MEETING REPORT

Excitation in Plant Membrane Biology

Very few substances can waltz right through cell membranes—indeed, could they do so, cells would lose their integrity rapidly. The control of ion and macromolecule passage through both plasma membranes and organelar membranes falls to various channel, pump, and carrier proteins, which are diagrammed in Figure 1. Not only do these proteins allow ions and other macromolecules to pass from one side of the membrane to the other, they also establish and maintain ion gradients that are essential for the control of metabolic processes, involved in storage processes, signal, and energy transduction, and, in some cases, render cells electrically excitable. The properties of these proteins alter in response to environmental and intracellular cues, allowing for controlled ion and macromolecule fluxes. These fluxes in turn initiate many of the cell’s responses to environmental stimuli as diverse as hormones, touch, and light.

With the development of the patch clamp technique, along with methods to isolate high quality plant protoplasts, it has become possible to analyze in exquisite detail the currents that pass across plant cell membranes. These and other technical advances have also paved the way for the characterization of other membrane-mediated processes, such as sugar and amino acid transport. The ninth of the triennial International Workshops on Plant Membrane Biology, which was held from July 19 to 24, 1992, in Monterey, California, bore witness to the dramatic progress that has seized the field of plant membrane biology in the last year or two. Many of the more than 300 participants at this year’s meeting, which was organized by Lincoln Taiz, together with Frances Dupont, Russell Jones, Rob Leonard, Bill Lucas, and Julian Schroeder, reported remarkable advances in the biochemical and molecular characterization of a wide array of plant membrane processes. In this report we will highlight a few of these advances, focusing on ion transport in higher plants. Space constraints make it impossible to discuss more than a fraction of the many exciting presentations on other topics of plant transport and membrane biology.

Proton Pumps

The plasma membrane potential of resting plant cells, at approximately −120 to −180 mV, is much more negative than that of animal cells. The establishment of ion gradients is mediated by the electrogenic H⁺-ATPase, which pumps protons out of the cell at the expense of ATP, creating both an electrical gradient and a pH gradient. Some of the negative potential also results from the selective permeability of the unstimulated membrane to K⁺ ions, which, as in animal cells, tend to diffuse from the inside to the outside down their electrochemical potential gradient. The electrical gradient, in conjunction with the pH gradient, is thought to provide the driving force for the transport and cotransport of nutrients such as K⁺, nitrate, phosphate, chloride, sugars, and amino acids. In the case of proton-coupled cotransporters, it has been suggested that when actively extruded protons move back down the electrochemical potential gradient and reenter the cell, other molecules are cotransported simultaneously, via the appropriate transporter proteins.

Because H⁺-ATPases drive so many transport processes in plant cells, as well as having a critical role in the regulation of intracellular pH, it is not surprising that they are encoded by multigene families whose members may be regulated and/or

Figure 1. Ion Transport and Signal Transduction in Biological Membranes.

Three-dimensional diagram of the fluid mosaic model of the membrane, showing integral transport proteins embedded in the lipid bilayer. From left to right are shown: (A) an ion pump, (B) an ion channel, (R, C) coupling proteins for signal perception and transduction, and (C) a carrier. Reprinted from Hedrich et al. (1987) and used with permission from Trends in Biochemical Sciences.
expressed differentially (Harper et al., 1990). In tobacco, four genes have so far been identified that encode putative plasma membrane H+-ATPases. Marc Boutry (University of Louvain) showed that three of these (PMA1-3) share high similarity, whereas the fourth (PMA4) is somewhat diverged. Using gene-specific probes and GUS reporter gene constructs, Boutry and his colleagues found that all four genes are expressed in many different organs but that the levels of expression vary from gene to gene and organ to organ. All of these genes have short open reading frames upstream of the actual translation start site, raising the possibility that they are subject to translational control.

