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Root Hair Electrophysiology
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1.1 Introduction

Root hairs are an excellent model cell for studies of ion transport in higher plant roots. They are accessible, extending out from the surface of the cell, so they are easily impaled with the micropipette(s) necessary for electrophysiological characterization of ionic currents. Imaging, including fluorescence microscopy is unimpeded by other cells, and external probes, such as ion-selective microelectrode and oxygen electrodes can be brought directly to the surface of the cell. After impalement, the micropipette tip location can be confidentially assigned to the cytoplasm, especially in younger (10–100 μm) cytoplasm-rich root hairs based on injection of fluorescent molecules. Alternatively, the root hair can be impaled in the vacuole by impaling at the vacuole-dominated base of the root hair, as indicated below. Root hair tip growth is relatively fast (about 1 μm min^{-1}), so that ion transport measurements can be made in the context of a dynamic, physiologically competent, growing cell (see Mulder, this volume). Much of this chapter will describe the electrophysiological properties of root hairs, and review studies of ion transport in which root hairs played a crucial role. However, to highlight the breadth of advantages that root hairs confer as a model cell, a case study (electrical properties of *in situ* vacuoles) will introduce the versatility of root hair research.

1.2. *In Situ* Vacuolar Electrophysiology using Root Hairs.

In plant cells, the vacuole often comprises 80 to 90% of the cell volume. It must play the *central role* in ionic homeostasis. Yet, much of our understanding of the nature of ion fluxes across the vacuolar membrane relies on vacuoles isolated from their normal milieu, the only way to access them for patch clamp (Sakmann and Neher, 1995) or other direct transport measurements. How transport properties change as a consequence of removal from the cell is unknown, but vacuolar properties may change dramatically. Growing root hairs are an ideal system for measuring the electrical properties of the vacuole *in situ*, because of the accessibility of the cytoplasmic and vacuolar compartments in a dynamic growing cell.

The electrical network of the root hair shown in Figure 1 indicates the complexity of plasma membrane and vacuole electrical properties in series, and the technique that allows the electrical properties of the vacuole to be measured *in situ*: voltage clamping while the cytoplasm is maintained at a virtual ground to isolate the electrical responses of the vacuole from those of the cytoplasm. An example of the impalements is shown in

Figure 2. The experiments uncovered increased ionic conductance in response to hyperosmotic stress in root hairs (Lew, 2004).

In situ measurements could be done in other cells, from other organisms. That root hairs can be used is provident, since they are often an integral part of the root system, and play a role in ion and water uptake during the growth and development of the plant. Research on root hairs enlightens our understanding of diverse aspects of plant physiology, and is relevant to the physiology of crop yield. They are accessible to a broad array of techniques in support of scientific enquiry.

For electrophysiological research, the electrical properties of the root hair are crucially important.

2.1 Electrical Model of the Root Hair

The electrical properties of cells, including root hairs, are normally described in the same terms as those used in electronics: Voltage, current, resistance, capacitance and impedance (which is related to both resistance and capacitance). The first three electrical properties are related through Ohm's Law, which describes the voltage created by a current passing through a resistance: E (voltage) = I (current) • R (resistance). An equivalent electrical circuit for the root hair is shown in Figure 1. The voltage across the plasma membrane is normally about –180 mV (negative inside), the resistance is normally about 20–40 MΩ (MegaOhms). The actual magnitude of the voltage varies dependent upon the state of the cell and the composition of the extracellular medium. Some of the voltage difference is caused by the asymmetric distribution of ions on either side of the plasma membrane. These ions will contribute to the voltage based on their relative permeability through the plasma membrane. Permeability will be affected by ion transport trhough the membrane. There is a relation between resistance (R) and ionic permeability (P), but not a simple one, since since resistance depends upon both the permeability and concentrations of each of the contributing ions (Schultz, 1980). In root hairs, K^+ permeability dominates. If K^+ were the sole permeant ion (which it is not), the voltage at *net* zero current would be described by the Nernst potential: $E_{Nernst} = (RT/F)\bullet ln(c_o/c_i)$, where R is the gas constant, T the temperature, F the Faraday constant, and, c_o and c_i are the ion concentrations outside and inside, respectively. At room temperature, the Nernst potential can be simplified to (in mV) : $E_{Nernst} = 55\bullet log_{10}(c_o/c_i)$. In addition to asymmetric distributions of ions, the plasma membrane H^+–ATPase generates a outward H^+ current which also contributes to the voltage (Felle, 1982; Lew, 1991). The relative contributions in *Arabidopsis* root hairs can be estimated from the effect of metabolic inhibitors which deplete cellular ATP (Lew, 1984): The H^+–ATPase contributes about –135 mV, the Nernst potential about –45 mV (Lew, 1991).

