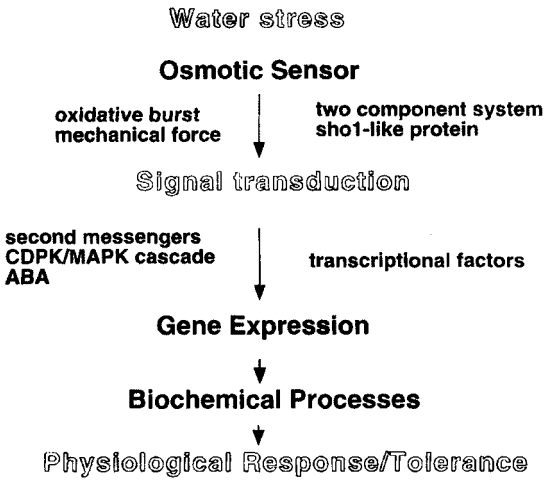


*Figure 8.* Regulation of stomatal closure resulting from water deficit or osmotic stress. The three or perhaps four pathways all lead to the opening of $K^+$ channels. The outflux of $K^+$ causes a drop in guard cell turgor and subsequent stomate closure.

gene expression is initiated. It is not hard to see the rationale because most developmental and physiological changes in a plant start with gene expression. Examples of how scientists approach this problem are given below.

*2. Molecular, genetic and cellular approaches have elucidated the signal transduction pathways involved in stress responses.*

When plants are exposed to water deficit or osmotic stress, they respond physiologically with adaptive processes. These processes have a biochemical basis that in turn depends on the expression of new genes. How the water deficit signal is perceived by the hypothesized osmotic sensor and transduced to the nucleus (Fig 9) is presently being investigated with molecular, genetic, and cellular techniques. The first approach uses molecular biology. A general strategy here is to first look for genes that are induced by water stress conditions, which can be done by a number of methods such as differential screening of cDNA libraries. The next step is to define the cis elements that are responsible for the stress induction. These cis-acting elements are usually located in the promoter region of the gene. Using this information, one can find the transcriptional factors that interact with the cis-acting elements and go upstream of the signaling pathway by finding components that regulate the transcriptional factors. One of the leaders who use this approach is Kazuo Shinozaki's group in Japan. Here are two reports that precisely describe the power of this approach. Yamaguchi-Shinozaki and Shinozaki (1994) describe how the initial cis-acting elements were identified. Liu *et al* (1998) continued to identify the transcriptional factors that act on the cis-acting elements.

More recently, a genetic approach was applied to the dissection of the water stress-pathway. This approach starts by fusing a water stress-responsive promoter to the luciferase reporter and then asking: Can we select for mutants that have abnormal expression pattern of this reporter gene in

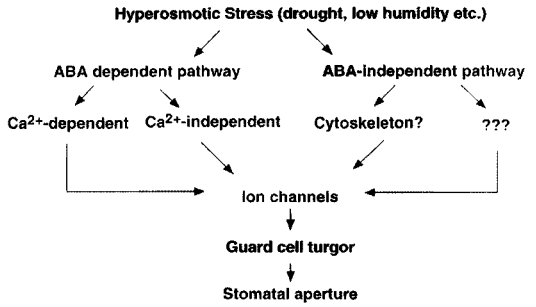


*Figure 9.* Components in water stress signaling that lead to the expression of new genes and the physiological response of the plants.

ransgenic *Arabidopsis*? A chemical mutagenesis procedure was used to mutagenize the seeds, and seedlings were monitored for luciferase expression under a fluorescent camera. Plants with aberrant expression patterns of reporter genes under various conditions (drought, cold, salinity) were isolated and tested further to identify those with true defects in their response to water stress. A number of mutants have been identified that are categorized into many types. Some are constitutive response mutants that express the reporter gene even under normal conditions, suggesting a mutation in negative regulators of the pathway. Some are less responsive to the stress condition, suggesting a mutation in positive regulators. Some are hypersensitive to the stress condition, also suggesting mutation in negative regulators (Ishitani *et al*, 1997).

A good example for a cellular approach that utilized a transient assay system was given by Sheen (1996). An ABA-responsive promoter was fused to the gene-encoding green fluorescent protein (GFP) and transformed into maize mesophyll protoplast by electroporation. The expression of GFP was monitored under fluorescence microscope after the cells were treated by ABA, cold, and high salt. All these treatments caused expression of GFP in the transformed cells. With the idea that calcium may serve as the second messenger for ABA, calcium ionophore was used to mimic the treatments. Indeed calcium increases in the cells led to expression of GFP. Then the author went on to show that

a calcium-dependent protein kinase (CDPK) may mediate calcium messenger and a protein phosphatase may antagonize the CDPK function.

Biochemical approaches were also used to tackle the early steps in the water stress pathway. Most noteworthy might be the finding of mitogen-activated protein kinase (MAPK) cascade in the early stage of the water stress signaling (Jonak *et al*, 1996). This approach involves a technique called in-gel kinase assay that can detect the activity of a protein kinase in the protein extract isolated from plant cells treated by various stress conditions. Using this technique, MAPK is found to activate rapidly and transiently following water stress. Activation of MAPK involves phosphorylation at both threonine and tyrosine residues and inactivation requires these residues to be dephosphorylated. Protein kinases and phosphatases involved in these processes have recently been identified and have opened up a new window to understanding stress-signaling pathways (Meskiene *et al*, 1998; Xu *et al*, 1998; Gupta *et al*, 1998; Luan, 1998).

In summary, studies using these approaches suggest that water stress signaling may have ABA-dependent and independent pathways. ABA-dependent pathways may have calcium-dependent and calcium-independent branches. ABA-independent pathways may involve osmosensing pathways that activate MAPK cascades. All pathways may play a role in rapid response, such as stomatal regulation and slow response involving gene expression. A combination of these approaches will be fruitful in dissecting the details of these pathways (Shinozaki and Yamaguchi-Shinozaki, 1997).

### *3. Abscisic acid and jasmonic acid mediate wound signal transduction.*

Wounding of plants, as often occurs when they are attacked by insects, causes the plants to mount a defense response. New genes are expressed both at the site of wounding and after the transmission of a systemic signal throughout the plant. When this phenomenon first began to be investigated, C. A. Ryan and his group established that wounding results in the induction of proteinase inhibitors (PINs), and the induction of the genes that encode these inhibitors (pin genes) has been used as a diagnostic to investigate the signal transduction pathway that leads from wounding to gene expression. The plant hormones abscisic acid (ABA) and jasmonic acid (JA) play an important role in the conversion of many environmental signals into changes in plant gene expression. The *in vivo* involvement of ABA and JA in the gene activation processes that follow mechanical damage of the plant tissue is supported by the finding that endogenous ABA and JA levels rise three- to six-fold upon wounding. This increase is, moreover, not restricted to the tissue damaged directly but can also be detected in non-wounded, systemically induced tissues (Peña-Cortés *et al*, 1995). This phenomenon is common to several plant species including potato, tomato and tobacco (Sánchez-Serrano *et al*, 1991). Furthermore, in all three plant species, a correlation has been established between the ABA and JA increase and either the expression of the pin2 gene family (in potato and tomato) or the activity of an introduced reporter gene driven by the pin2 promoter in transgenic potato or tobacco plants (Peña-Cortés and Willmitzer, 1995).

