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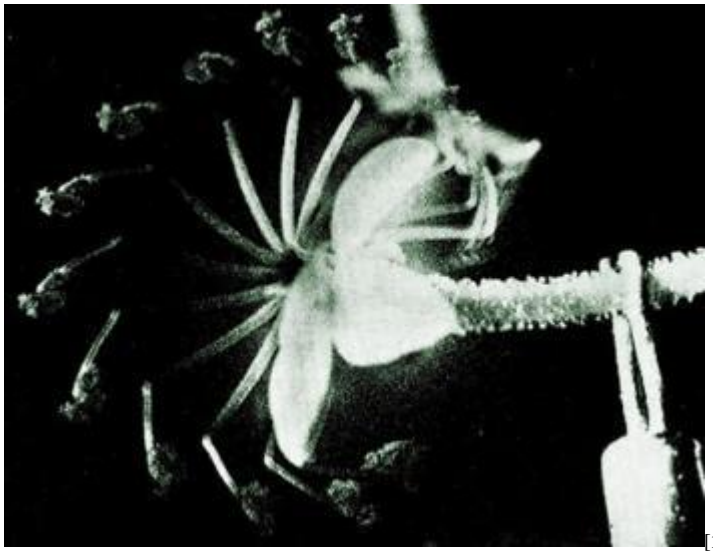
Chapter 4 - Using water and nutrients: cell growth

 [1]

A sequence of superimposed images captures the flower column of a trigger plant (*Stylidium crassifolium*) as it 'fires' in response to a physical stimulus (in nature, an insect). A 1 cm column rotates through more than 200° from a 'cocked position' (a) in 10—30 ms to a relaxed position (b) (photographs taken at 2 ms intervals). The kinetic energy manifested in this rapid firing is derived from events controlled at a membrane level. Ions transported into specialised cells cause hydrostatic (turgor) pressure to develop which is suddenly dissipated following mechanical stimulation. Similar rapid movements occur in mimosa (sensitive plant) and some carnivorous plants (Based on Findlay and Findlay 1975)

The almost infinite variety of vegetable forms, which have been grouped into no less than 82,606 distinct species, is formed of but one elementary material, made up of multitudes of little vesicles or bladders, called CELLS. The tissue of which they are composed, when first formed, is called cellulose. The different forms of this TISSUE are held together by a living mucus, a gummy fluid, out of which the tissue itself is made.

(C. Baker, Plants, the Earth and Minerals, mid-nineteenth century)

Introduction

Plant cells commonly expand 100-fold on the way to attaining final form. Such spectacular change is the basis of primary growth, including cell expansion, organ formation or biomass accumulation at a plant community level. Events at a single cell level must be perfectly coordinated for plants to achieve functional and repeatable structures. Up to 18 000 cells are recruited from a root meristem each hour, and most of these cells undergo various degrees of expansion over the hours following division. What events bear on a cell emerging from a meristem or embryo as it undergoes expansive growth?

Cells emerging from a seed embyro, shoot apical meristem, lateral cambium of a tree or a root apex all encounter different fates. Therefore, the dynamics of cell expansion must reflect the characteristics of each tissue that will differentiate from these cell lines. For example, longitudinal versus radial expansion rates of cells giving rise to layers of epidermis and cortex in a coleoptile are sharply contrasting, resulting in long, slender epidermal cells and thicker, shorter cortical cells. In spite of their distinct morphological fates, growth is perfectly coordinated between adjacent cell files to produce a slim, straight coleoptile. On the other hand, differential rates of epidermal and cortical cell expansion in developing leaves result in large intercellular spaces developing in the spongy mesophyll (Section 1.2).

Transition from near-spherical, small cells (about 1 pL) arising from recent cell divisions to enlarged vacuolated and generally elongated cells of a mature plant is therefore subject to genetic and environmental constraints. These constraints manifest themselves in cell expansion through variations in (1) *rate* of expansion and (2) *direction* of growth (cell shape and polarity). The genome of each cell sets a programmed pattern of growth which is modulated by environmental factors. For example, formation of an inflorescence proceeds by expansive growth of new floral organs coordinated within a tight develop-mental framework encoded by homeotic genes. In contrast, the exceptional thickening of leaves in response to CO_2 enrichment and salinity is achieved by environment signals that modify cellular growth rather than development.

Cell expansion requires maintenance of disequilibria at the cell level: ions must be imported, water must flow along free energy gradients and cell wall bonds must yield to turgor pressure. Cells must therefore use energy-yielding pathways and membrane-bound compartments to acquire ions at the correct osmotic and nutritional levels (Sections 4.1 and 4.2), regulate water inflow and stretch their walls (Section 4.3). Maintenance of electrical and chemical gradients is made possible by active (energised) transport across membranes. Dispersing the free energy stored in electrochemical gradients is controlled largely through channels located in membranes. Transport mech-anisms for ion and water uptake will be discussed in Section 4.1, while Section 4.2 will describe experimental approaches which are leading to a rapid expansion in our knowledge of membrane transport. Finally, the universally important process of cell-wall yielding will be discussed in

Section 4.3, showing how resource acquisition and cell enlargement are coordinated.

4.1 Membrane transport and ion balance

4.1.1 Osmotic engines for plant function

Plants move, and when viewed at different organisational levels, such movement ranges from cellular (opening and closing of stomata) to whole organs (opening and closing of a flower). Growth can also be considered a directed movement, enabling roots to obtain nutrients (Chapter 3) and leaves to absorb light (Section 1.2). Growth is possible through co-ordination of solute and water transport at a membrane level, sustaining resource supply for biosynthesis and generating turgor pressure. Time scales of cell and organ movement can be staggeringly fast (thousandths of a second) or so slow that they can only be visualised over days with time-lapse photography.