The fourth electrical property, capacitance is the ability of the root hair to hold charge, just as a battery is capable of holding charge. Capacitance determines the net imbalance of positive versus negative ions in the cell required to generate a voltage difference (*cf* Noble, 1991). The net charge, Q (in coulombs) is related to capacitance (C, coulombs $volt^{-1}$) by: $Q = C \bullet \Delta E$, where ΔE is the voltage difference. The charge, Q, depends upon

the volume of the cell: $Q = V_{root\ hair} \bullet c_{ion} \bullet F$, where $V_{root\ hair}$ is the root hair volume, c_{ion} is the concentration of ions resulting in the net charge imbalance, and F is the Faraday constant (96,480 coulombs $mole^{-1}$, to convert concentration to charge). Assuming root hair dimensions (cylindrical) of 15 by 87.5 μm for the epidermal part and 10 by 75 μm for the hair, the root hair area is $6.57 \bullet 10^{-5}$ cm^2, and the volume is $21.35 \bullet 10^{-12}$ liters. The root hair capacitance is about 66 picoFarad (based on the specific capacitance for biological membranes, about 1 μF cm^{-2}, multiplied by the root hair area). The net charge imbalance required to create a voltage difference of –180 mV would be: $c_{ion} = (66 \bullet 10^{-12} \bullet -0.18V)/(21.35 \bullet 10^{-12} \bullet 96{,}480)$, or 5.8 μM, a very small ion concentration difference. Thus, very small *net* charge movements across the membrane will have large effects on the voltage difference: A single ion channel with a flux of $1 \bullet 10^6$ molecules sec^{-1} (1 pA current) would have to remain open only 12 seconds to cause this large a voltage difference in the root hair. In a 'real' root hair, there are an ensemble of transporters functioning simultaneously, yet the potential is remarkably constant.

Capacitance has another impact on electrophysiological measurements. It affects the time dependence of voltage changes in response to changes in ionic current flow across the membrane. Formally, we first take the derivative with respect to time of the basic equation, $Q = C \bullet \Delta E$: $dQ/dt = C \bullet (dE/dt)$. Since current (I, coulombs sec^{-1}) is equal to dQ/dt, then $I = C \bullet (dE/dt)$. Substituting Ohm's law, $E = I \bullet R$ (or $I = E/R$), we obtain $E/R = C \bullet (dE/dt)$. This last equation can be solved to yield: $E = Ae^{(-t/RC)}$, where A is the voltage at time zero. The solution describes how a change in voltage will occur, as an exponential change over time. The rapidity of the change will depend upon the product of the resistance and capacitance of the cell ($R \bullet C$, with units of time, often abbreviate as $t = R \bullet C$, the time constant). For the root hair, the time constant, $R \bullet C$, is about 2–40 msec. This becomes important in some measurement techniques, such as the discontinuous voltage clamp, which rely upon differences in time responses to separate the resistance of the cell from the resistance of the microelectrode impaled into the cell. It can also be important in instrumentation, because it places limits on the measurability of fast events. The fifth electrical property is impedance, a measurement of resistance that accounts for the effect of capacitance on time-varying changes. It is normally used in the context of the frequency dependence of changes in voltage or current.

Ion transport across the membrane, as long as net charge movements are occurring, is the fundamental act that can be explored in electrophysiological measurements.

2.2 Electrophysiological Measurements

Voltage differences and resistances are measured by inserting microelectrodes into the cell. Microelectrode construction and use is described by Thomas (1978), Purves (1981) and Blatt (1991). Ion-selective microelectrodes are described in detail by Ammann (1986). The Plymouth Workshop Handbook (Ogden 1994) includes contributed chapters describing a variety of experimental techniques and analysis. Volkov (2006) is a recent compilation of microelectrode (and other) techniques.

Impalements with a single microelectrode can be used to measure the root hair voltage, but measurements of resistance and capacitance are complicated by the fact that current injection occurs through the same micropipette as the measurement of voltage. Since micropipette tips have high resistances (often 20–40 MΩ), the tip resistance obscures any measurement of the resistance across the root hair plasma membrane. Therefore, the 'standard' for measurements of voltage *and* resistance is impalements with two microelectrodes (Etherton et al., 1977). One microelectrode is used to inject current, while the second microelectrode monitors voltage changes. This is a feasible approach with root hairs (Lew, 1994, 1996, 2004), but technically challenging. With a single microelectrode, it is possible to use a technique called discontinuous voltage clamp to obtain estimates of both voltage and resistance (Finkel and Redman, 1984), a technique used successfully in a characterization of Nod factor effects on multiple ion transporters (H^+–ATPase, K^+ and anion channels) of clover root hairs (Kurkdjian et al, 2000), part of the signaling pathway prior to root nodulation (Kurkdjian, 1995), which also involves intracellular Ca^{2+} spiking (Kanamori et al., 2006)(see Bisseling, this volume and Cardenas et al., this volume). The discontinuous voltage-clamp technique relies upon the differences in the response times of the microelectrode and cell, which in turn depend upon their resistance and capacitance (the time constant R • C described above). In general, it is difficult to assure that resistance and capacitance of the microelectrode won't be changed during the impalement; this is not a problem with multiple impalements. Double barrel micropipettes offer the ability to impale only once, yet be able to inject current and monitor voltage in separate microelectrodes. Their fabrication and technical aspects of their use are described by Lew (2006). Current and voltage measurements are normally performed using voltage clamp, to characterize the voltage dependence of ion transport.

2.3 Cable Properties and Electrical Coupling

When current is injected into an *Arabidopsis* root hair, and the voltage deflection measured in that root hair (root hair I) and the adjacent root hair (II), the voltage deflections in root hair II are quite large (*ca* 50% of the deflection in root hair I)(Lew, 1994). This is direct evidence for electrical coupling between adjacent root hairs, presumably due to ion flux through the plasmodesmata. The cells act like an electrical cable, through which ionic currents flow freely from cell to cell; essentially an ionic syncytium. The universality of ionic syncytia is not known. Their biological significance is unclear. Electrical signals will readily propagate cell-to-cell, but ionic compositions would likely be heterogenous, because of the time required for ion diffusion through the relatively long distances of an interconnected network of multiple cells.