Further evidence for the involvement of ABA and JA in wound-induced pin2 gene expression was provided by a series of experiments in which potato plants were sprayed with ABA or JA and pin2 mRNA accumulated in the absence of any wounding (Peña-Cortés *et al*, 1991; Hildmann *et al*, 1992). Both non-sprayed and directly-sprayed leaves showed increased pin2 mRNA levels with a pattern identical to the one described for wounded plants. Conclusive evidence for the involvement of ABA in wound-induced activation was obtained from mutants impaired in ABA biosynthesis. In those mutants, wound induction of pin2 was not observed (Peña-Cortés *et al*, 1989). However, in these mutants treatment with ABA caused a return of the accumulation of pin2 mRNA to levels normally found in wild-type plants upon wounding

(Peña-Cortés *et al*, 1995). Unexpectedly, exogenous application of JA to these mutants also lead to the accumulation of pin2 mRNA, suggesting that the JA site of action is located downstream from the site affected by ABA in the signal transduction pathway mediating wound response (Peña-Cortés *et al*, 1996).

*4. An electrical current may mediate the systemic activation of wound-induced genes.*

The local and systemic activation of pin2 genes following wounding suggest the existence of a signal that moves from the injured tissue to the upper or lower leaves and leads to the systemic induction of pin2 gene expression. Several mediators, both chemical and physical, have been suggested as the putative systemic signal. Phytohormones such as ABA (Peña-Cortés *et al* 1989; Hildmann *et al*, 1992), JA (Farmer and Ryan, 1990, 1992; Peña-Cortés *et al*, 1993), ethylene (O'Donnell *et al*, 1996), the peptide systemin (Pearce *et al*, 1991), and oligosaccharides (Ryan, 1987) all have been demonstrated to be chemical signals. Moreover, hydraulic and electrical signals, classified as physical signals, have been implicated in wound-induced gene expression. The appearance of variation potentials throughout most of the shoot has been reported following localized wounding by heating or burning in several plants (Wildon *et al*, 1989; Malone and Stankovic, 1991). Furthermore, Wildon *et al* (1992) reported that mechanical wounding and localized burning generate electrical signals that are propagated through the plant, thereby inducing pin2 expression systemically.

Like wounding, the application of electrical current was able to initiate ABA and JA accumulation in wild-type plants but not in ABA-deficient plants (Herde *et al*, 1996). These results suggested that, like wounding, electrical current requires the presence of ABA for the induction of pin2 gene expression. In contrast to wounding and electrical current, the burning of leaves activated pin2 gene expression in ABA-deficient plants by directly triggering the biosynthesis of JA via an alternative pathway that is independent of endogenous ABA levels (Herde *et al*, 1996). Analyzing tomato ABA mutants that had progressively lower ABA levels it was found that there is a threshold level of ABA for stress-induced gene expression (Herde *et al*, 1999). Since pin2 mRNA was not detected upon wounding or current application in mutants with progressive attenuation in ABA content, it was suggested that a threshold value of ABA exists and functions as an on/off switch for wound-induced pin2 gene expression. Therefore, the ABA level functioning as the threshold value for the ABA-dependent pathway that leads to pin2 gene expression upon wounding and current application was between 21% and 47% of that found in wild-type tomato plants.

The ABA level that accumulated in wounded and current-treated plants was roughly reflected by the respective JA content of wild-type and mutant plants after these treatments (Peña-Cortés *et al*, 1995). Unwounded wild-type and ABA-deficient plants had almost identical levels of JA, suggesting that JA biosynthesis is not influenced by endogenous levels of ABA. Harms *et al* (1995) reported that high endogenous level of JA in transgenic plants overexpressing a flax allene oxide synthase (AOS) cDNA did not induce constitutive pin2 gene expression. One possible explanation for these phenomena is that endogenous ABA and JA are stored in cellular compartments different from those where ABA and JA accumulate upon wounding. JA biosynthesis or at least part of it, is suggested to occur in the chloroplast (Herde *et al*, 1995), which implies that accumulation of JA in this organelle is not sufficient to lead to changes in the expression of JA-responsive genes such as pin2.

*5. The biosynthesis of JA during stress may involve two different cellular compartments, the chloroplast and the peroxisomes.*

The biosynthetic pathway for JA, called the octadecanoic pathway, was first elucidated by Vick and Zimmerman (1987). The

synthesis of JA starts with linoleic acid and involves the sequential activities of lipoxygenase to make fatty acid hydroperoxide, hydroperoxide dehydratase or allene oxide synthase (AOS) to produce allene oxide, and allene oxidase to produce 12-oxo-phytodienoic acid (PDA). 12-oxo-PDA then undergoes chain shortening via ß-oxidation. It is assumed that these final steps of the pathway take place in the peroxisomes, because that is where the ß-oxidation enzymes are located in plant cells. Kleiter and Gerhardt (1998) recently provided evidence that long-chain fatty acids can be degraded completely into their constituent acetyl units by higher-plant peroxisomes. But where are the first steps in the pathway carried out? When Herde *et al* (1995) examined the location of the flax AOS in the transformed tobacco plants (see above) they found it to be located in the chloroplasts. Subsequently, Blée and Joyard (1996) showed that the first three enzymes in the pathway, up to the production of DPA, are located in the chloroplast envelope (Fig 10).

The failure of the increased levels of JA in the AOS transgenic plants to activate the