One vital outcome of *solute movement* is a hydrostatic pressure difference between the inside and outside of plant cells, termed turgor pressure (*P*; Section 4.3). This substantial pressure (commonly 0.5–0.8 atmospheres) is exerted through a 5–10 nm thick plasma membrane appressed against a cell wall. Structural integrity of both plasma membranes and cell walls are vital to withstand *P* but these matrices are also modified during changes in cell shape or volume. For example, cell walls relax, stretch and rigidify, and new membranes are synthesised as cells grow. Cell movements might occur independently of those of their neighbours, such as the fine, turgor-driven move-ments of guard cells in a stomatal complex, or cooperatively, such as roots lifting a hefty concrete overburden (Section 16.1).

Ultimately, osmosis generates the hydrostatic gradients to sustain these processes. Osmotic fluxes in plants are based on proteins which catalyse solute and water transport; these proteins are embedded in membranes like components on an electronic circuit board. In this section we explore the mem-brane circuit board, the function of components and the physical principles by which they are governed.

4.1.2 Transport of molecules across cell membranes

(a) Diffusion and selectivity

δ Transport proteins play a critical part in solute movement because membranes constitute a barrier to free diffusion of molecules. If membranes allowed rapid diffusion of both ions and water, gradients of ions would be slight and osmotic

pressure could only be achieved with enormous energy consumption. Instead, some molecules, such as water and gases, move rapidly across membranes. Unrestricted movement of water relative to solutes is the basis of osmosis, and in plants the generation of *P*. Similarly, if the movement of CO_2 and O_2 to and from the sites of photosynthesis and respiration were substantially impeded, steep concentration gradients would reduce the efficiency of these vital processes. The principles of *diffusion* and *selectivity*, which are used to describe differential rates of molecular movement, provide a physical rationale for osmosis.

In a homogeneous medium, net movement of molecules down their concentration gradient is described by Fick's First Law of diffusion. The molecule and medium may be a solute in water, a gas in air or a molecule within the lipid bilayer; a version of this equation describing water movement in soil appeared in Section 3.2. Fick's Law holds when the medium is homogeneous in all respects except for the concentration of the molecule. If there was an electric field or a pressure gradient then Fick's Law may not be appropriate (Section 4.1.2(b)). Considering the case of a solute in water, say, sugar, Fick's Law states that net movement of this solute, also called the net flux (J_s), is proportional to the concentration gradient of the solute $\Delta C_s/\Delta x$:

$$J_s = -D_s \frac{\Delta C_s}{\Delta x} \qquad (4.1)$$

The diffusion coefficient (D_s) is a constant of proportionality between flux, J_s, and concentration gradient (mM m^{-1}), where solute concentration (C_s) varies over a distance (Δx). Flux is measured as moles of solute crossing a unit area per unit time (mol m^{-2} s^{-1}), so D_s has the units m^2 s^{-1}. D_s has a unique value for a particular solute in water which would be quite different from D_s for the same solute in another medium, for example the oily interior of a lipid membrane.

When we consider diffusion of a molecule across a mem-brane from one solution to another, Fick's Law can be applied to each phase (solution 1–membrane–solution 2). However, flux across the membrane also depends on the ability of the molecule to cross boundaries (i.e. to partition) from solution into the hydrophobic membrane and then from the mem-brane back into solution. Another difficulty is that the thickness of membranes is relatively undefined and we need to know this for Fick's equation above (Δx). The two solutions might differ in pressure and voltage and these can change steeply across a membrane; however, if for simplicity we consider a neutral solute at low concentration, these factors are not relevant (see below for charged molecules). A practical quanti-tative description of the flux of neutral molecules across membranes uses an expression intuitively related to Fick's Law stating that flux across a membrane (J_s) of a neutral molecule is proportional to the *difference in concentration* (ΔC_s):