Arabidopsis also has multiple H+-ATPase genes—at least 10, according to Jeff Harper (Scripps Research Institute), who has used the polymerase chain reaction to amplify these AHA genes. Using translational fusions to a GUS reporter gene followed by both histochemical and biochemical analysis of GUS expression, Harper, Michael Sussman, and their colleagues (University of Wisconsin) have found that at least some of the AHA genes are differentially expressed. For example, the most divergent of the 10 isoforms, AHA10, appears to be expressed preferentially in developing seeds. This isoform is particularly divergent in the conserved regions near its C terminus. The C-terminal regions of plasma membrane H+-ATPases have been proposed to carry out autoinhibitory functions, and the sequence alterations of AHA10 in this region raise the possibility of a distinct mode of regulation for this isoform.

Proton pumps are found not only in the plasma membrane, but also in the vacuolar membrane, or tonoplast. The vacuolar H+-ATPase, or V-ATPase, and the tonoplast H+-translocating pyrophosphatases (H+-PPases) pump protons into the vacuole; proton export and membrane potential are then coupled to the import of inorganic and organic ions and sugars into the vacuole. Phil Rea (University of Pennsylvania) reported that H+-PPase genes have been cloned from Arabidopsis and beet (Sarafian et al., 1992); although the derived proteins are very similar to one another, they are unlike any known ion pump. They may turn out to be related to the H+-PPI synthases of several photosynthetic bacteria of the genus Rhodospirillum, which share a common ancestor with plant mitochondria. Rea speculated that during the evolution of plants, the enzyme gained its present dependence on K+ and lost its free reversibility. Dale Sanders (University of York), in collaboration with Rea and Ron Poole (McGill University), reported that the vacuolar H+-PPase transports K+ directly into the vacuole. This finding points to a novel and perhaps central mechanism for K+ uptake into vacuoles.

**Tonoplast Channels**

Ion channels in the vacuolar membrane are central to tonoplast transport, storage processes, homeostasis, and cellular signal transduction. For example, in plants that show crassulacean acid metabolism (CAM), malate, which is synthesized in the cytosol as a byproduct of nocturnal CO2 fixation, accumulates in the vacuole during the night. During the day, this stored malate is transported back to the cytoplasm, where it is decarboxylated. Andrew Smith (University of Oxford) described a novel type of ion channel that may provide an important pathway for malate uptake into the vacuole of the CAM plant Kalanchoe. This channel is activated by positive potentials inside the vacuole. Smith suggested that vacuolar proton pumps that charge the inside of the vacuole could drive passive malate uptake into the vacuole through this channel.

Plant vacuoles are also an important source of cytosolic Ca2+, and Ca2+-induced Ca2+ release is thought to play an important role in plant signal transduction. Eva Johannes (University of York) described Ca2+-permeable ion channels in beet root vacuoles whose Ca2+- and voltage-dependence suggest that they may contribute to Ca2+-mediated Ca2+ release. Johannes and researchers from several other groups pointed out that they have not been able to reproduce earlier results reporting inositol 1,4,5-trisphosphate-activated Ca2+ channels in plant vacuoles (Alexandre et al., 1990).

**K+ Regulation**

In animals, the role of K+ fluxes through voltage-dependent K+ channels is to reset the membrane potential after it is changed, for example by an action potential speeding along a neuron. In plants, by contrast, K+ channel activity can lead to long-term changes in intracellular K+ concentration. Such changes influence plant nutrition, enzyme activities, and turgor pressure: as water accompanies K+ into or out of the cell by osmosis, the resulting volume changes lead to cell expansion, growth, and plant, leaf, and stomatal movements. At least two classes of K+ channels, both voltage gated, have been identified in guard cells and other plant cells: an inward-conducting channel, which opens when the membrane is hyperpolarized, and an outward-conducting channel, which opens with depolarization (Schoeder et al., 1987).