Technically, cell to cell electrical coupling causes problems with quantitation of voltage clamping. Voltage clamping requires clamp fidelity, in which the voltage is clamped to the same value throughout all regions of the cellular space. Because ionic currents flow between adjacent cells, a complete space clamp cannot be achieved. It's a technical drawback that must be acknowledged, and won't be solved until a quantitative map of cable properties (including sub-epidermal cells) is created.

2.4 Beyond Potential and Resistance

There are a number of electrophysiological techniques available beside intracellular microelectrodes. These include electrophysiological techniques for the measurement of cytoplasmic ion activities, for the measurement of diffusive ion gradients extracellularly, which can be converted into ion fluxes, direct measurements of turgor with a pressure probe, and even measurements of other molecules, such as O_2 and metabolites such as auxin. These techniques will be described in the following sections.

2.4.1 Ion-selective microelectrodes

Ion-selective microelectrodes are normally constructed by placing an ion-sensitive liquid ion-exchanger (Ammann, 1986; Thomas, 1978) in the tip of the microelectrode. To avoid displacement due to turgor when the microelectrode is impaled into the cell, polyvinylchloride (PVC) is often included with the ion-selective cocktail so that the ion-exchanger is held firmly in place (Felle, 1993). The resistance of the resulting membrane is very high, so a high input impedance electrometer ($>10^{15}$ Ω) must be used for measurements. The ion-selective microelectrode usually exhibits a close to Nernstian response to the ion it is specific for. When the ion-selective microelectrode is impaled into a cell, it measures both the cell potential and the Nernst potential for the ion. To separate the two voltages, the cell must be impaled with another microelectrode to measure the cell potential. This may be a separate impalement, or a double barrel microelectrode can be used (Miller and Wells, 2006). Root hairs are very suitable for these types of measurements, since the cytoplasmic compartment can be impaled with ease, obviating the need for any indirect assignment of tip location, based for example, on the measured activity of the selected ion.

2.4.2 Extracellular measurements of net ion fluxes

Not only can ion-selective electrodes be used to measure intracellular (and extracellular) free ion concentrations, but with modifications to the measuring technique, they can be used to measure ion fluxes. This is done by measuring the ion concentration at two distances from the cell or tissue being examined. The fundamental basis for inferring flux from two measurements of ion concentration (c_1 and c_2 measured Δx distance apart) is that a diffusive gradient will be created, either inward or outward depending on whether influx or efflux is occurring. This technique was one of the methods used by Felle and Hepler (1997) to demonstrate a tip-localized Ca^{2+} influx in growing root hairs. The method has been described in detail (Volkov, 2006). The general procedure of measuring diffusive gradients created by net fluxes across the cell membrane has been extended to include a wide variety of substances that can be sampled using microelectrode technology, including oxygen.

2.4.3 Measurements of turgor

Measurements of the cell hydrostatic pressure (turgor) would not normally be considered an *electro*physiological technique. However, the required micromanipulation methods used to impale the cell are the same, and turgor is closely allied with transport of both water and ions across cellular membranes (both the plasma membrane and vacuolar membrane). In this technique, micropipettes are pulled to coarser tip, to minimize the obstruction to mass flow through the tip. The micropipette is filled with a low viscosity silicone oil, which improves the ease of hydraulic flow and does not mix with the cell cytoplasm. Upon impalement, the internal pressure of the cell pushes the oil/cytoplasm meniscus into the micropipette. Pressure is applied to the micropipette via a piston: the pressure required to 'push' the meniscus back to the tip is the initial turgor of the cell. The pressure is measured with a transducer in contact with the silicone oil. Typical turgors of *Arabidopsis* root hairs are about 680 ± 200 kiloPascals (Lew, 1996). Turgor is regulated rapidly: within 40–50 minutes of a hyperosmotic treatment that causes a rapid decrease in turgor, the turgor recovers to near its original level. At least in *Arabidopsis*, turgor recovery occurs in concert with activation of the plasma membrane H^+–ATPase and uptake of osmotically-active ions (mostly K^+ and Cl^-) (Shabala and Lew, 2002).

3.1 Ion Transport in Root Hairs

Having summarized some of the cell biophysical techniques used to measure the physiological dynamics of root hairs, what roles would ion transport play in the growth and differentiation of the root hair?

Maintaining osmotic balance of the root hair during cellular expansion is essential to maintain a constant turgor. With growth rates of 1 μm min^{-1}, a root hair of 8 μm diameter will increase its volume about 50 fl min^{-1}. If osmotic balance is to be maintained by ion influx, the root hair must take up about 25 fmol min^{-1}. Root hairs may also be a pathway for ion uptake to support the osmotic balance of other cells within the plant body. Thus ionic balance is crucial to the root hair. In addition, it is common for plant cells to accumulate ions selectively. That is, K^+ accumulation is preferred to Na^+; Ca^{2+} is actively excluded from the cytoplasm; Cl^- is normally taken up as the counterion.