$$J_s = P_s \times \Delta C_s \qquad (4.2)$$

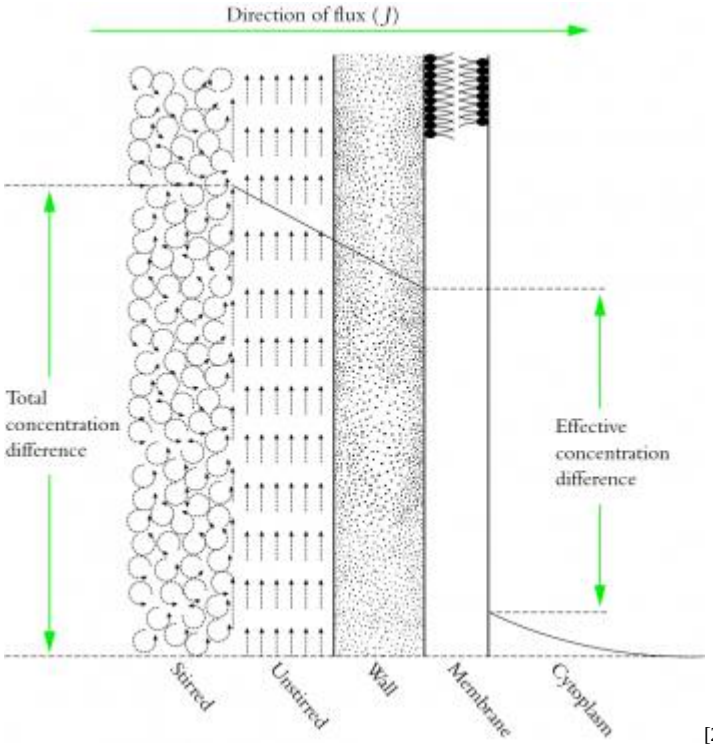


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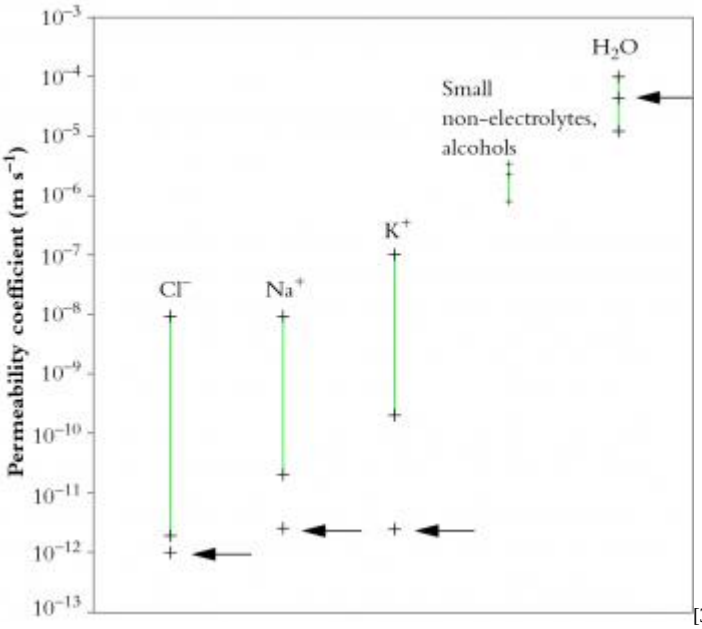
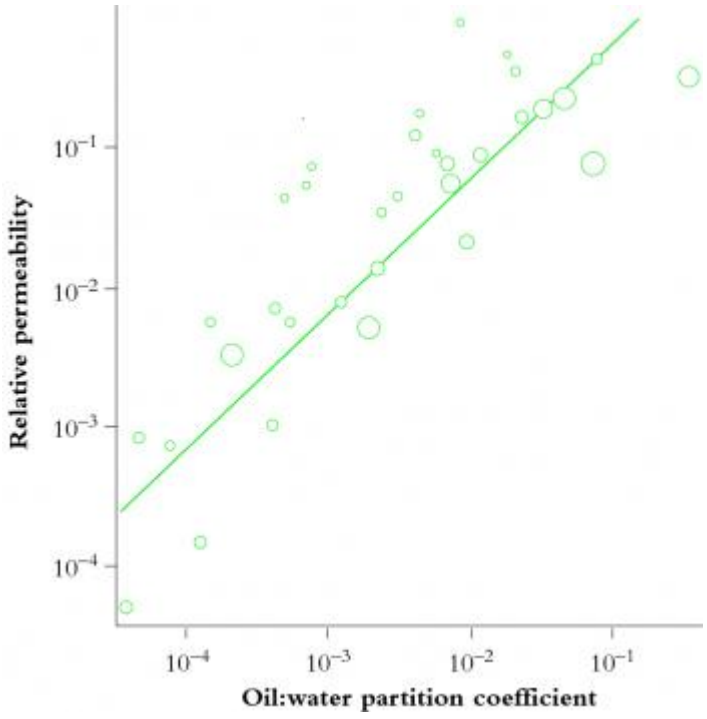
Figure 4.1 Concentration profiles across a plant cell wall and membrane when solutes diffuse from outside into the cytoplasm. Imposed concentration gradients will always be greater than gradients driving diffusion across the lipid membrane. Unstirred layers immediately adjacent to the cell wall, in the wall and in cytoplasm will result in measurements of permeability coefficients being underestimates of their true value for the membrane. Corrections can be made, for example by measuring solute diffusion across the wall without a membrane present and then subtracting wall effects (Based on Hope and Walker 1975; reproduced with permission of Oxford University Press)

The constant of proportionality in this case is the perme-ability coefficient (P_s), expressed in m s^{-1}. When P_s is large, solutes will diffuse rapidly across a membrane under a given concentration gradient. P_s embodies several factors: partitioning between solution and membrane, membrane thickness and diffusion coefficient of the solute in the membrane. It can be used to compare different membranes and to compare treat-ments that might alter the ability of a solute to move across the membrane. Note that Equation 4.2 assumes that the *mem-brane limits the rate of solute flux* and that concentration gradients leading to diffusion in solutions adjacent to the membrane will not be significant. If the two solutions are stirred rapidly then this will help to justify this assumption (Figure 4.1). How-ever, there is always an unstirred layer adjacent to the membrane through which diffusion occurs, and for molecules that can permeate the membrane very rapidly the unstirred layer can be a problem for the correct measurement of permeability.



[3]

Figure 4.2 The range of permeability coefficients for various ions, solutes and water in both membranes (bars) and artificial phospholipids (arrows). Note that the permeability of ions as they cross membranes is higher than that through a lipid bilayer, especially for potassium, indicating the presence of specialised permeation mechanisms in plant membranes. Water permeabilities are high in both membranes but can range over an order of magnitude in plant membranes. This variability may be partially accounted for by the activity of aquaporins.



[4]

Figure 4.3 Correlation between the permeability of a range of non-electrolytes in living cells of *Chara ceretophylla* and their ability to partition from water into oil (a high value of partition coefficient means that the solute easily enters the oil; note the log scale).The diameter of the circles

are indicative of the size of the molecule (Based on Nystrom 1973; with permission of Prentice—Hall)