Rick Gaber (Northwestern University) and Hervé Sentenac (CNRS, Gif-sur-Yvette) described an exciting breakthrough in the field of plant K+ transport, the cloning of different Arabidopsis genes that share structural similarity to animal K+ channels (Anderson et al., 1992; Sentenac et al., 1992). Both genes were cloned by complementation of yeast mutants deficient in K+ uptake. The derived KAT1 (Gaber) and AKT1 (Sentenac) proteins show homologies to the Shaker superfamily of K+ channels, and both are especially similar to cyclic nucleotide-gated channels. In addition, AKT1 has an ankyrin-like repeat region at its C terminus. This domain may mediate linkage to the cytoskeleton or may be involved in some other sort of protein–protein interaction. Because both AKT1 and KAT1
confer K⁺ uptake ability to yeast and research on guard cells has suggested that inward-conducting K⁺ channels provide a major pathway for K⁺ uptake in plants, both genes may encode this type of channel. This has been demonstrated for KAT₁; Xenopus oocytes injected with KAT₁ mRNA produce inward K⁺ currents upon hyperpolarization (Schachtman et al., 1992). Sentenac also showed preliminary data in which AKT₁ expressed in insect cells gave rise to inward currents.

As Gaber pointed out, however, Shaker-type K⁺ channels carry outward K⁺ currents, and it is not clear what makes KAT₁ an inward-conducting channel. Perhaps the orientation of the core channel region in the membrane is opposite to that of the Shaker channel. At any rate, the finding that KAT₁, and possibly AKT₁ as well, encodes an inward-conducting K⁺ channel represents a significant advance in membrane biology because genes encoding this important class of K⁺ channels have not yet been cloned in animal systems.

Sentenac showed that yeast cells expressing AKT₁ are able to take up K⁺ even when the extracellular concentration is as low as 0.6 μM. This finding supports conjectures from guard cell studies that inward K⁺ channels can contribute to K⁺ uptake at very low external K⁺ concentrations. Additional high affinity K⁺ uptake transporters have been suggested to provide the major mechanism for K⁺ uptake during starvation for external K⁺. These other high affinity transporters have not yet been identified, but based on analogies to bacterial, fungal, and animal K⁺ uptake systems, carriers or pumps have been suggested as possible transport mechanisms (Hesse et al., 1984; Rodriguez-Navarro et al., 1986).

Given the many functions of K⁺ channels, it is not surprising that there are at least two isoforms in Arabidopsis. In fact, Karen Ketchum and Carolyn Slayman (Yale University) used the polymerase chain reaction to amplify Shaker-type sequences in Arabidopsis and have identified a third sequence that is similar, but not identical, to KAT₁ and AKT₁. These genes may well turn out to be expressed in specific organs or cell types, and it will be interesting to see whether they have unique properties or functions. K⁺ channels are also being purified biochemically. Carsten Zeilingher and his colleagues (Universität Hannover) purified a protein from fava bean mesophyll that confers tetraethylammonium-sensitive Rb⁺ uptake to reconstituted vesicles, a property of K⁺ channels, and produces K⁺ channel activity in bilayers. The partial sequence of tryptic peptides shows that this protein is similar to KAT₁.

No outward-conducting K⁺ channel has yet been cloned from plants, but Julian Schroeder (University of California, San Diego) and colleagues have been able to detect a voltage-dependent outward K⁺ current in Xenopus oocytes injected with either total poly(A)+ mRNA from several plant tissues or transcripts from a maize shoot cDNA library (Cao et al., 1992). By fractionating the cDNA library into smaller and smaller pools that encode outward channel activity, it should be possible to isolate a gene for this K⁺ channel.

Control of Stomatal Function

The guard cells, which form the stomatal openings, have become especially popular subjects for signal transduction studies in higher plant cells. At least several of the membrane transduction mechanisms identified in guard cells are likely to be of general importance to higher plant physiology and cell biology. During stomatal opening, which is induced by conditions such as red or blue light, high humidity, or low CO₂, the guard cells accumulate large quantities of K⁺ and synthesize or take up anions. The resulting increase in osmotic pressure draws water in, and the cells swell. Because of physical constraints, swelling of the cells opens the stomatal aperture. Stomatal closing is triggered by effectors such as a decrease in light or the presence of the phytohormone abscisic acid (ABA), which is induced by water stress, and requires the efflux of K⁺ and anions from the guard cells. Water then flows out, the cells become less turgid, and the stomata close.