Ions may also play a role in signalling. Ca^{2+} influx to maintain a tip-high Ca^{2+} gradient during tip growth of the hair has already been noted (section 2.4.2). It may act as a mediator of the vesicle fusion required for continued cell expansion, and is known to activate both kinases and phosphatases in the cytoplasm. Protons are crucial to the life of the cell. Not only in the context of cytoplasmic pH regulation, but modulation of extracellular pH, and as a signalling molecule analogous to Ca^{2+} during onset of nodulation (Felle and Herrmann, 2000). The ubiquitous role of protons is further supported by the central role of the H^+-ATPase in nutrient transport (Palmgren, 1998, 2001; Sondergaard et al., 2004).

3.2 The Plasma Membrane H^+-ATPase

The plasma membrane H^+-ATPase is a P-type active ion pump (Lauger, 1991) common in walled cells of the plant and fungal kingdoms, and many of the algae. Due to the electrogenic nature of the ATP-dependent H^+ extrusion, the H^+-ATPase contributes significantly to the negative-inside potential of the cell, a driving force for uptake of a number of nutrients. In *Arabidopsis* root hairs, direct evidence for the role of the H^+-ATPase was based upon the depolarizing effects of an inhibitor of P-type active pumps, vanadate, and cytoplasmic ATP depletion by cyanide (Lew, 1991). The magnitude of the depolarization, about 135 mV from an initial value of about –180 mV is evidence for its dominant role (Lew, 1991). The gene(s) for the H^+-ATPase have been cloned from a number of species, it is known to exist in various isoforms (Michelet and Boutry, 1995). A number of gene sub-families (Arango et al., 2003) exhibit differential expression that suggests different functional roles (Moriau et al., 1999; Lefebvre et al., 2005). In *Nicotiana plumbaginifolia*, one of the genes (pma4) of a sub-family that appears to be expressed in tissues that have a role in ion uptake is strongly expressed in root hairs of seedlings, implicating root hairs as a major site of ion uptake for young roots (Moriau et al., 1999). The voltage dependent conductance of the membrane before and after vanadate inhibition of the H^+-ATPase is relatively unchanged at voltages from -200 to 0 mV (Lew, 1991). Thus, the pump exhibits a voltage-independent 'constant current' activity. Estimates of the current density are problematic due to the problems discussed regarding the root hair cable properties and cell-to-cell coupling, but it appears to be quite high, indicating a high level of H^+-ATPase activity in root hairs (Lew, 1991).

It is unclear whether root hair H^+ ATPase activity is regulated by intracellular ions, such as calcium (known to activate the *Neurospora crassa* H^+ ATPase [Lew, 1989]) or protons, one of the substrates for the enzyme (although the H^+ ATPase does not appear to regulate cytosolic pH [Felle, 1996]). Experiments ionophoresing either Ca^{2+} or H^+ ions directly into the root hair revealed no compelling evidence for regulation by either ion. Increasing cytoplasmic [Ca^{2+}] rapidly inhibits cytoplasmic streaming; only at higher levels does Ca^{2+} depolarize the potential and inhibit cell-to-cell coupling (Lew, 1994). Increasing cytoplasmic [H^+] causes a rapid increase in vacuolar area (Lew, unpublished) but has no effect on the membrane potential and minimal effects on cell-to-cell coupling (Lew, 1994).

The plant hormones auxin (Tretyn et al., 1991; Ayling et al., 1994) and cytokinin (Silverman et al., 1998) affect the membrane potential of root hairs. Exogenous, but not intracellular injection, of cytokinin hyperpolarizes the potential, consistent with H^+-ATPase activation, and stimulates root hair growth (Silverman et al., 1998). Auxin either depolarizes (Ayling et al., 1994; Tretyn et al., 1991) or hyperpolarizes the potential, dependent on the auxin concentration (Tretyn et al., 1991). In addition to effects on the electrical properties, auxin is implicated in root hair initiation (Masucci and Schiefelbein, 1994).

Because of the central role of the H^+-ATPase in nutrient uptake, its regulation is of considerable interest (Palmgren, 2001). The fungal toxin fusicoccin is known to activate the H^+-ATPase and does so by activating an endogenous transduction pathway of H^+-ATPase phosphorylation to cause binding of a 14–3–3 protein (*cf* Kanczewska et al.,

2005). Root hairs presumably contain the appropriate signalling components, since fuscoccin hyperpolarizes the potential of *Limnobium stolonifera* root hairs (Ullrich and Novacky, 1990). There is a recent report of fusicoccin activation of a MAP kinase cascade in tomato leaves (Higgins et al., 2006). A MAP kinase cascade directly activates the H^+-ATPase in the fungus *Neurospora crassa* in a pathway separate from activation of gene expression (Lew et al., 2006). It is part of a turgor regulating system in the fungus; a similar system may exist in higher plants (Shabala and Lew, 2002). In plant root hairs, it is likely that continuing research will reveal a web of pathways controlling H^+-ATPase activity, either directly or via regulation of expression of H^+-ATPase gene(s).

As noted above, on the basis of inhibitor studies, the H^+-ATPase is a major contributor to the membrane potential of the cell. The potential is one component of the proton motive force (pmf), which is the sum of the potential and pH differences between the inside of the cell and the extracellular environment: pmf (mV) = ΔVoltage + 55 • ΔpH. Either the potential, the pH difference, or both, can be used to drive the uptake of other nutrients and ions into the cell. Of these ions, K^+ and Cl^- are accumulated to high levels inside the cell.