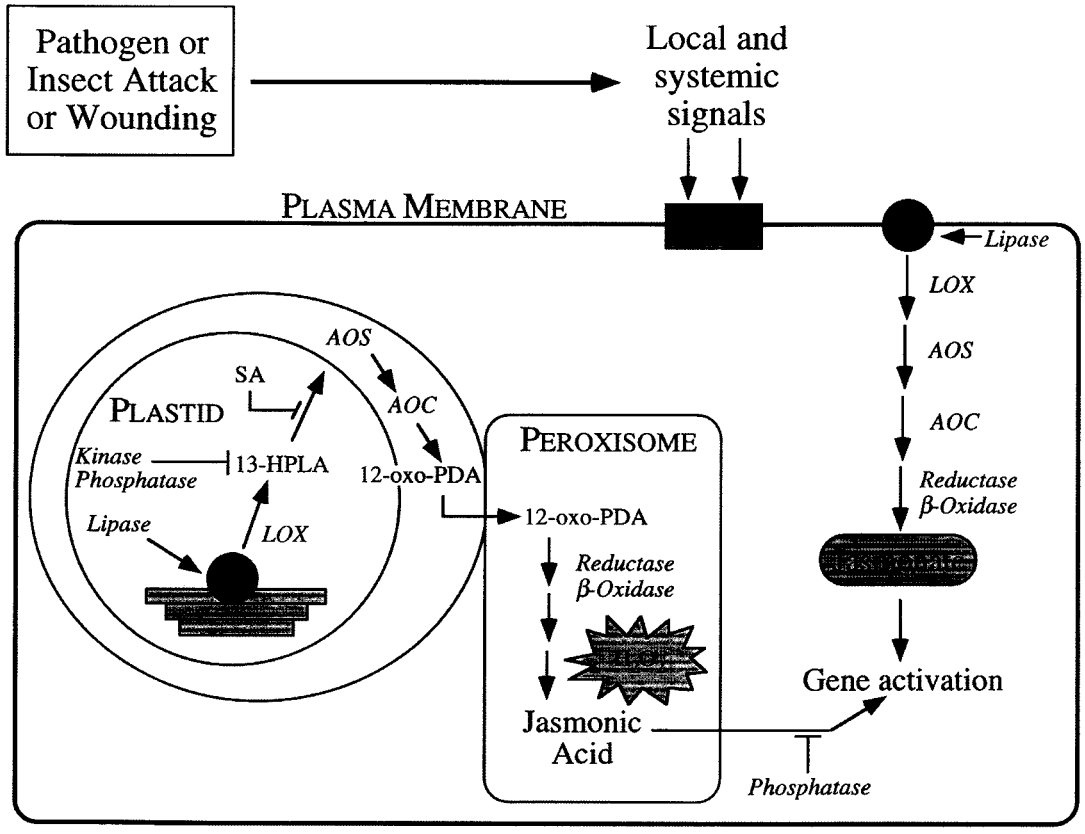


*Figure 10.* Alternative pathway for jasmonic acid biosynthesis in plants. Over-expression of the flax allene oxide synthase (AOS) cDNA leads to an increase of endogenous jasmonic acid (JA) levels in transgenic potato plants, the transgenic protein being accumulated in the chloroplast. Blée and Joyard (1996) showed the presence of this protein in the plastidial envelope allowing the formation of 12-oxo-PDA in this organelle. The last steps of JA biosynthesis involve three ß-oxidation reactions that may take place in the peroxisomes. Acyl-CoA oxidase activity involved in the decarboxylation of fatty acids generates $H_2O_2$. Plastid and cytosolic phosphatases are involved in the signaling pathway mediating JA biosynthesis and subsequently gene activation. Kinase activity seems also to be required for the wound-induced accumulation of JA but not for gene activation. Salicylic acid inhibits JA biosynthesis by preventing the wound-induced AOS gene expression (Harms *et al*, 1998). LOX, lipoxygenase; AOC, allene oxide cyclase; 12-HPLA, hydroperoxylinolenic acid.

pin2 genes could be explained by an accumulation of JA in a compartment (possibly the peroxisomes) that avoids the interaction of JA with its putative receptor and subsequent gene activation. Although six-week old AOS plants show JA levels 6-10 times higher than those found in untransformed plants, they do not show phenotypic changes. However, in older plants, the older leaves of the transgenic lines show symptoms that resemble the leaf lesion found in pathogen infected leaves. The lesions become necrotic and expand on the surface leading to leaf-death and abscission. This phenotype expands to the upper part of the plant, and after 13-weeks the transgenic lines have only a few healthy leaves at the top of the plant (the youngest leaves). At this time untransformed plants show the first senescence symptoms in the older leaves. The phenotype observed in the transgenic plants correlates with an even higher JA content, reaching levels 12-20 higher than those observed in normal plants. In these older leaves pin2 gene expression has been turned on and other JA-responsive genes such as AOS, lipoxygenase have been activated as well. In addition, the expression of pathogen-related genes (PRs) is also upregulated in the transgenic plants (Peña-Cortés unpublished results). More interestingly, the transgenic plants with increased levels of JA also show elevated levels of $H_2O_2$. This increase correlates with the appearance of the symptoms and with the activation of the JA-responsive genes. Treatment of control plants with $H_2O_2$ also results in the accumulation of pin2-, AOS-, LOX- and PR-mRNA. Furthermore, mechanical wounding or JA application leads to an increase of endogenous levels of $H_2O_2$ in potato leaves. There are at least two possible explanations for this observed increase in $H_2O_2$. The rise in $H_2O_2$ could simply be a byproduct of the increased JA biosynthesis because the reaction in the pathway catalyzed by acyl-CoA oxidase generates $H_2O_2$. It is also possible the JA activates other reactions that lead to the production of $H_2O_2$ or inhibits reactions that scavenge $H_2O_2$. Further work is clearly needed to determine how the wound signal is transmitted in plants.

## V. PLANTS RESPOND TO BIOTIC STRESSES FROM PATHOGENS AND PESTS WITH A MULTITUDE OF DEFENSE RESPONSES THAT INVOLVE COMPLEX SIGNALING NETWORKS

### *1. Plant pathogen interactions are governed by single genes in a gene for the gene system.*

Genetic analysis of many plant pathogen interactions has demonstrated that plants contain single resistance genes (or R genes) that confer resistance against a specific race of the pathogens that expresses a specific and complementary avirulence (avr) gene. If either the plant R gene mutates or the pathogen avr gene mutates, then resistance of the plant to the pathogen is lost. This is generally referred to as the gene for gene system of plant pathogen resistance. Resistance of a plant to a pathogen manifests itself in a variety of ways, but is often accompanied by a hypersensitive response (HR). HR is the localized induction of cell death at the site of infection, often resulting in numerous visible necrotic spots on the infected plant organ. Pathogenicity occurs when the plant does not detect the presence of the pathogen soon enough to mount an effective defense response. Resistance of a plant to a pathogen sets off a series of reactions that include a rapid oxidative burst at the plasma membrane, ion fluxes characterized by $H^+$-$K^+$ exchange, crosslinking of cell wall proteins and synthesis of phenolics, production of antimicrobial compounds (phytoalexins), and induction of the pathogen related (PR) genes that encode proteins such as chitinases, glucanases, osmotin, etc. These responses of the plant occur in many different plant pathogen interactions (bacteria, viruses, fungi) suggesting that there is a common signal transduction network for the response. In addition to this local necrotic response, the pathogen also induces a systemic response that results in a reduction of the severity of disease symptoms after infection by any pathogen, including a normally virulent one. This phenomenon is referred to as systemic acquired resistance (SAR).