Patch clamp and fluorescent imaging studies have begun to reveal some of the secrets of guard cell signal transduction, particularly the response to ABA. ABA has two effects on guard cells: it induces open stomata to close, and it prevents closed stomata from opening. By loading guard cells with Ca²⁺ indicator dyes, several groups have shown that ABA treatments induce repetitive rises in Ca²⁺ levels. Julian Schroeder reported that these rises are accompanied by an inward current whose characteristics show that it is carried by Ca²⁺ and other cations. Several groups have demonstrated that Ca²⁺ is also recruited from intracellular stores. The increase in free Ca²⁺ then activates anion channels, and the resulting anion efflux depolarizes the membrane. This in turn activates voltage-gated K⁺ efflux channels. Schroeder pointed out that depolarization and cytosolic Ca²⁺ increases apparently activate not one but two distinct types of anion current, one that activates slowly and leads to a sustained depolarization (the S current) (Schroeder and Hagiwara, 1989) and one that activates and deactivates rapidly (the R current) (Keller et al., 1989). Based on the biophysical properties of the S-type anion current, Schroeder suggested that it may bear central responsibility for driving long-term ion efflux during stomatal closing (Schroeder and Keller, 1992).

It is becoming more and more evident that second messengers other than Ca²⁺ also play important roles during ABA-mediated stomatal closure. Michael Blatt (Wye College) and Enid MacRobbie and Fouad Lemtiri-Chlieh (Cambridge University) presented data suggesting that ABA-induced alkalization may be an important second messenger process for ABA-mediated enhancement of outward K⁺ channel activity (Blatt, 1992). Both microelectrode (Blatt) and fluorometric (Irving et al., 1992) measurements of cytosolic pH show that ABA induces alkalization of guard cell cytosol.
Ca\textsuperscript{2+} increases also inhibit stomatal opening. Ca\textsuperscript{2+}-induced deactivation of inward K\textsuperscript{+} channels can contribute to this inhibition (Schroeder and Hagiwara, 1989). One of Ca\textsuperscript{2+}’s many known effects on eukaryotic cells is to activate protein phosphatase 2B (PP2B), which it does in the presence of calmodulin. To ask whether Ca\textsuperscript{2+} might deactivate inward K\textsuperscript{+} channels by causing their dephosphorylation, Sarah Assmann (Harvard University) and her colleagues treated guard cell protoplasts with the immunosuppressants cyclosporin A (CsA) and FK506. The complex formed when either compound binds to its endogenous receptor in animal cells inactivates PP2B. Assmann’s group showed that when applied to guard cell protoplasts, CsA reverses the effects of high Ca\textsuperscript{2+}; FK506 does so as well, but only if its receptor, FKBP, is also supplied. This effect of CsA and FK506/FKBP suggests that Ca\textsuperscript{2+}-activated dephosphorylation of K\textsuperscript{+} channels may contribute to their deactivation. These results also imply that guard cells contain cyclophilins, and, indeed, Assmann’s collaborators in Stuart Schreiber’s group found that guard cells contain two small proteins with biochemical similarity to animal cyclophilins.

Several lines of evidence show that outward as well as inward K\textsuperscript{+} channels are highly regulated by cytosolic modulators. Nava Moran (Weizmann Institute) presented data suggesting that competing phosphorylation processes may both activate and inactivate outward K\textsuperscript{+} channels in pulvinus cells. Assmann reported that GTP\textsubscript{y}S, which tends to stimulate G protein activity, inhibits outward K\textsuperscript{+} channels in mesophyll cells.

Another plant growth regulator that affects guard cells is auxin, which has two effects: it induces a transient depolarization followed by a sustained hyperpolarization and concomitant stomatal opening. Rainer Hedrich (Universität Hannover) showed that the characteristics of auxin regulation of R-type anion channels suggest that these anion channels are responsible for auxin-induced depolarization (Marten et al., 1991). Auxin affects the opening of anion channels only when it is applied extracellularly; internally supplied auxin does not induce the depolarization, showing that the site of auxin action is on the extracellular side. Hedrich and colleagues showed that auxin activates the H\textsuperscript{+}-ATPase after anion channel activation has subsided, and this stimulation of H\textsuperscript{+} extrusion most likely drives auxin-mediated stomatal opening.