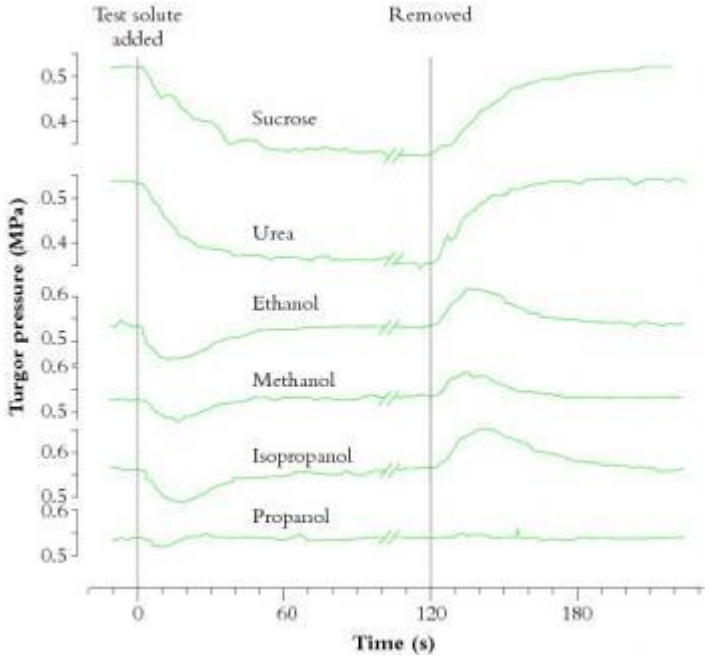
P_s differs markedly for different molecules passing through lipid-based membranes. Permeabilities can differ by eight orders of magnitude (Figure 4.2), reflecting the selectivity of native lipid bilayers. Differences in membrane permeabilities between molecules are much larger than differences in diffusion coefficients in free solution, the latter depending on the size of molecules rather than membrane properties. Collander (1954) showed that partitioning between water and oil phases (expressed as partition coefficient) was a determining factor for membrane permeability (Figure 4.3). Charged molecules and large polar molecules do not easily partition into the oily membrane while some polar molecules such as water, methanol and urea permeate faster than predicted from their partition coefficients. This indicates that there are special pathways for the movement of these small molecules other than through the lipid phase. A comparison of artificial lipid membranes with biological mem-branes supports this notion because it shows that many molecules and ions permeate biological membranes much faster than would be predicted on the basis of oil solubility and size (Figure 4.2). For these solutes there are *transport proteins* in biological membranes that increase solute permeability.

Section 4.3 describes how ΔP (the difference in pressure across the membrane and wall) in a plant cell is equivalent to the osmotic pressure difference ($P_i - P_o$) across the membrane when there is no water flow:

$$\Delta P = \Pi_i - \Pi_o \qquad (4.3)$$

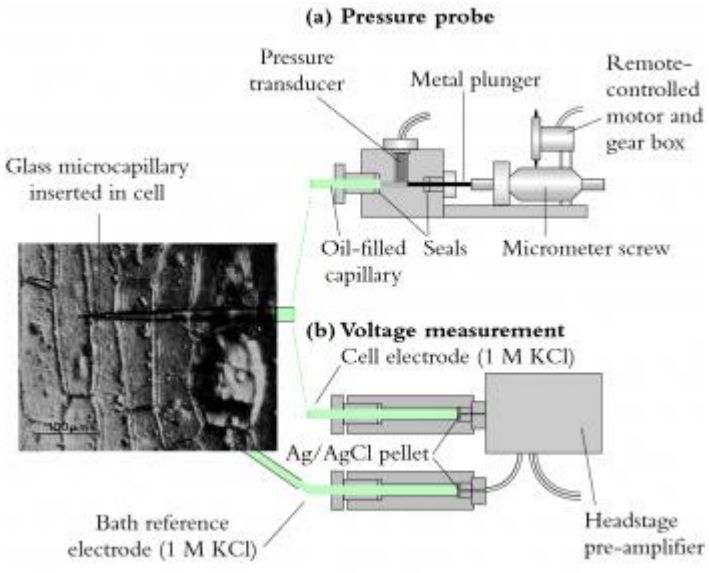
where i = inside and o = outside. This is the case only if a membrane is ideally semipermeable, that is, water permeability is much larger than solute permeability. The degree of semi-permeability that a membrane shows for a particular solute is measured as the reflection coefficient (s).

$$\sigma = 1 - \frac{\text{Solute permeability}}{\text{Water permeability}} \qquad (4.4)$$



[5]

Figure 4.4 Turgor pressure *(P)* in a *Tradescantia virginiana* epidermal cell as a function of time after the external osmotic pressure was changed with different test solutes. Measurements were made with a pressure probe (see Figure 4.5a). The initial decrease in *P* is due to water flow out of the cell and is larger for solutes with a reflection coefficient near one (sucrose and urea). Propanol induces no drop in *P*, indicating that its reflection coefficient is zero. Subsequent increase in *P* is due to penetration of particular solutes such as alcohol across the cell membrane. Water flows osmotically with the solute thereby increasing *P* to its original value. Removing solutes reverses osmotic effects.That is, a decrease in *P* follows the initial inflow of water as solutes (e.g. alcohols) diffuse out of cells (Tyerman and Steudle 1982; reproduced with permission of CSIRO)



[6]

Figure 4.5 Techniques employing fine glass capillaries to probe plant cells. Glass capillaries are heated and pulled to a fine point with an opening as small as 1 µm. (a) A miniaturised pressure probe. An oil-filled capillary is inserted into a cell whose turgor pressure (*P*) is transmitted

through the oil to a miniature pressure transducer. The voltage output of the transducer, which is proportional to *P*, can be read off on a computer or chart recorder. A metal plunger acting as a piston can be used via remote control to vary cell *P*. (b) A probe for measureing membrane voltage. The capillary is filled with 1 mol L^{-1} KCl and connected to a silver/silver chloride electrode that acts as an interace between solution voltage and input to the amplifier. A voltage is always measured with respect to a reference (in this case, a bath electrode). The headstage amplifier is close to the electrodes to minimise noise.

The reflection coefficient usually ranges between zero and one. Some substances such as mercuric chloride decrease water permeability of plant membranes so much (Section 4.1.3(a)) that s becomes negative. Close consideration of *P* in the presence of different solutes (Figure 4.4) reinforces the importance of s for osmosis. Using a pressure probe (Figure 4.5a) to measure *P*, the membrane is found to be ideally semipermeable for sucrose (s = 1); that is, the membrane almost totally 'reflects' sucrose. Over long periods, sucrose is taken up slowly but permeability relative to water is negligible. In this case, the change in *P* would be equivalent to the change in P (Figure 4.4, sucrose). If s is near zero, then water and the solute (say, propanol) are equivalent in terms of permeability. No change in *P* can be generated across a cell wall if s is zero (Figure 4.4). If s is negative, this leads to the intriguing situation where *P* changes in the opposite way to that which we normally expect.