*2. Molecular genetic approaches led to the identification and cloning of pathogen resistance (R) genes.*

In the past several years, disease resistance (R) genes have been isolated from diverse species such as tomato, *Arabidopsis*, tobacco, flax, barley and rice. Interestingly, the proteins they encode share certain motifs that all suggest a role in signal transduction. For example, the tomato *Pto* gene that confers resistance to a bacterial disease encodes a serine/threonine kinase, suggesting a role in a phosphorylation cascade. The largest group of R proteins carries leucine-rich repeats (LLR), putative cytoplasmic signaling domains and nucleotide binding sites. These proteins are thought to reside entirely within the cell. The tomato disease R gene *Cf9*, on the other hand, is an extracytoplasmic glycoprotein with an LRR motif in the extracellular domain. Such LRR domains may be instrumental in bringing about dimerization of the proteins. Song *et al* (1995) cloned the rice *Xa21* gene which confers resistance to *Xanthomonas campestris oryzae* pv. *oryzae*, the organism that causes rice bacterial blight. Xa21 encodes a receptor-like kinase with an LRR in its extracellular domain. The domain structures of different R genes are shown in Figure 11. This domain is thought to transduce an extracellular signal through activation of the intracellular kinase domain (Ronald, 1997). Yet another class is represented by the rice gene *Xa21D*, encoding a presumed secreted LRR (Wang *et al*, 1998). Although these genes have highly conserved structural motifs, virtually nothing is known about the subcellular location of the proteins, the identity of the ligands or the domains essential for pathogen recognition. Once the molecular determinants governing these interactions are known we can begin to develop novel strategies for disease control.

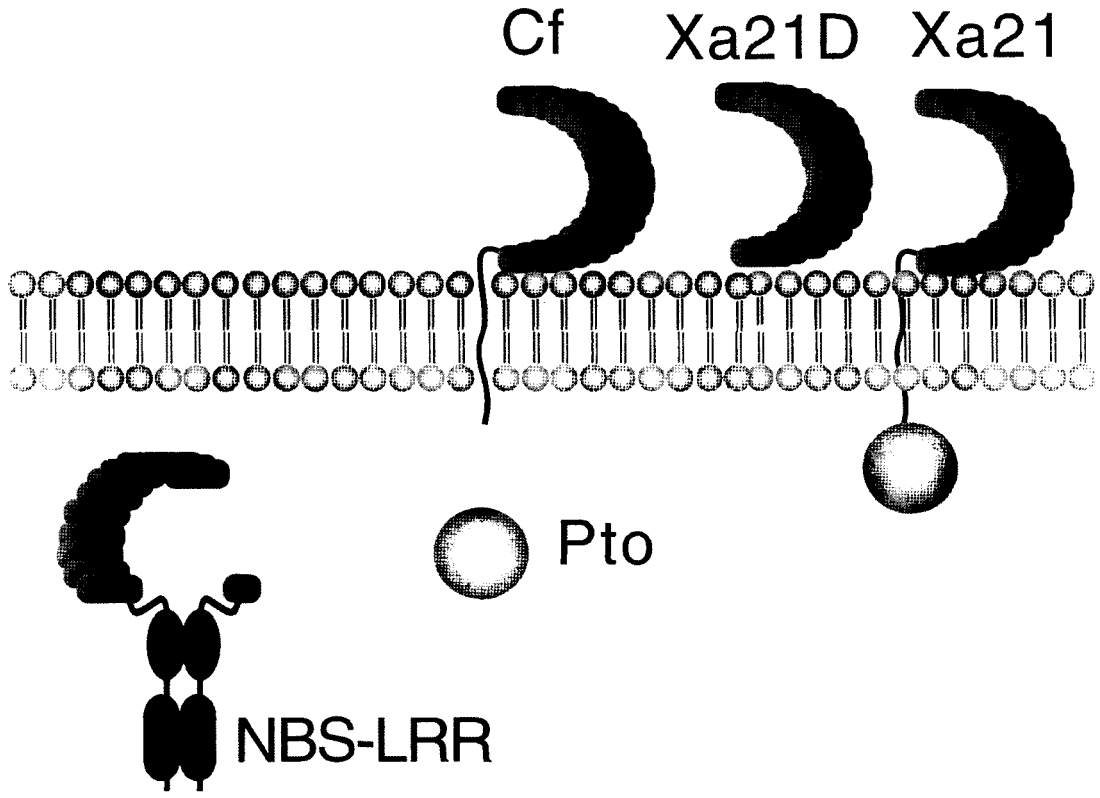


*Figure 11.* Domain structure of different resistance (R) gene products involved in plant disease resistance.

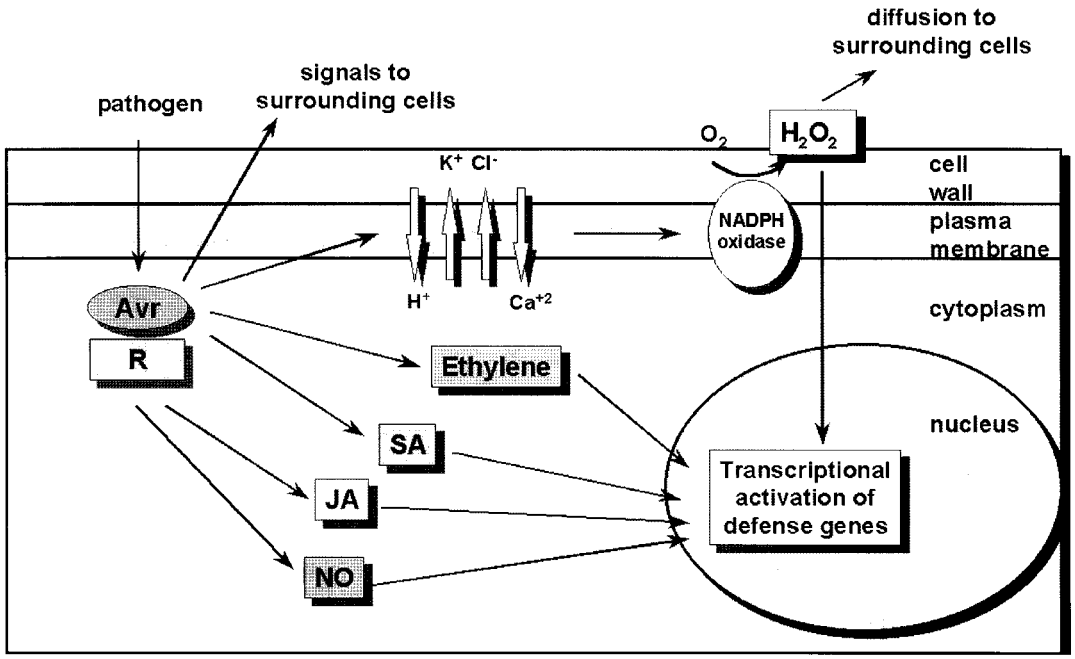


*Figure 12.* Signals in plant defense response against pathogens. The defense response is triggered by specific recognition between the product of the avirulence gene from the pathogen (Avr), and the product of the resistance gene from the plant (R). After this recognition, several plant metabolites like $H_2O_2$, ethylene, salicylic acid (SA), jasmonates (JA) and nitric oxide (NO) are accumulated. These metabolites act as secondary signals for the transcriptional activation of defense genes.