Some growth regulators have different effects on different cell types. Although ABA increases cytosolic Ca\textsuperscript{2+} levels in guard cells, Simon Gilroy and Russell Jones (University of California, Berkeley) showed that it decreases Ca\textsuperscript{2+} levels in barley aleurone cells. Treatment of aleurone cells with gibberellic acid (GA), by contrast, increases cytosolic Ca\textsuperscript{2+}; it also stimulates α-amylase secretion (Gilroy and Jones, 1992). α-Amylase synthesis and stability require Ca\textsuperscript{2+} in the lumen of the endoplasmic reticulum, and GA treatment also increases the activity of the Ca\textsuperscript{2+}-ATPase that pumps Ca\textsuperscript{2+} into the ER. This GA effect, which may be mediated by calmodulin, is also reversed by ABA. A possible explanation for the opposite effects of ABA on Ca\textsuperscript{2+} levels in guard cells and aleurone cells is that the site of ABA reception is not an ion channel itself. Rather, as has been suggested to be the case in guard cells, ABA binding may trigger second messenger events, such as G protein activation. These second messengers might then target different membrane transporters, depending on the cell type.

Root Ion Transport

Many higher plant cell types, in addition to guard cells, lend themselves well to the identification and characterization of specialized membrane transport and signal transduction mechanisms. Initial patch clamp studies of a number of plant cell types were reported at the meeting, including several focused on ion transport in roots.
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Cotransporters—Their Isolation and Characterization

One of the highlights of the meeting was the molecular cloning of a growing group of H+/coupled cotransporters, which is setting the stage for the molecular dissection of their functions. Many different routes to gene isolation can be, and have been, taken. One approach is genetic. Mutants that tolerate a cytotoxic compound that enters through, or blocks the function of, a transporter or channel may define a gene that encodes that transporter or channel. Nigel Crawford (University of California, San Diego) used this approach to clone from Arabidopsis the first higher plant nitrate transporter gene.

The mutation that defined the nitrate transporter is a member of one of eight complementation groups that confer to Arabidopsis the ability to grow on chlorate. Chlorate is taken up by a nitrate transporter and, once internalized, is normally metabolized by nitrate reductase to chlorite, which is toxic. Any number of mutations could theoretically render plants tolerant to chlorate, but because chl1 mutants have normal nitrate reductase activity and reduced uptake of both chlorate and nitrate, they were good candidates for lesions in a nitrate transporter. The CHL1 gene, which Crawford and his colleagues cloned using a T-DNA-tagged allele isolated by Ken Feldmann (University of Arizona), has no significant similarity to any other protein, including known fungal and bacterial nitrate transporters. However, like many other transporters, the predicted protein has 12 membrane-spanning segments. Crawford and colleagues showed that external exposure of voltage-clamped Xenopus oocytes injected with CHL1 transcripts to nitrate and protons evokes an inward current into the oocytes. This inward current can probably be attributed to multiple protons entering the cell for every nitrate molecule taken up.

As more and more channels and transporters are cloned, it is clear that multiple isoforms are the rule rather than the exception. Norbert Sauer (Universität Regensberg) reported, for instance, that there are at least 12 distinct but similar sugar transporter genes in Arabidopsis, and Dan Bush has cloned three additional Arabidopsis sequences on the basis of their similarity to a generalized sugar transporter sequence. The predicted proteins encoded by the latter sequences share only 30% identity with the Arabidopsis STP1 sugar transporter (Sauer et al., 1990). In a similar vein, Heven Sze (University of Maryland) showed that multiple genes in both Arabidopsis and oat encode the 16-kD proteolipid subunit of V-type ATPases.

Maarten Chrispeels (University of California, San Diego) described the cloning and characterization of an entirely different type of transporter, a putative water channel. This protein, tonoplast integral protein (TIP), is very abundant in the tonoplast. Chrispeels's lab cloned the bean TIP gene using anti-TIP antibodies and then isolated homologous sequences from Arabidopsis. The bean TIP gene is 25 to 30% identical to bovine MIP, plant NOD26, and bacterial GlpF, all of which have six transmembrane domains and have been proposed to function in some sort of transport process. Recently, another family member, CHIP28, which was cloned from erythrocytes, was suggested to encode a water channel (Preston et al., 1992). Chrispeels reported that Xenopus oocytes expressing Arabidopsis γ-TIP, like those expressing CHIP28, swell rapidly and burst when placed in a hypo-osmotic medium. Mercuric chloride, which inhibits water channels in mammalian cells, blocks this TIP-induced swelling.