3.3 K^+ Transport

K^+ is an essential nutrient, actively accumulated from the soil solution, which normally contains low concentrations (0.3 to 5 mM) compared to 100-200 mM in the cell. Much of the identification of K^+ transporters has relied on functional complementation of yeast K^+ transport mutants using cDNA library screens (Fox and Guerinot, 1998). There are at least five K^+ transporter families which include K^+ channels, co-transporters, and anti-porters (Maser et al., 2001; Ashley et al., 2006).

Uptake through channels would be energized by the negative-inside potential of the cell (Gassmann and Schroeder, 1994; Maathuis and Sanders, 1993, 1995). If the potential is about –170 mV and internal [K^+] is 100 mM, then active accumulation through the channel can occur at soil solution [K^+] greater than about 0.1 mM. One example of an inward channel is AKT1, known to be expressed in *Arabidopsis* roots (Legarde et al., 1996). The *akt1* mutant exhibits reduced potassium uptake (using ^{86}Rb as the radioactive tracer) and apparently lacks the inward K^+ channel (Hirsch et al., 1998; Ivashikina et al., 2001) as measured by patch clamp. Direct evidence for its role in K^+ uptake in intact root hairs was obtained by using extracellular ion-selective microelectrodes to monitor K^+ sequestration after oligochitin-elicitor treatment. The elicitor first induced K^+ release, followed by re-uptake in wildtype root hairs, but re-uptake was delayed in the *akt1* mutant (Ivashikina et al., 2001). The kinetics of the inward K^+ channel (AKT1) are modulated by AtKC1, a regulatory subunit (Reintanz et al., 2002); it is regulated by a Ca^{2+}-activated protein kinase pathway (Xu et al., 2006; Li et al., 2006).

Outward K^+ channels (GORK) are also expressed in root hairs (Ivashikina et al., 2001). Because the voltage-sensitive gating of this outward K^+ channel is affected by extracellular [K^+] (activation shifts to more positive voltages at higher [K^+]), it is possible that the channel functions in K^+-'sensing' and may have a role in the polar growth of the

cell (Ivashikina et al., 2001). Alternatively, the outward channel may maintain the potential within well-defined limits: at low external [K^+], the potential will be more negative, hence the channel would activate at more negative potentials should the potential depolarize; at high external [K^+], when the potential is more positive, activation at a more positive potential would avoid excessive K^+ loss from the cell.

Another K^+ permease family plays a role in K^+ uptake: KT/KUP. In *Arabidopsis*, 10 of the 13 AtKT/KUP genes are expressed in root hairs (Ahn et al., 2004), which is indirect evidence for the role of root hairs in K^+ uptake. Functional complementation of the genes in an *E. coli* mutant deficient in K^+ uptake demonstrated the protein products function in K^+ transport (Ahn et al., 2004). A knock-out mutant of the AtKT3/KUP4 member (Ahn et al., 2004) of the KT/KUP family causes a 'tiny root hair' (*trh1*) phenotype, specifically the absence of root hair elongation after initiation (Rigas et al., 2001). Whether the phenotype is caused by a disruption of K^+ transport is unclear, since elevating extracellular [K^+] did not rescue the phenotype. However, even in wildtype, elevated [KCl] causes a decrease in root hair length, a response also observed in the already short root hairs of the *trh1* mutant (Desbrosses et al., 2003).

Much of the research described above has relied upon characterization of the ion channels with the patch clamp technique. Root hairs are special as a model system for examining issues related to transport of K^+ and other osmotically active ions because uptake can be examined in the context of cellular growth, *in situ*. Time-dependent inward and outward currents, inhibitable by quaternary ammoniums (tetraethylammonium and tetrapentylammonium) and Cs^+ ('standard' K^+ channel inhibitors, Hille, 1984) have been measured in intact root hairs using a discontinuous single-electrode voltage clamp technique (Bouteau et al., 1999), vindicating *in vitro* characterizations. The inward K^+ current does appear to have a role in cellular expansion. To maintain turgor during growth, the root hair must accumulate osmotically active substances. K^+ uptake plays a role: inhibition of the inward K^+ channel with tetraethylammonium inhibits an inward K^+ current and *Arabidopsis* root hair growth (Lew, 1991). The inhibition of growth is transient (growth resumes after about 4 minutes), so K^+ uptake is not an obligatory mechanism for maintaining the internal osmolarity at a level sufficient to 'drive' cellular expansion.

Net K^+ influx was measured in growing *Limnobium stoloniferum* root hairs using the vibrating ion-selective probe technique (Jones et al., 1995). The root hairs have TEA-sensitive inward (and outward) K^+ currents (Grabov and Bottger, 1994). Root hair growth is inhibited by Al^{3+} (half-maximally at about 7 μM), but K^+ influx continued after inhibition of growth by 20 μM Al^{3+} (Jones et al., 1995). Aluminum does inhibit inward K^+ channels of wheat root hairs (half-maximally at about 8 μM; Gassmann and Schroeder, 1994). So, Al^{3+} levels sufficient to inhibit an inward K^+ channel measured on root hair protoplasts with the patch clamp technique do not affect K^+ influx in intact root hairs, even though growth inhibition is observed. The conclusion is that there is no one-to-one correspondence between K^+ uptake and growth. This may be unfortunate for scientists but certainly beneficial for plants subjected to diverse stresses during their growth and survival, such that multiple mechanisms for maintaining growth are crucial.