For plant and animal cells, most major osmotic solutes have a s of about one so Equation 4.3 can be presented without s as a variable. However, s could be important when highly permeable solutes like ethanol, urea and ammonia reach sig--n-ificant levels, for example when membranes of waterlogged plants are exposed to ethanol and ammonia.

(b) Chemical potential

Diffusion of neutral molecules at low concentrations is driven by differences in concentrations across membranes (Section 4.1.2(a)). There are other forces that may influence solute diffusion, including the voltage gradient when considering move-ment of charged molecules (ions) and the hydrostatic pressure when considering movement of highly concentrated molecules (such as water in solutions). These forces can be added to give the total potential energy of a particular molecule relative to a reference value:

Total potential = Reference potential + Concentration + Electrical + Pressure

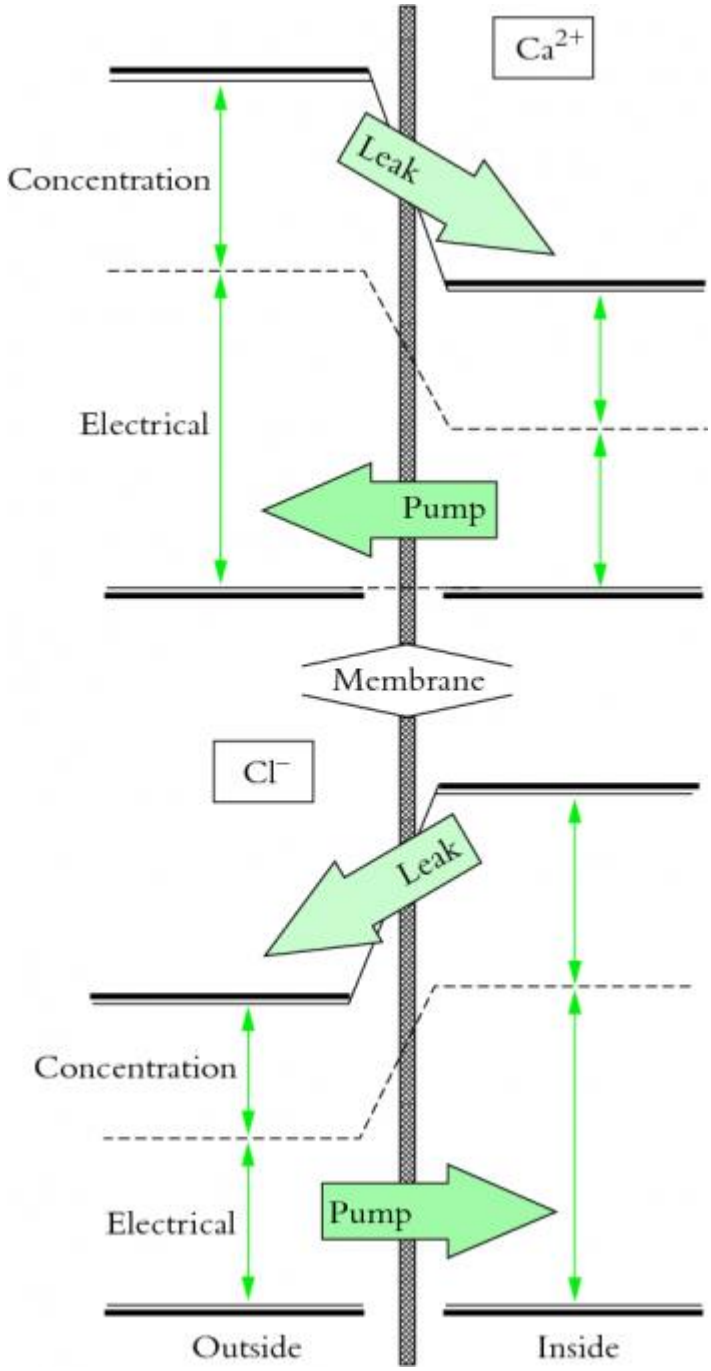
Gravitational potential energy could also be added to this equation if we were to examine the total potential over a substantial height difference, but for movement of molecules across membranes this is not relevant. The formal relationship for the chemical potential of a molecule *j* (u_j), measured as energy content per mole (joules mol^{-1}) and using the same order of terms as the expression above, is:

$$\mu_s = \mu_s^\star + RT \ln C_s + z_s F E + \bar{V}_s P \qquad (4.5)$$

The *concentration term* is a measure of the effect on chemical potential of the concentration (actually the activity which is usually somewhat less than total concentration). The gas con-stant, *R* (8.3143 joules mol^{-1} K^{-1}), and absolute temperature, *T* (equals 273.15 plus temperature in degrees Celsius, expressed in degrees Kelvin), account for the effects of temperature on chemical potential. Incidentally, from this term and the pressure term, the well-known van 't Hoff relation can be derived for osmotic pressure: P = *RTC*.

The electrical term is a measure of the effect of voltage (*E*) on chemical potential. The charge on a solute (*z*) is obviously relevant since if it was zero this term would not contribute to the total potential. The sign also determines whether an ion is repelled or attracted by a particular voltage. Electrical charge and concentration are related by the Faraday constant (*F*) which is 96 490 coulombs mol^{-1}. The electrical and con-centration terms form the basis of the Nernst equation (see below).

The *pressure term* measures the effect of hydrostatic pressure on chemical potential, where *P* = pressure and $\bar{V}_s$ is the partial molar volume of the solute.



[7]

Figure 4.6 Illustration of how electrical and concentration terms contribute to electrochemical potential of ions. Calcium (top) commonly tends to leak into cells and must be pumped out whereas chloride tends to leak out and must be pumped in to be accumulated.