Promoter-GUS fusions and in situ analysis of RNA accumulation indicate that one of the TIP homologs, γ-TIP, is expressed in regions of cell enlargement, for example the zone of cell elongation just behind the root tip. This is consistent with a putative function in cells in which vacuoles are forming and water accumulation
would be rapid. Another isoform is induced by water stress. This TIP might allow rapid and regulated water transfer from the vacuole to the cytoplasm. It will be interesting to see whether any of the TIPs are associated with the plasma membrane of plant cells instead of (or in addition to) the tonoplast.

**Biochemical Purification**

Some transporters and channels have been purified biochemically, allowing their properties to be studied in reconstituted vesicles and providing a route to their functional characterization and future molecular cloning. This approach provides insight into the structure and organization of transporters composed of multiple subunits. For example, Heven Sze and her collaborators used this approach to show that 10 different subunits are required to obtain functional reconstitution of the vacuolar H+-ATPase (Ward and Sze, 1992).

Another example was provided by Patrice Thuleau (University of California, San Diego), who described the purification of a 75-kD protein that may be a component of a voltage-dependent Ca$^{2+}$ channel from carrot plasma membrane. Thuleau purified this protein based on its ability to bind known, azido-labeled Ca$^{2+}$ channel blockers. The purified protein, when reconstituted into liposomes, behaves as a Ca$^{2+}$-selective channel. This channel is unstable, however, and over time degenerates into an apparently new type of channel that is permeable to Cl$^{-}$ as well as Ca$^{2+}$ but is still sensitive to Ca$^{2+}$ channel blockers. Perhaps additional components of the channel are required to stabilize the 75-kD subunit. As Thuleau pointed out, the Ca$^{2+}$ channel in rabbit muscle has five subunits, one of which, like the protein he described, binds Ca$^{2+}$ channel blockers and has channel activity on its own.

Thuleau also presented the first direct recordings of voltage-dependent Ca$^{2+}$ channels in higher plants by patch clamping the plasma membrane of the cell line used for isolation of the 75-kD protein. His results are particularly exciting because, despite the widely assumed importance of Ca$^{2+}$ channels to plant cell function, direct evidence for the existence of voltage-dependent Ca$^{2+}$ channels in the plasma membrane of higher plant cells has been lacking. In fact, although pharmacological Ca$^{2+}$ channel blockers are often used to suggest the involvement of Ca$^{2+}$ channels in plant signal transduction, Geoffrey Findlay (The Flinders University of South Australia) showed that some Ca$^{2+}$ channel blockers can actually inhibit outward K$^{+}$ channels in Amaranthus protoplasts.

Serge Defrot (Université de Poitiers) described a similar type of approach—that is, purification of blocker binding proteins—to identify a sucrose transporter from sugar beet. Sucrose uptake is sensitive to NEM, and plasma membranes contain a 42-kD protein that becomes labeled with radioactive NEM except when it is pretreated with sucrose. Under non-denaturing conditions, this protein is associated with a larger fraction, and it may, therefore, function as a multimer or in association with another protein(s). When reconstituted into vesicles, this fraction indeed drives sucrose uptake but not valine uptake.

Still another transporter that was purified using this approach is the tonoplast Na$^{+}$-H$^{+}$ antiport from sugar beet (Eduardo Blumwald, University of Toronto). Sugar beet is a moderate halophyte, and it may, therefore, function as a multimer or in association with another protein(s). When reconstituted into vesicles, this fraction indeed drives sucrose uptake but not valine uptake.

A rate-limiting step in molecular biological studies of plant genes is often lies in the characterization of the function of the encoded protein. For example, as Jeff Harper pointed out, although proton-pumping ATPase genes from plants are inferred to encode functional proton pumps because of their homology to fungal and mammalian ATPase genes, such a biochemical function has not yet been demonstrated directly. Expression in heterologous systems such as Xenopus oocytes or yeast can provide a means to unequivocally and accurately determine the function of plant transport genes. For example, Michael Palmgren (Copenhagen University) presented data showing that yeast vesicles can be used to study the function of higher plant H$^{+}$-ATPases (Villalba et al., 1992).