Because of their large size, the electrical properties of *Limnobium* root hairs are easily measured (*cf* Ullrich and Novacky, 1990) with a technique uncommon in higher plant electrophysiology: the sucrose gap (Purves, 1981). The sucrose gap electrically isolates two regions of the root hair, so that the voltage dependence of ionic currents can be measured by voltage clamping. With this technique, Grabov and Bottger (1994) identified TEA-sensitive inward and outward K^+ channels which were active at potentials more negative and more positive than the normal membrane potential, respectively. Modifying the redox potential of the root hairs by adding the electron acceptor hexacyanoferrate III to the external solution activated the inward K^+ channels and inhibited the outward K^+ channels. The physiological role of redox modulation is not known, but it may be related to a redox system located on the plasma membrane that mediates iron uptake (Moog et al., 1995; Robinson et al., 1999). Redox modification (with reductive agents) is known to control root hair morphogenesis (Sanchez-Fernandez et al., 1997).

Other factors beside redox poise may regulate K^+ transport. Many K^+ channels are regulated by ATP. It would not be surprising to uncover a linkage between 'energy charge' of the cell and ion transport, both direct effects (for example the ATP dependence of the H^+-ATPase activity) and indirect effects (regulation of channel activity). Such an 'energy charge' linkage exists between the Na^+-K^+ ATPase and K^+ channel activity in renal proximal tubules (Tsuchiya et al., 1992). In yeast, Ramirez et al (1989) reported altered K^+ channel activation by cytoplasmic ATP in a H^+-ATPase mutant (*pma1–105*) . In higher plants, there is no direct biochemical linkage between K^+ uptake and the H^+-ATPase (Briskin and Gawienowski, 1996), but indirect linkages may eventually be discovered. Certainly, nutritional status regulates potassium transport (Ashley et al., 2006). Root hairs are a relatively 'simple' system in which to explore such regulation.

K^+ uptake alone cannot account for osmotic balance in growing cells; it must be accompanied by a counterion to assure the maintenance of electrical balance. Cl^- is the major anion actively accumulated in plant cells.

3.4 Cl^- Transport

In growing *Arabidopsis* root hairs, Cl– influx was measured directly with the ion-selective vibrating probe (Lew, 1998). Cl^- uptake cannot be passive through a Cl^- channel, because the negative inside voltage of the cell would electrophoretically 'expel' the chloride anion from the cell. Instead, uptake relies upon a nH^+/Cl^- symport, so that Cl^- uptake is coupled to the proton motive force generated by the H^+-ATPase. Evidence for a symport mechanism is based upon measurements of the electrical potential and pH, both cytoplasmic and extracellular, in *Limnobium* root hairs (Ullrich and Novacky, 1990). The extracellular addition of Cl^- causes a large depolarization of the potential (about 60-100 mV) along with acidification of the cytoplasm and alkalinization of the extracellular medium. This is consistent with cotransport of more than one H^+ with each Cl^- ion: the net positive charge influx would cause the depolarization. The pH changes are consistent with the fact that HCl is being transported. More extensive experiments supporting the

presence of a H^+/Cl^- symport were performed by Felle (1994), who measured Cl^- accumulation directly with an intracellular Cl^--selective microelectrode in *Sinapis alba* root hairs. Felle (1994) also measured pH with a H^+-selective microelectrode as well as the potential and resistance of the root hair. A shift to an acid extracellular pH (from 9.5 to 4.5) was sufficient to increase intracellular [Cl^-] about 2.5 fold, even though the membrane potential depolarizes under these conditions. This indicates a kinetic dependence of H^+/Cl^- symport activity on the ΔpH component of the proton motive force, separate from the ΔVoltage component.

Chloride channels would function in Cl^- efflux from the cell. Nod factor signaling includes a transient depolarization of the potential which occurs concomitant with the appearance of Cl^- in the extracellular medium and a decrease in cytoplasmic [Cl^-]. The Cl^- efflux is believed to be caused by a Ca^{2+}-activated Cl^- channel in the root hair (Felle et al., 1998). Anion channels from root epidermal cells have been characterized using patch clamp (Diatloff et al., 2004), the channels are permeable to a wide range of anions (including citrate). Organic anion selective channels are regulated by extracellular Al^{3+} and the phosphate nutritional status of the plant; these and other physiological roles of anion channels in roots (e.g., osmoregulation) have been recently reviewed by Roberts (2006).

3.5 Nitrogen (NH_4^+ and NO_3^-), Sulphate and Phosphate Transport.

Although they are not normally accumulated to high levels in the plant, nitrogen species (usually NH_4^+ and NO_3^-), sulphate and phosphate are essential ionic nutrients and must be actively imported by the cell.