*3. Signaling for transcriptional activation of genes in the defense response against pathogens.*

A number of genes that encode for defense proteins are activated at different times after infection (Hammond-Kosack and Jones, 1996). These defense proteins have different functions in the defense reaction, such as antimicrobial activity, cell wall reinforcement, synthesis of phenolic compounds, and protection against oxidative stress. Temporal coordination in the transcription of these defense genes is controlled by a complex network of signaling pathways (Yang *et al*, 1997; Blumwald *et al*, 1998). In recent years, significant advances have been obtained in the identification of components involved in this network. Thus, several plant metabolites have been identified as secondary signals, which are transiently accumulated in the plant cell at different times after the pathogen signal is perceived. These metabolites are reactive oxygen species (ROS), such as $H_2O_2$, salicylic acid (SA), jasmonates (JA), ethylene, and nitric oxide (NO) (Fig 12) (Durner *et al*, 1997; Van Camp and Van Montagu, 1998). Furthermore, classical components for intracellular signal transduction, such as ion fluxes, G-proteins, protein phosphorylation cascades, cyclic AMP and cyclic ADP-ribose, have been also identified (Zhu *et al*, 1996; Blumwald *et al*, 1998; Durner *et al*, 1998).

One of the signaling pathways that has been most studied in recent years is the SA-mediated pathway (Durner *et al*, 1997). This pathway leads to the transcriptional activation of genes encoding for acidic pathogenesis-related proteins (PR proteins) and for oxidative stress-detoxifying enzymes (like glutathione-S-transferase, GST). The biosynthesis of SA occurs via the phenylpropanoid pathway, through benzoic acid (BA). Other signals such as ethylene, jasmonates, NO and $H_2O_2$ activate this biosynthetic pathway, through the transcriptional activation or the enzymatic activation of two key enzymes (phenylalanine ammonia-lyase, PAL, and benzoic acid 2-hydroxylase, BA2H). SA is accumulated in the vacuole as a glucoside (SAG), which is inactive (Fig 13).

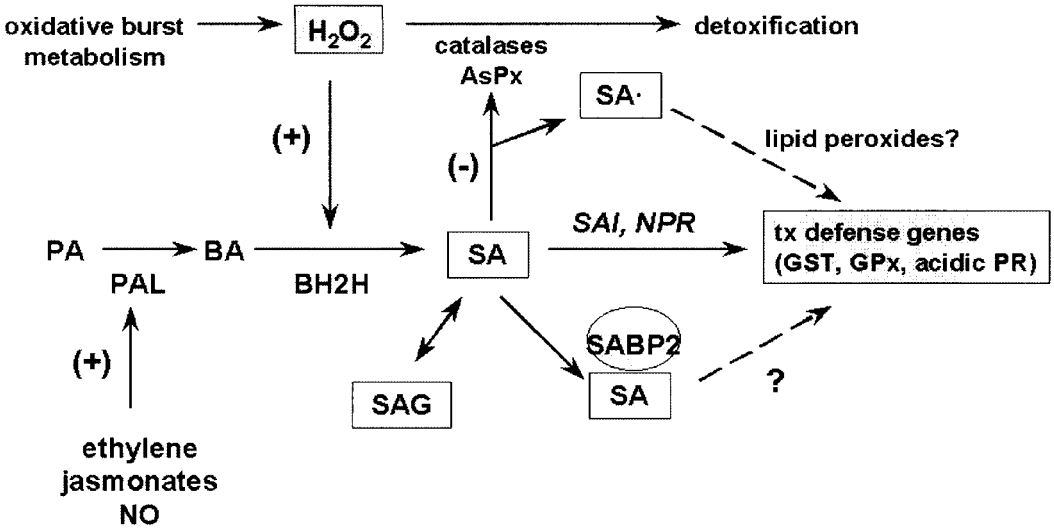


*Figure 13.* Perception and transmission of SA signal. Salicylic acid (SA) is synthesized from phenylalanine (PA) and benzoic acid (BA). This biosynthetic pathway is activated by ethylene, jasmonates and NO (which induce the expression of the gene for the enzyme phenylalanine ammonia-lyase, PAL), and by $H_2O_2$ (which activates the enzyme benzoic acid 2-hydroxylase, BA2H). SA can be accumulated as the inactive glucoside form (SAG). SA activates transcription of genes encoding for acidic pathogenesis-related proteins (acidic PR) and for the oxidative stress-detoxifying enzymes glutathione-S-transferase (GST), and glutathione peroxidase (GPx). This effect of SA can be mediated by free radicals (SA)produced by inhibition of catalases and ascorbate peroxidase (AsPx)by the product of genes *npr1/sai1/nim1*, or by a SA-binding protein (SABP2).

Different approaches have been used to elucidate the mechanism by which SA is able to transduce the signal through the nucleus and activate transcription of genes. Although some of the components involved have been identified, the mechanism is still unclear. Genetic approaches have led to the isolation of the npr1/sai1/nim1 class of *Arabidopsis* mutants (npr, nonexpresser of PR gene; sai, salicylic acid insensitive; nim, noninducible immunity). Recently, the NPR1 protein was identified as a 60kDa-soluble protein containing ankyrin repeats, that are thought to function in protein-protein interactions (Cao *et al*, 1997). Over-expression of NPR1 leads to enhanced resistance to diverse pathogens (Cao *et al*, 1998).

Biochemical and molecular approaches have lead to the characterization of SA-binding proteins in tobacco (Durner *et al*, 1997). One group of these SA-binding proteins are heme-containing enzymes involved in detoxification of $H_2O_2$, such as catalases and ascorbate peroxidase (AsPx). Binding of SA to these proteins inhibits their enzymatic activity, leading to an increase in the endogenous concentration of $H_2O_2$. The hypothesis that $H_2O_2$ and other ROS could act as second messengers downstream of SA in the transcriptional activation of genes is now being debated. It has been also suggested that the radical SA, produced by the interaction of SA with catalases and peroxidases, could participate as a secondary signal through lipid peroxidation. Recently, a new soluble SA-binding protein was identified in tobacco, although its nature and function remain to be elucidated (Du and Klessig, 1997). In the nucleus, the SA signal is able to activate transcription of immediate early and late genes. Several immediate early genes, which transcription does not require *de novo* protein synthesis, have been isolated. A homologue to myb gene (Yang and Klessig, 1996), a gene with homology to glucosyltransferase genes (Horvath and Chua, 1996), and GST genes (Ulmasov *et al*, 1994) are among this class. Cis-elements that respond to SA with an immediate early kinetics have been identified in

the promoter of some of these and other viral and bacterial genes (Qin *et al*, 1994). *In vitro* DNA-protein binding assays and *in vivo* transcriptional assays using transgenic plants have given some information about the mechanism by which these elements are activated by SA. Results obtained from these studies indicate that SA increases the binding of transcription factors (from the TGA family of bzip factors) to these cis elements, mediated by protein phosphorylation (Jupin and Chua, 1996; Stange *et al*, 1997). Interestingly, other signals like MeJA and auxins are also able to activate transcription of GST genes, using the same cis-elements and the same mechanism. Therefore, it seems that these nuclear events of transcriptional activation of oxidative stress-protective genes are the convergence point of different signal transduction pathways.