Kathryn Boorer and Roger Leigh (Rothamsted Experimental Station, UK) reported membrane potential and flux studies that show that the H$^{+}$-coupled glucose transporter previously cloned and characterized by Norbert Sauer (Sauer et al., 1990) can be functionally expressed in Xenopus oocytes. Xenopus oocytes have been further adapted for direct voltage clamp characterization of plant channel and transporter function by Julian Schroeder's laboratory and used to study the expression and function of K$^{+}$ channels and in collaborative efforts to characterize transporters, as discussed above.

Although Xenopus oocytes have only recently been adapted for studies of plant cell transport processes, voltage clamp analysis of Xenopus oocytes expressing heterologous membrane proteins has already allowed direct determination of the function of important plant transport genes, and it will likely be instrumental in gaining a detailed understanding of plant membrane transport. The potency of this approach was amply clear from the keynote lecture, which was given by Bert Sakmann (Max-Planck-Institut, Heidelberg), who shared with Erwin Neher the 1991 Nobel Prize in Physiology and Medi-
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cine for the development of the patch clamp technique and their many pioneering studies aimed at elucidating the molecular mechanisms of cell signaling. By expressing mutant versions of the acetylcholine receptor in Xenopus oocytes, Sakmann and his colleagues were able to identify the protein regions and amino acid residues involved in ion permeation and cation selectivity. These studies provided the first detailed experimental data for the construction of atomic models of the structure–function relationship of ion channels.

Sakmann also reported on recent studies with Peter Seeburg (University of Heidelberg) on the Ca$^{2+}$-permeable, non-selective NMDA receptor channel. The Mg$^{2+}$ block of this channel plays a major role in its physiological function and in neuronal modulation, and it has been suggested to contribute to long-term potentiation. Sakmann reported that molecular sites responsible for this Mg$^{2+}$ block and Ca$^{2+}$ permeability have now been identified (Burnashev et al., 1992).

Structure–function studies have also been carried out with great success in yeast. Carolyn Sluyman described her lab’s ongoing mutagenesis studies to delineate functional domains of the yeast plasma membrane H$^+$/ATPase. Because the wild-type PMA1 gene is essential, expressing mutant proteins in a background free of the wild-type protein was not a simple matter. Therefore, Sluyman and her colleagues devised an expression system that makes use of a temperature-sensitive allele, which may indicate that protein–protein interactions occur during biogenesis.

Mutants that have conservative substitutions in residues adjacent to Asp-378 are expressed with low activity. All of these enzymes have nearly normal $K_m$ for ATP binding but much reduced sensitivity to the inhibitor vanadate, a phosphate analog. Sluyman pointed out that a total of 45 residues of PMA1 have been altered by many groups, including that of Ramón Serrano (Villalba et al., 1992), and that 13 of these residues, all in the cytoplasmic region of the molecule, are necessary for vanadate—and presumably phosphate—binding.

Yeast expression systems can also be used to assay mutant derivatives of plant membrane proteins. For example, Norbert Sauer described the use of a yeast system to study the activity of a H$^+$/monosaccharide symporter from Chlorella. When transformed into Schizosaccharomyces pombe or Saccharomyces cerevisiae, the Chlorella gene (HUP1), as well as the homologous Arabidopsis (STP1 and STP4) and tobacco (MST1) genes, allow uptake of the nonmetabolizable glucose analog 3-OMG. Because the plant sugar transporters are energized by pH gradients, whereas structurally similar yeast sugar transporters catalyze facilitated diffusion, Sauer and his colleagues asked whether any mutations can change the Chlorella carrier from an active to a passive carrier. So far, no mutation accomplishes this feat. For instance, three conserved His residues were implicated in active transport, but all of these can be removed without affecting H$^+$-coupled transport.