Ammonium uptake must involve multiple mechanisms which would depend upon pH, and thus the relative contributions of the unprotonated ammonia (NH_3) and protonated NH_4^+. The two forms are interconvertible by the following reactions: $NH_3(aq) + H_2O <–> NH_4^+ + OH^-$ (pK_b 4.75) and $NH_4^+ <–> NH_3 + H^+$ (pK_a 9.25). At neutral pH, the dominant form would be the ammonium ion (NH_4^+), but NH_3 may still play a role as a membrane-permeable form. However, the addition of NH_4^+ extracellularly causes depolarization of the potential in barley and tomato root hairs (Ayling, 1993). This is consistent with positive charge entry into the cell and implies that NH_4^+ is the transported form. Because K^+ channels are often significantly permeable to NH_4^+, the ammonium ion could be transported by an inward K^+ channel, but ammonium transporters have been identified in root hairs (Lauter et al., 1996). The LeAMT1 was discovered by screening a root hair-specific cDNA library of tomato; its functional identification was based of rescue of a yeast mutant deficient in ammonium transport (Lauter et al., 1996). Four AMT genes that are expressed in roots have been identified in *Arabidopsis* (Loque et al., 2006), some are expressed in response to nitrogen deficiency, and have been assigned a role in high affinity uptake by analyzing T-DNA insertion mutants. When the tomato genes LeAMT1;1 and LeAMT1;2 are expressed in oocytes, the addition of NH_4^+ caused inward currents, consistent with uptake of the positively charged ammonium ion (Ludewig et al., 2003).

In general, NH_4^+ is a preferred nitrogen species, because NO_3^- must be reduced to ammonium for production of amino acids, but NO_3^- is more common in the soil solution. As an anion, it is expected that active accumulation would rely on a nH^+/NO_3^- symport. Using *Limnobium* root hairs, Ullrich and Novacky (1990) examined the effect of NO_3^- on the membrane potential and cytoplasmic pH. Nitrate addition depolarized the potential, but, unexpectedly, it caused an alkalinization of both the cytoplasm and extracellular solution. This is not consistent with a nH^+/NO_3^- symporter, which should acidify the cytoplasm, but could be due to rapid reduction of NO_3^- in the cytoplasm, consuming the imported H^+. Using *Arabidopsis* root hairs, Meharg and Blatt (1995) voltage clamped to assess the voltage dependence of the ionic currents induced by NO_3^- transport. They observed larger NO_3^--induced currents at voltages more negative than the normal membrane potential and at acid extracellular pH, supporting the presence of a voltage dependent nH^+/NO_3- symporter. Nitrate (NO_3^-) transporters have been cloned (*cf* Wang and Crawford, 1996; Wang et al., 1998; Lauter et al., 1996), some are preferentially expressed in root hairs (Lauter et al., 1996). Heterologous expression of a nitrate transporter (CHL1) in *Xenopus* oocytes caused pH dependent inward currents consistent with a nH^+/NO_3- symporter (Tsay et al., 1993). Mutations in both the *Arabidopsis* CHL1 (NRT1) and NRT2 nitrate transporters minimize the membrane potential changes observed after additions of low levels of NO_3^- (Wang and Crawford, 1996; Wang et al., 1998).

As is true for other anions, phosphate appears to be taken up via a nH^+/Pi symporter (Ullrich and Novacky, 1990). Phosphate transporters have been cloned, and do appear to be expressed in root epidermal cells, including root hairs (Daram et al., 1998; Liu et al., 1998). The promoter for the phosphate transporter gene has been proposed as a biotechnological tool since it causes induction of heterologous genes in roots, including root hairs (Schunmann et al., 2004).

Sulfate is normally reduced in photosynthetic tissues, but must first be transported into the plant. A number of genes encoding sulfate transporters have been cloned from a variety of plant species and function established by heterologous expression in yeast (*cf* Yoshimoto et al., 2002). The transporters are expressed in root hairs, and are induced sulfate deficiency (Maruyama-Nakashita et al., 2004). Sulfate deficiency also induces expression of enzymes responsible for reduction of SO_4^-, but since most reduction and assimilation occurs in the leaves, regulatory mechanisms are complex (Hopkins et al., 2005), and include sulfate transport into vacuoles as a mechanism controlling cytoplasmic concentrations (Kataoka et al., 2004) (the regulation of sulfate assimilation was recently reviewed by Kopriva et al., 2006). The transport mechanism is as yet uncharacterized *in situ*, but since sulfate is taken up as the anion, a nH^+/SO_4^- symport is very likely, and supported by the observation that uptake is stimulated by acid pH when plant sulfate transporters (SHST1 and SHST2 from *Stylosanthes hamata*) are expressed in yeast (Smith et al., 1995).

4.0 Ion Transport and Root Hair Morphogenesis

As a tip-growing cell, root hairs offer the ability to explore potential roles that ion transport may have during root hair initiation and elongation. As osmotically active agents accumulated to high levels, K^+ and Cl^- may function to maintain intracellular osmolarity during growth. In addition, transported ions may act as a second messenger during signal transduction. Hormones and other intracellular components of signal transduction may regulate ion transport as part of their regulation of morphogenesis.

4.1 Ca^{2+} Transport and Root Hair Morphogenesis.

Ca^{2+} is very important to the physiological function of plant cells, primarily because of its role as a second messenger. The importance of Ca^{2+} in signal transduction will be detailed elsewhere in this book (Gilroy, calcium in root hair growth; Barker and Esseling, endosymbiotic N-fixing Rhizobium-legume association; Cardenas et al., root hair imaging).