REFERENCES


ALEXANDRE J, LASSALES JP, KADO RT (1990) Opening of calcium channels in isolated red beet root vacuole membrane by inositol 1,4,5 triphosphate. Nature 343: 567-570

ALLEN GJ, MUIR SR, SANDERS D (1995) Release of calcium from individual plant vacuoles by both $InsP_3$ and cyclic ADP-ribose. Science 268: 735-737

ARIOLI T, PENG L, BETZNER A, BURN J, WITTKE W, HERTH W, CAMILLERI C, HÖFTE H, PLAZINSKI J, BIRCH R, CORK A, GLOVER J, REDMOND J, WILLIAMSON R (1998) Molecular analysis of cellulose biosynthesis in Arabidopsis. Science 279: 717-720

ASSMANN (1993) Signal transduction in guard cells. Annu Rev Cell Biol 9: 345-375

BAR-PELED M, BASSHAM DC, RAIKHEL NV (1996). Transport of proteins in eukaryotic cells: more questions ahead. Plant Mol Biol 32: 223-249

BARRIEU F, CHAUMONT F, CHRISPEELS MJ (1998). High expression of the tonoplast aquaporin ZmTIP1 in conducting tissues of maize. Plant Physiol 17 :1153-1163

BASSHAM DC, RAIKHEL NV (1997) Molecular aspects of vacuolar biogenesis. Adv Bot Res 25: 43-58

BEEVERS L, RAIKHEL NV (1998) Transport to the vacuole: receptors and trans elements. J Exp Bot 49: 1271-1279

BLEE E, JOYARD J (1996) Envelope membranes from spinach chloroplast are a site of metabolism of fatty acid hydroperoxides. Plant Physiol 110:445-454

BLUMWALD E, AHARON GS, LAM BC-H (1998) Early signal transduction pathways in plant-pathogen interactions. Trends Plant Sci 3: 342-346

BOUDET AM (1998) A new view of lignification. Trend Plant Sci 3: 67-71

CARPITA N, GIBEAUT DM (1993) Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. Plant J 3: 1-30

CAO H, GLAZEBROOK J, CLARKE JD, VOLKO S, DONG XN (1997) The Arabidopsis NPR1 gene that controls systemic acquired resistance encodes a novel protein containing ankyrin repeats. Cell 88: 57-63

CAO H, LI X, DONG XN (1998) Generation of broad-spectrum disease resistance by overexpression of an essential regulatory gene in systemic acquired resistance. Proc Natl Acad Sci USA 95: 6531-6536

CHAUMONT F, BARRIEU F, HERMAN EM, CHRISPEELS MJ (1998) Characterization of a maize tonoplast aquaporin expressed in zones of cell division and elongation. Plant Physiol 117: 1143-1152

CHEREL I, DARAM P, GAYMARD F, HOREAU C, THIBAUD J-B, SENTENAC H (1996) Plant K+ channels: structure, activity and function. Biochem Soc Trans 24: 964-970

CHRISPEELS MJ, RAIKHEL NV (1992) Short peptide domains target proteins to plant vacuoles. Cell 68: 613-616

DOYLE DA, CABRAL J.M, PFUETZNER RA, KUO A, GULBIS JM, COHEN SL, CHAIT BT, MACKINNON R (1998) The structure of the potassium channel: molecular basis of $K^+$ conduction and selectivity. Science 280: 69-81

DRIOUICH A, FAYE L, STAEHELIN A (1993) The plant Golgi apparatus: a factory for complex polysaccharides and glycoproteins. Trends Biochem Sci 18: 210-214

DU H, CLARKE AE, BACIC A (1996) Arabinogalactan proteins: a class of extracellular matrix proteoglycans involved in plant growth and development. Trends Cell Biol 6: 411-414

DU H, KLESSIG DF (1997) Role for salicylic acid in the activation of defense responses in catalase-deficient transgenic tobacco. Mol Plant Microbe-Int 7: 922-925

DURNER J, SHAH J, KLESSIG DF (1997) Salicylic acid and disease resistance in plants. Trends Plant Sci 2: 266-274

DURNER J, WENDEHENNE D, KLESSIG DF (1998) Defense gene induction in tobacco by nitric oxide, cyclic GMP, and cyclic ADP-ribose. Proc Natl Acad Sci USA 95: 10328-10333

FARMER EE, RYAN CA (1990) Interplant communication: Airborne methyl jasmonate induces synthesis of proteinase inhibitors in plant leaves. Proc Natl Acad Sci USA 87: 7713-7716

FARMER EE, RYAN CA (1992) Regulation of expression of proteinase inhibitor genes by methyl jasmonate and jasmonic acid. Plant Physiol 98: 995-1002

FOX TC, GUERINOT ML (1998) Molecular biology of cation transport in plants. Annu Rev Plant Mol Biol 49: 669-696

GAYMARD F, PILOT G, LACOMBE B, BOUCHEZ D, BRUNEAU D, BOUCHEREZ J, MICHAUX-FERRIERE H (1998) Identification and disruption of a *Shaker*-like outward channel involved in $K^+$ release into the xylem sap. Cell 94: 647-655

GILROY S, READ ND, TREWAVAS AJ (1990) Elevation of stomatal cytosol calcium by photolysis of loaded caged probes initiates stomatal closure. Nature 346: 769-771

GUPTA R, HUANG Y, KIEBER JJ, LUAN S (1998) Identification of a dual-specificity protein phosphatase that inactivates MAP kinase from Arabidopsis. Plant J 16: 581-590

HAHN MG (1996) Microbial elicitors and their receptors in plants. Annu Rev Phytopathol 34: 387-412

HAMMOND-KOSACK KE, JONES JD (1996) Resistance gene-dependent plant defense responses. Plant Cell 8: 1773-1791

HARMS K, ATZORN R, BRASH A, KÜHN H, WASTERNACK C, WILLMITZER L, PEÑA-CORTES H (1995) Expression of a flax allene oxide synthase cDNA leads to increased endogenous jasmonic acid (JA) levels in transgenic potato plants but not to a corresponding activation of JA-responding genes. Plant Cell 7: 1645-1654

HERDE O, FUSS H, PEÑA-CORTES H, FISAHN J (1995) Proteinase inhibitor II gene expression induced by electrical stimulation and control of photosynthetic activity in tomato plants. Plant Cell Physiol 36: 737-742

HERDE O, ATZORNS R, FISANH J, WASTERNACK C, WILLMITZER L, PEÑA-CORTES, H (1996) Localized wounding by heat initiates the accumulation of proteinase inhibitor II in ABA-deficient plants by triggering jasmonic acid biosynthesis. Plant Physiol 112: 853-860

HERDE O, PEÑA-CORTES H, WASTERNACK C, WILLMITZER L, FISANH J (1999) Electric signaling and pin2 gene expression on different stimuli depend on a distinct threshold level of endogenous abscisic acid in several abscisic acid-deficient tomato mutants. Plant Physiol 119: 213-218