A different type of approach to probing the function of plant membrane proteins is disrupting them with antisense RNA and assaying the effect on the plants. Peter Gogarten (University of Connecticut) and Lincoln Taiz (University of California, Santa Cruz) used this approach to examine the function of the A subunit of the vacuolar ATPase from carrot (Gogarten et al., 1992). At a gross morphological level, antisense plants look relatively normal, although their growth rate is reduced. However, the tonoplast-enriched vesicle fraction of antisense plants shows a dramatic reduction in ATPase activity; moreover, uptake of OMG into tonoplast vesicles, which is driven by the H$^+$ gradient across the tonoplast, is almost completely eliminated. Golgi-enriched vesicles, remarkably enough, show normal (or even enhanced) ATPase activity. This result suggests that Golgi contain a distinct form of the A subunit of the V-ATPase.

Membrane Receptors

Just as this year’s meeting was studded with breakthroughs in our understanding of membrane transport, so the next Plant Membrane Biology meeting, to be held in Regensburg, Germany, in 1995 may witness advances in the characterization and isolation of membrane receptors involved in signal perception. Several dispatches from this exciting front were presented. For example, Winslow Briggs (Carnegie Institution of Washington) and his colleagues have found that exposure of plants to blue light leads to the phosphorylation of a membrane protein of approximately 120 kD (the exact size depends on the plant species) (Short et al., 1992). The phosphorylated form migrates more slowly on denaturing protein gels than the unphosphorylated form. This substrate protein, the kinase that phosphorylates it, and the photoreceptor are closely associated, and it is possible that a single polypeptide includes all three functions.

Peptide sequences from the 120-kD protein revealed similarities to intermediate filament (IF) proteins, and a monoclonal antibody to the IF rod region cross-reacts with a plant protein of ~120 kD whose mobility on SDS gels also decreases with blue light treatment, suggesting that it may be identical to the protein that undergoes blue light-dependent phosphorylation. The IF antibody also cross-reacts with a smaller protein that shows a blue light-
dependent increase in abundance in the plasma membrane. The kinetics of this increase are consistent with a role for this smaller protein in blue light-mediated growth inhibition.

Michael Hahn (University of Georgia) described his lab's studies to understand how plant cells perceive oligosaccharide signals such as the fungal wall saccharide hepta-β-glucoside. Using the binding of radiolabeled hepta-β-glucoside as an assay, Hahn and his colleagues have purified a binding protein that cofractionates with the plasma membrane. The next step is to learn how ligand binding to this receptor leads to defense responses such as phytoalexin biosynthesis. Nod factors are another class of microbial oligosaccharides that elicit dramatic plant responses. These compounds, secreted by root-nodulating bacteria, lead to the formation of nodules on plant roots. Sharon Long (Stanford University) showed that an early host plant-specific response to the nod factors is the sustained depolarization of root hair cells (Enhardt et al., 1992). Although exciting advances have been made in identifying the precise chemical nature of the nod factors, Long pointed out that plant cell surface receptors for these factors have not yet been identified.

Another eagerly sought-after receptor is that for auxin. Hélène Barbier-Brygoo (CNRS, Gif-sur-Yvette) described an auxin binding protein, ABP1, that is targeted to the endoplasmic reticulum. She suggested, based on microelectrode studies of tobacco mesophyll cells, that this protein plays a role in auxin signal transduction (Barbier-Brygoo et al., 1991). These heavily discussed data and conclusions have now found support from patch clamp studies of maize coleoptile protoplasts (Hubert Felle, Universität Giesen). Felle and coworkers found that auxin activates the proton pump and that anti-ABP1 antibodies block this response. Moreover, an agonist antibody that Barbier-Brygoo showed enhances the electrical response of tobacco protoplasts to auxin also stimulated the pump current in Felle's patch clamp experiments. This ninth International Workshop on Plant Membrane Biology made apparent the immense recent progress in understanding the molecular mechanisms of membrane transport and signaling in higher plants. Membrane transport and transduction are of central importance to plant cell physiology, and with the characterization and cloning of plant membrane proteins—ion channels, pumps, cotransporters, and receptors—we will ultimately learn even more about both the external events and signals that control the activity of these proteins and the effects that their activation or inhibition have on intracellular processes such as cellular metabolism and gene regulation.

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