Molecules diffuse across a membrane down a chemical potential gradient, that is, from higher to lower chemical potential. Diffusion continues until the difference in chemical potential equals zero, when equilibrium is reached. The direction of a chemical potential gradient relative to transport of a molecule across that membrane is important because it indicates whether energy is or is not added to make transport proceed (Figure 4.6). Osmotic 'engines' must actively pump solutes against a chemical potential gradient across membranes to generate *P* in a

cell. Sometimes ions move against a con-centration gradient even when the flux is entirely passive (no energy input) because the voltage term dominates the con-centration term in Equation 4.5. In this case, ions flow according to gradients in electrical and total chemical potential. For this reason, the chemical potential of ions is best referred to as the *electrochemical potential*.

(c) Ions, charge and membrane voltages

Ions such as potassium and chloride (K^+ and Cl^-) are often major osmotic solutes in plant cells. In fact, deficiency of either of these two inorganic nutrients can increase a plant's susceptibility to wilting. In addition, most other inorganic nutrients are acquired as ions and some major organic metabolites involved in photosynthesis and nitrogen fixation bear a charge at physiological pH. For example, malic acid is a four-carbon organic acid that dissociates to the divalent malate anion at neutral pH. Besides the role in generation of *P*, ionic fluxes and the associated electrical effects of these fluxes are components of signalling in plants. Calcium (Ca^{2+}) fluxes across cell membranes are involved in cell signalling and although not osmotically significant they play a crucial role in the way cells communicate and self-regulate. Finally, some ions are used to store energy but need not occur at osmotically significant concentrations. Cell membranes from all kingdoms use hydrogen (H^+) ions (protons) in one way or another to store energy that can be used to move other ions or to manu-facture ATP (Section 1.2). The highest concentration of H^+ that occurs is only a few millimoles per litre yet H^+ plays a central role in energy metabolism. Other ions such as sodium (Na^+) can also be used to store energy in plant cells (see the discussion of secondary active transport below).

To understand ion movement across membranes, two crucial points must be understood: (1) ionic fluxes alter and at the same time are determined by voltage across the membrane; (2) in all solutions bounded by cell membranes, the number of negative charges is balanced by the number of positive charges. Membrane potential is attributable to a minute amount of charge imbalance that occurs on membrane surfaces. So at constant membrane potential the flux of positive ions across a membrane must balance the flux of negative ions. Most biological membranes have a capacitance of about 1 microFarad cm^{-2} which means that to alter membrane voltage by 0.1 V, the membrane need only acquire or lose about 1 pmol of univalent ion cm^{-2} of membrane. A univalent ion is one with a single positive (e.g. K^+) or negative (e.g. Cl^-) atomic charge. In a plant cell of about 650 pL, this represents a change in charge averaged over the entire cell volume of 12 nmol L^{-1}!

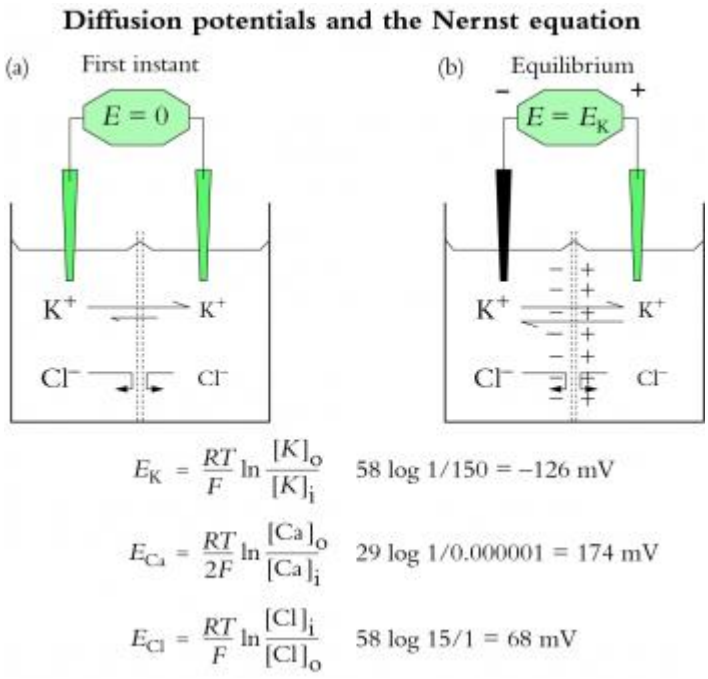
The membrane voltage or membrane potential difference, as it is sometimes called, can be measured by inserting a fine capillary electrode into a plant cell (Figure 4.5b). Membrane voltage is measured with respect to solution bathing the cell and in most plant cells the voltage is negative across the plasma membrane. That is, the

cytoplasm has a charge of –0.1 to –0.3 V (–100 to –300 mV) at steady state with occasional transients that may give the membrane a positive voltage. The tonoplast membrane that surrounds the central vacuole is generally 20 to 40 mV more positive than the cytoplasm (still negative with respect to the outside medium).

Cell membrane voltages can be affected by ion pumps, diffusion potential and fixed charges on either side of the membrane.

Special mention needs to be made of one such fixed charge which arises from galacturonic acid residues in cell walls. Al-though cations move to neutralise this fixed negative change, there is still a net negative potential associated with cell walls (Donnan potential). In spite of being external to the plasma membrane, the Donnan potential is in series with it and probably adds to what we measure as the membrane potential with electrodes.

Most charge on macromolecules in the cytoplasm is also negative (e.g. nucleic acids, proteins) and because of their size it can be regarded as a fixed negative charge. This has osmotic consequences. Macromolecules and their balancing cations result in a relatively high osmotic pressure (up to 200 mmol L^{-1} of total solutes) that results in inflow of water by osmosis and generation of *P* in most freshwater environments. In animal cells, where a cell wall is not present, body fluids must be regulated so that cell membranes do not rupture. For wall-less eukaryotes living in fresh water, a considerable amount of energy is expended in excreting water, for example by contractile vacuoles (see Case study 4.1). Walled eukaryotes have the luxury of letting water enter the protoplasm by osmosis where *P* is developed rather than the cell expanding until it ruptures.