HILDMANN T, EBNEHT M, PEÑA-CORTES H, SANCHEZ-SERRANO J, WILLMITZER L, PRAT S. (1992) General roles of abscisic acid and jasmonic acids in gene activation as result of mechanical wounding. Plant Cell 4: 1157-1170

HIRSCH RE, LEWIS BD, SPALDING EP, SUSSMAN MR (1998) A role for the AKT1 potassium channel in plant nutrition. Science 280: 918-921

HODGKIN AL, KEYNES RD (1957) Movements of labelled calcium in giant squid axons. J Physiol 138: 253-281

HORVATH D, CHUA N-H (1996) Identification of an immediate-early salicylic acid-inducible tobacco gene and characterization of induction by other compounds. Plant Mol Biol 31: 1061-1072

ISHITANI M, XIONG L, STEVENSON B, ZHU J-K (1997) Genetic analysis of osmotic and cold stress signal transduction in Arabidopsis: interactions and convergence of abscisic acid-dependent and abscisic acid-independent pathways. Plant Cell 9: 1935-1949

JAN LY, JAN YN (1992) Tracing the roots of ion channels. Cell 69: 715-718

JOHANSSON I, KARLSSON M, SHUKLA VK, CHRISPEELS MJ, LARSSON C, KJELLBOM P (1998) Water transport activity of the plasma membrane aquaporin PM28A is regulated by phosphorylation at two different sites. Plant Cell 10: 451-460

JONAK C, KIEGERL S, LIGTERINK W, BARKER PJ, HUSKISSON NS, HIRT H (1996) Stress signaling in plants: a mitogen-activated protein kinase pathway is activated by cold and drought. Proc Natl Acad Sci USA 93: 11274-11279

JUPIN I, CHUA N-H (1996) Activation of the CaMV as-1 cis-element by salicylic acid: differential DNA-binding of a factor related to TGA1a. EMBO J 15: 5679-5689

KLEITER A, GERHARDT B (1998) Glyoxysomal and beta-oxidation of long-chain fatty acids: completeness of degradation. Planta 206: 125-130

LIU Q, KASUGA M, SAKUMA Y, ABE H, MIURA S, YAMAGUCHI-SHINOZAKI K, SHINOZAKI K (1998) Two transcription factors, DREB1 and DREB2, with an EREBP-AP2 DNA binding domain separate two cellular signal transduction pathways in drought- and low-temperature-responsive gene expression, respectively, in Arabidopsis. Plant Cell 10: 1391-1406

LIU K, LUAN S (1998) Voltage-dependent $K^+$ channels as targets of osmosensing in guard cells. Plant Cell 10: 1957-1970

LUAN S (1998) Protein phosphatases and signaling cascades in higher plants. Trends Plant Sci 3: 271-275

MALHO R, MOUTINHO A, VAN DER LUIT A, TREWAVAS AJ (1998) Spatial characteristics of calcium signalling: the calcium wave as a basic unit in plant cell calcium signalling. Phil Trans Roy Soc B 3: 1463-1473

MALONE M, STANKOVIC B, (1991) Surface potentials and hydraulic signals in wheat leaves following localized wounding by heat. Plant Cell Envir 14: 431-436

MARCHANT JS, TAYLOR CW (1997) Cooperative activation of $IP_3$ receptors by sequential binding of $IP_3$ and $Ca^{2+}$ safeguards against spontaneous activity. Curr Biol 7: 510-518

MAUREL C, REIZER J, SCHROEDER JI, CHRISPEELS MJ (1993) The vacuolar membrane protein γ-TIP creates water specific channels in *Xenopus* oocytes. EMBO J 12: 2241-2247

McCABE PF, VALENTINE TA, FORSBERG LS, PENNELL RI (1997) Soluble signals from cells identified at the cell wall establish a developmental pathway in carrot. Plant Cell 9: 2225-2241

MESKIENE I, BOGRE L, GLASER W, BALOG J, BRANDSTOTTER M, ZWERGER K, AMMERER G, HIRT H (1998) MP2C, a plant protein phosphatase 2C, functions as a negative regulator of mitogen-activated protein kinase pathways in yeast and plants. Proc Natl Acad Sci USA 95: 1938-1943

MUÑOZ P, NORAMBUENA L, ORELLANA A (1996) Evidence for a UDP-Glucose transporter in Golgi apparatus-derived vesicles from pea and its possible role in polysaccharide biosynthesis. Plant Physiol 112: 1585-1594

NECKELMANN G, ORELLANA A (1998) Metabolism of uridine 5'-diphosphate-glucose in Golgi vesicles from pea stems. Plant Physiol 117: 1007-1014

NEUHAUS JM, ROGERS JC (1998) Sorting of proteins to vacuoles in plant cells. Plant Mol Biol 38: 127-144

NICOL F, HIS I, JAUNEAU A, VERNHETTES S, CANUT H, HÖFTE H (1998) A plasma membrane-bound putative endo-1,4-ß-D-glucanase is required for normal wall assembly and cell elongation in Arabidopsis. EMBO J 17: 5563-5576

O'DONNELL OJ, CALVERT C, ATZORN R, WASTERNACK C, LEYSER HMO, BOWLES DJ (1996) Ethylene as a signal mediating the wound response of tomato plants. Science 274: 1914-1917

PAPAZIAN DM, BEZANILLA F (1997) How does an ion channel sense voltage? News Physiol Sci 12: 203-210

PEAR J, KAWAGOE Y, SCHRECKENGOST W, DELMER D, STALKER D (1996) Higher plants contain homologues of the bacterial celA genes encoding the catalytic subunit of cellulose synthase. Proc Natl Acad Sci USA 93: 12637-12642

PEARCE G, STRYDOM D, JOHNSON S, RYAN CA (1991) A polypeptide from tomato leaves induces the synthesis of wound-inducible proteinase inhibitor proteins. Science 253: 895-898

PEÑA-CORTES H, SANCHEZ-SERRANO JJ, MERTENS R, WILLMITZER L, PRAT S (1989) Abscisic acid is involved in the wound-induced expression of the proteinase inhibitor II gene in potato and tomato. Proc Natl Acad Sci USA 86: 9851-9855

PEÑA-CORTES H, WILLMITZER L, SANCHEZ-SERRANO JJ (1991) Abscicic acid mediates wound induction but not developmental-specific expression of the proteinase inhibitor II gene family. Plant Cell 3: 963-972

PEÑA-CORTES H, ALBRECHT T, PRAT S, WEILER EW, WILLMITZER L, (1993) Aspirin prevents wound-induced gene expression in tomato plants by blocking jasmonic acid synthesis. Planta 191: 123-128

PEÑA-CORTES H, FISAHN J, WILLMITZER L (1995) Signals involved in wound-induced proteinase inhibitor II gene expression in tomato and potato plants. Proc Natl Acad Sci USA 92: 4106-4113

PEÑA-CORTES H, WILLMITZER L (1995) Role of hormones in gene activation in response to wounding. In: DAVIES PJ (ed) Plant Hormones: Physiology, Biochemistry and Molecular Biology. Dordrecht, The Netherlands: Kluwer Academic Publisher. pp 495-514