4.2 Control of Root Hair Morphogenesis by Ionic Nutrients

Besides the signaling role of Ca^{2+}, other ions also affect morphogenesis, or more accurately, root hair initiation and growth appears to be induced under conditions of nutrient deficiency. The general role of root hairs is believed to be an increase in the soil volume in near proximity to the root, increasing the effective zone of diffusive supply of ions to the root (Jungk, 2001). Root hair length and/or number are increased by nutrient deficiencies (Peterson and Stevens, 2000): for example, phosphorus (Bates and Lynch, 1996, nitrate (Jungk, 2001), potassium (Desbrosses et al., 2003), iron (Moog et al., 1995), and manganese (Konno et al., 2006). The regulation of root hair initiation and growth under nutrient deficiencies is unclear, and the 'sensing' of nutrient deficiency is unlikely to be localized specifically to root hairs. However, Shin et al. (2005) suggest that reactive oxygen species may play a role in response to nutrient deficiencies, H_2O_2 is induced within 30 hours of nutrient deprivation, and absent in *Arabidopsis* root hair mutants (for potassium and nitrogen, but not for phosphorus deprivation), so it is possible root hairs have a role in sensing potassium and nitrogen deficiencies. The hormone auxin may control root hair formation in response to iron or phosphorus deficiency, since root hair and transfer cell formation is inhibited in auxin mutants (*axr2*), completely for iron deficiency and partially for phosphorus deficiency (Schikora and Schmidt, 2001).

The concept that root hairs can play a role in nutrient uptake is supported by their induction under deficient conditions, but they may not be *obligatory* for nutrient uptake. Comparisons of rice mutants lacking root hairs or lateral roots revealed that lateral roots are more important for silicon uptake (Ma et al., 2001). The density of the root network ramifying through the soil would be expected to compensate for the absence of root hairs.

5.0 Concluding Remarks

Even if root hairs are not crucial for the survival of the plant, they can play important roles in nutrient uptake. They are a remarkable model system for intracellular

manipulations, electrophysiology and cell imaging. It's probably fair to say that no other cell type in the plant can be used with such remarkable experimental sophistication *in situ*, intact and growing. They are an excellent single cell testtube; in conjunction with molecular biological manipulations, they can be used to unravel molecular mechanisms of ion transport that underpin the growth and development of a plant cell.

6.0 References

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Figure 1. The electrical networks of the root hair. The plasma membrane and vacuolar membrane network of resistances (R_m and R_v, respectively), capacitance (C_m and C_v) and potentials (E_m and E_v) are shown to the left. The electrical circuits required to measure the electrical properties of the vacuolar membrane *in situ* are shown to the right, overlaid on a diagram of the root hair.

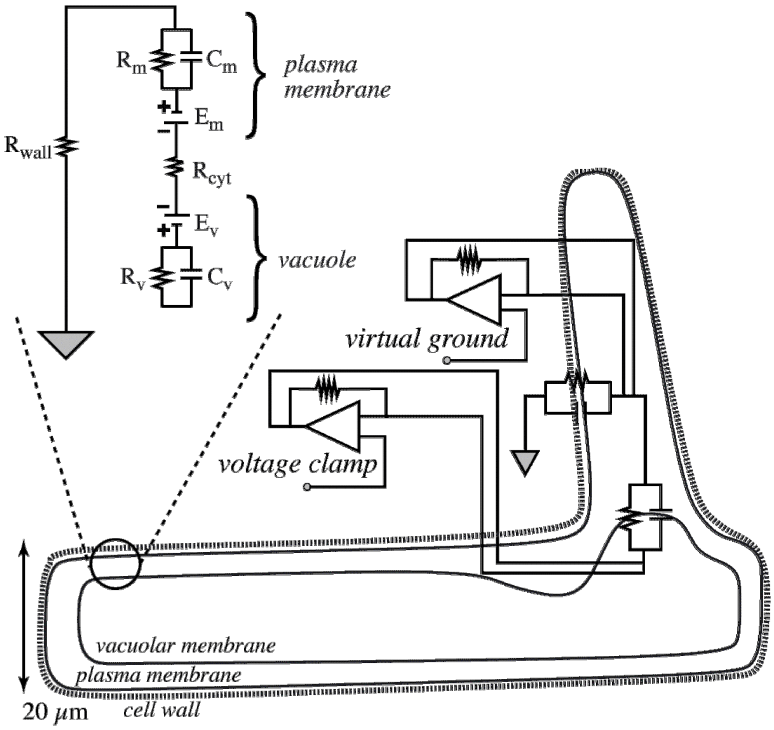

Figure 2. An example of *in situ* measurement of the electrical properties of the vacuolar membrane. Impalements into the cytoplasm and vacuole are shown in A, as marked. A photograph of the dual impalements is shown in B. The current voltage relations for the vacuolar membrane before (circles) and after (squares) a hyperosmotic treatment are shown in C. These were measured while the cytoplasm was maintained at a 'virtual ground' by the electrical circuitry shown in Figure 1. Note that hyperosmotic treatment causes a hyperpolarization of the plasma membrane, recorded by both the cytoplasmic and vacuolar microelectrodes (since the vacuolar membrane is in electrical series with the plasma membrane). The vacuole membrane response to hyperosmotic treatment is increased conductance without a change in the positive electrical potential of the vacuole (C).