[8]

Figure 4.7 How a diffusion potential develops through differential movement of an ion across a membrane. This is achieved here by separating a concentrated KCI solution from a dilute KCI solution by using a membrane which is permeable to K^+ but not Cl^-. Letter sizes indicate concentrations. Initially (a), a minute amount of K^+ crosses the membrane along its concentration gradient.This K^+ movement creates a positive charge in the right-hand compartment as K^+ concentration there rises above Cl^- concentration. At equilibrium (b), a diffusion potential is established, reflecting movement of charge (as K^+) to the right-hand chamber. Further inflow of K^+ is prevented. Concentrations never equalise on both sides because K^+ is the only species able to move through the membrane. Ditfusion potentials of K^+, Ca^{2+} and Cl^- in typical plant cells, calculated using the Nernst equation, are shown below. Energised transport must be invoked to explain the large disparity between actual membrane potentials (normally around -100 mV) and the diffusion potentials of Ca^{2+} and Cl^-

Different ions have different permeabilities in membranes. Potassium, for example, is usually the most permeable ion, entering under most conditions about 10 to 100 times faster than Cl^- (Figure 4.2). Since ions diffuse at different rates across membranes, a slight charge imbalance occurs and gives rise to a membrane voltage (Figure 4.7). This voltage in turn slows down movement of the rapidly moving ion so that the counter-ion catches up. The result is that when net charge balance is achieved, a diffusion potential has developed that is a function of the permeabilities (P_{ion}) of all diffusible ions present and concentrations of each ion in each compartment. The Goldman equation describes this phenomenon and gives the membrane voltage (ΔE) that would develop due to diffusion of ions. The Goldman equation for the ions that mostly determine this diffusion potential (K^+, Na^+ and Cl^-) is given by:

$$\Delta E = \frac{RT}{F} \ln \frac{P_K C_K^o + P_{Na} C_{Na}^o + P_{Cl} C_{Cl}^i}{P_K C_K^i + P_{Na} C_{Na}^i + P_{Cl} C_{Cl}^o} \qquad (4.6)$$

The superscripts refer to the inside (i) or outside (o) of the mem-brane and R, T, F and C are defined elsewhere (Equation 4.5). Note that the concentration terms for Cl^- are reversed in the numerator and denominator compared to the cations. This is because Cl^- is the only anion represented. Many texts do not include H^+ in the Goldman equation because, in spite of high permeability of H^+, diffusion of H^+ is unlikely to have a strong effect on ΔE at such low (micromolar) concentrations. However, membrane potential is occasionally dominated by the diffusion of H^+, indicating that H^+ permeability must be exceedingly high. For example, local variations in pH cause alkaline bands to form on *Chara corallina* cells and in the leaves of aquatic plants at high pH.

The Nernst equation

When one ion has a very high permeability compared to all other ions in the system the membrane will behave as an ion-sensitive electrode for that ion (e.g. Figure 4.7). A pH electrode which is sensitive to H^+ flux across a glass membrane

serves as an analogy. In the case of a single ion, the Goldman equation can be reduced to the simpler Nernst equation that yields the equilibrium membrane potential which would develop for a particular concentration gradient across a membrane.

$$\Delta E = \frac{RT}{zF} \ln \frac{C_o}{C_i} \qquad (4.7)$$

where R and T are the gas constant and temperature (degrees Kelvin) and F is the Faraday constant. Typical charges on ions (z) would be –1 (Cl^-), +1 (K^+) and –2 (divalent anions) and so on. This term in the Nernst equation gives the correct sign for the calculated membrane potential.

Table 4.1 Examples of comparisons between Nernst potentials (E_K) and recorded membrane voltages. In most cases an electrogenic pump is inferred from a recorded membrane voltage that is more negative than the most negative Nernst potential, in these cases for potassium. For Arabidopsis *(bottom) the fact that the Nernst potential for potassium is mostly more negative than the resting membrane potential indicates that potassium must be pumped in, probably by a proton symporter*

Cells	E_K (mV)	Membrane voltage (mV)	Medium (mM)
Chara corallina	–160	–220	0.1 K^+, pH 7
Beta vulgaris	–139	–209	0.6 K^+, pH 7.2
Elodea densa	–110	-135	1 K^+, pH 7.0
Avena	–90	–130	1 K^+
Pisum	–55	–102	10 K^+
Riccia fluitans	–106	–200	1 K^+
Arabidopsis thaliana	–231	–147	0.01 K^+
	–172	–134	0.1 K^+
	–113	–108	1 K^+

(Data from Findlay and Hope 1976; Maathuis and Sanders 1993) [9]

The Nernst equation is routinely used by electro-physiologists to calculate the equilibrium potential for each ion. Theoretical equilibrium potentials can then be compared with the actual membrane potential in order to decide whether the membrane is highly permeable to one particular ion. For example, in many plant cells there are K^+ channels that open under particular circumstances. When this occurs, the mem-brane becomes highly permeable to K^+ and the measured membrane potential very nearly equals the Nernst potential for K^+. The Nernst equation can also be used as a guide in deciding whether there is active transport through a membrane. For example, when the measured membrane potential is more negative than the most negative Nernst potential there must be active movement of charge across that membrane (Table 4.1).

Equation 4.7 can be rewritten with constants solved and $\log_{10}$ substituted for the natural logarithm. This yields a useful form as follows:

$$\Delta E = \frac{58}{z} \log_{10} \frac{C_o}{C_i} \qquad (4.8)$$

showing that 10-fold differences in concentration across a membrane are maintained by a 58 mV charge separation for monovalent ions. For example, –58 mV inside a cell will keep K^+ concentrations 10 times higher inside a cell than in the external medium and Cl^- concentrations 10 times lower.