PEÑA-CORTES H, PRAT S, ATZORN R, WASTERNACK C, WILLMITZER L (1996) Abscisic acid-deficient plants do not accumulate proteinase inhibitor II mRNA following system in treatment. Planta 198: 447-451

PERRIN R, DeROCHER A, BAR-PELED M, XENG W, NORAMBUENA L, ORELLANA A, RAIKHEL N, KEEGSTRA K (1999) Xyloglucan fucosyltransferase an enzyme involved in plant cell wall biosynthesis. Science 284: 1976-1979

PRESTON GM, CARROLL TP, GUGGINO WB, AGRE P (1992) Appearance of water channels in *Xenopus* oocytes expressing red cell CHIP28 protein. Science 256: 385-387

QIN X-F, HOLUIGUE L, HORVATH DM, CHUA N-H (1994) Immediate early transcription activation by salicylic acid via the Cauliflower Mosaic Virus as-1 element. Plant Cell 6: 863-874

RONALD PC (1997) The molecular basis of disease resistance in rice. Plant Mol Biol 35: 179-186

ROTHMAN JE, SOLLNER TH (1997) Throttle and dampers: controlling the engine of membrane fusion. Science 276: 1212-1213

RYAN CA (1987) Oligosaccharide signaling in plants. Ann Rev Cell Biol 3: 295-317

SANCHEZ-SERRANO JJ, AMATI S, EBNETH M, HILDMANN T, MERTENS R, PEÑA-CORTES H, PRAT S, WILLMITZER L (1991) The involvement of abscisic acid in wound responses of plants. In: DAVIES WJ, JONES HG (eds) The Physiology and Biochemistry of ABA. London, England: Bios Scientific Publishers Limited ("Bios")

SANDERFOOT AA, AHMED SU, MARTY-MAZARS D, RAPOPORT I, KIRCHHAUSEN T, MARTY F, RAIKHEL NV (1998) A putative vacuolar cargo receptor partially colocalizes with AtPEP12p on a prevacuolar compartment in Arabidopsis roots. Proc Natl Acad Sci USA 95: 9920-9925

SAXENA I, BROWN M, FEVRE M, GEREMIA R, HENRISSAT B (1995) Multidomain architecture of ß-glycosyltransferases: implications for mechanism of action. J Bacteriol 177: 1419-1424

SCHROEDER JI, WARD JM, GASSMAN W (1994) Perspectives on the physiology and structure of inward rectifying K+ channels in higher plants: biophysical implications for $K^+$ uptake. Ann Rev Biomol Struct 23: 441-471

SHEEN J (1996) $Ca^{2+}$-dependent protein kinases and stress signal transduction in plants. Science 274: 1900-1902

SHINOZAKI K, YAMAGUCHI-SHINOZAKI K (1997) Gene expression and signal transduction in water-stress response. Plant Physiol 115: 327-334

SONG W-Y, WANG G-L, CHEN L, KIM H-S, PI LI-YA, GARDNER J, WANG B, HOLSTEN T, ZHAI W-X, ZHU L-H, FAUQUET C, RONALD PC (1995) A receptor kinase-like protein encoded by the rice disease resistance gene *Xa21*. Science 270: 1804-1806

STACEY NJ, ROBERTS JC, CARPITA NC, WELLS B, McCANN MC (1995) Dynamic changes in cell surface molecules are very early events in the differentiation of mesophyll cells from *Zinnia elegans* into tracheary elements. Plant J 8: 891-906

STANGE C, RAMIREZ I, GOMEZ I, JORDANA X, HOLUIGUE L (1997) Phosphorylation of nuclear proteins directs binding to salicylic acid-responsive elements. Plant J 11: 1315-1324

TEMPEL BL, PAPAZIAN DM, SCHWARZ TL, JAN Y-N, JAN LY (1987) Sequence of a probable potassium channel component encoded at *Shaker* locus of *Drosophila*. Science 237: 770-775

TRUDEAU MC, WARMKE JW, GANETZKY B, ROBERTSON G (1995) HERG, a human inward rectifier in the voltage-gated potassium channel family. Science 269: 92-95

ULMASOV Y, HAGEN G, GUILFOYLE T (1994) The ocs element in the soybean GH2/4 promoter is activated by both active and inactive auxin and salicylic acid analogues. Plant Mol Biol 26: 1055-1064

VAN CAMP W, VAN MONTAGU M (1998) $H_2O_2$ and NO: redox signals in disease resistance. Trends Plant Sci 3: 330-334

VICK BA, ZIMMERMANN DC (1987) Oxidative systems for the modification of fatty acids. In: STUMPF PK, CONN EE (eds) The Biochemistry of Plants, Vol 9. New York: Academic Press. pp 53-90

WANG GL, RUAN DL, SONG WY, SIDERIS S, CHEN LL, PI LY, ZHANG SP, ZHANG Z, FAUQUET C, GAUT BS, WHALEN MC, RONALD PC (1998) Xa21D encodes a receptor-like molecule with a leucine-rich repeat domain that determines race-specific recognition and is subject to adaptive evolution. Plant Cell 10: 765-779

WARD JM, PEI Z-M, SCHROEDER JI (1995) Roles of ion channels in initiation of signal transduction in higher plants. Plant Cell 7: 833-844

WEIG A, DESWARTE C, CHRISPEELS, MJ (1997) Molecular analysis of the aquaporin gene family in *Arabidopsis thaliana*. Plant Physiol 114: 1347-1357

WILDON DC, DOHERTY HM, EAGLES G, BOWLES DJ, THAIN JF (1989) Systemic responses arising from localized heat stimuli in tomato plants. Ann Bot 64: 691-695

WILDON DC, THAIN JF, MINCHIN PEH, GUBB IR, REILLY AJ, SKIPPER YD, DOHERTY HM, ODONNELL PJ, BOWLES DJ (1992) Electrical signaling and systemic proteinase inhibitor induction in the wounded plant. Nature 360: 62-65

XU Q, FU HH, GUPTA R, LUAN S (1998) Molecular characterization of a tyrosine-specific protein phosphatase encoded by a stress-responsive gene in Arabidopsis. Plant Cell 10: 849-857

YAMAGUCHI-SHINOZAKI K, SHINOZAKI K (1994) A novel cis-acting element in an Arabidopsis gene is involved in responsiveness to drought, low-temperature, or high-salt stress. Plant Cell 6: 251-264

YANG Y, KLESSIG DF (1996) Isolation and characterization of a tobacco mosaic virus-inducible myb oncogene homologue from tobacco. Proc Natl Acad Sci USA 93: 14972-14977

YANG Y, SHAH J, KLESSIG DF (1997) Signal perception and transduction in plant defense responses. Genes Develop 11: 1621-1639

ZHU Q, DRÖGE-LASER W, DIXON RA, LAMB C (1996) Transcriptional activation of plant defense genes. Curr Opin Genet Develop 6: 624-630