(d) Active and passive transport

Plant cells acquire solutes and water across their membranes (plasma membrane and tonoplast) through the combined action of passive and active transport. During *passive transport*, a solute moves down its electrochemical potential gradient with no expenditure of energy. In *active transport* a solute moves against its electrochemical potential and hence energy input is required. The extra energy may be derived from the chemical energy released from hydrolysis of ATP or pyrophosphate (PP_i, a high-energy polymer of phosphate) or it may be derived from the movement of a cotransported solute or coupled solute down its electrochemical gradient. Coupling of downhill movement of one solute to uphill movement of another is a common feature of membrane transport.

Both the tonoplast and plasma membrane of plants and fungi contain pumps that move H^+ across membranes. These pumps use chemical energy from the hydrolysis of ATP or PP_i. By moving positive charge out of the cytoplasm they establish a large membrane voltage (inside negative) and a steep pH gradient. The electrochemical gradient for H^+ can be very large, the equivalent of about 400 mV. Bioenergetic analyses suggest that the energised proton pump in plasma membranes moves one H^+ per ATP hydrolysed.

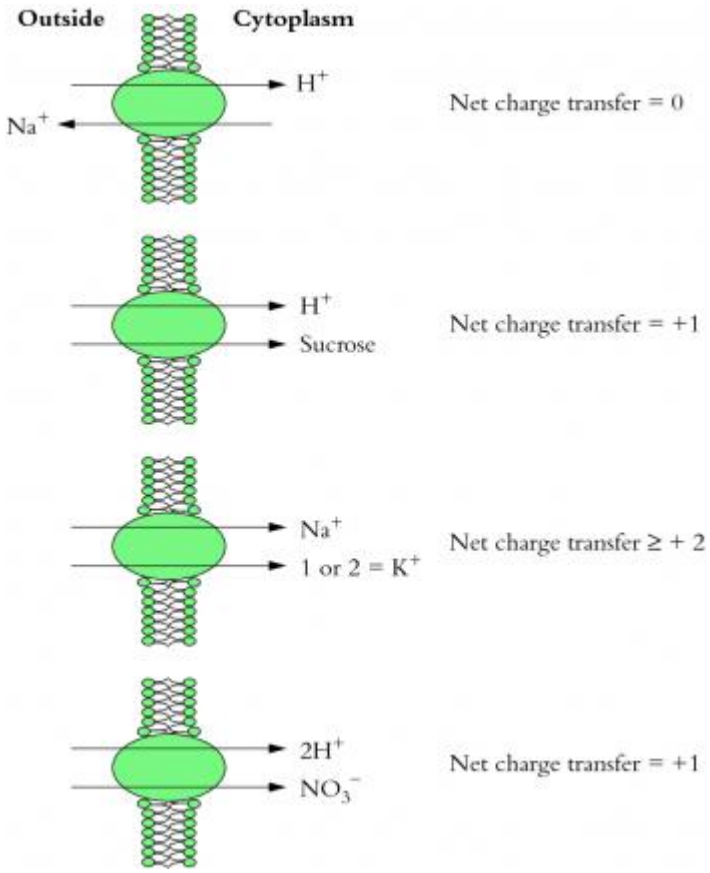


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Figure 4.8 Examples of cotransport systems in plant membranes. From top to bottom: *Sodium* (Na^+)/proton antiport occurs across both tonoplast and plasma membrane. It is required to pump Na^+ out of the cytoplasm, either into vacuoles across the tonoplast, or across plasma membranes into the external medium (Chapter 18). *Sucrose/proton symport* occurs across the plasma membrane and tonoplast of sucrose loading cells (Figure 5.33). *Sodium potassium symport*: it is not known at which membrane this transport system occurs since it was cloned from a gene from wheat and expressed in yeast and *Xenopus* oocytes. It might use an Na^+ gradient to accumulate K^+ from low concentrations (Gassmann *et al.* 1996). Nitrate (NO_3^-)/proton symport occurs across plasma membranes, particularly of roots where it drives NO_3^- uptake from soil. Up to three NO_3^- symporters might participate in NO_3^- uptake over a wide range of soil concentrations

The H^+ gradient established by the *primary active* H^+ pumps is then used to drive coupled active movement of other solutes across membranes. These coupled transport systems can be referred to as *secondary active* transport to indicate that they rely on a previously established gradient of another ion. There are many examples of H^+-coupled transport in plants (Figure 4.8). One example is the uptake of a $Cl^–$ ion, which is coupled to the influx of two H^+ ions. Chloride must be actively tran-sported across the plasma membrane because the membrane potential is usually so much more negative than the equili-brium potential for $Cl^–$. Coupling each $Cl^–$ entering the cell to the inflow of two H^+ ions means that there is a net charge transfer of +1 into the cell as each $Cl^–$ enters. This is more energetically favourable than zero net charge and definitely more favourable than a net negative

charge. Another example is H^+/sucrose cotransport which is important in the process of phloem loading (Chapter 5).

Secondary active transport in plants is not exclusively driven by H^+. Na^+ ion gradients sometimes drive secondary transport. This was first discovered in *Chara* but also occurs in some higher plants. A gene has been cloned from wheat roots for an Na^+-powered K^+ transporter and when expressed in frog oocytes (see Section 4.1.3(a)) the transporter uses an inwardly directed Na^+ gradient to drive uptake of K^+. Na^+-driven transport may have evolved in plants because under alkaline conditions (high external pH), the H^+ gradient may not be sufficient to drive transport. Such conditions exist in seawater which is well buffered at a pH of about 8.3 but contains abundant Na^+. The discovery of Na^+-driven transport is especially interesting because as yet no one has identified a primary Na^+ pump in plants. In animal cells, where Na^+ gradients are generally used in secondary active transport, there is a primary Na^+pump that pumps three Na^+ *out* and two K^+ *in* for every ATP molecule hydrolysed. This pump does not seem to exist in plant cells but an Na^+–H^+ exchanger (Na^+–H^+ antiporter) has been shown to occur on the plasma membrane and tonoplast of some plants.

Solute movement across membranes by either active or passive processes can be regarded as analogous to an enzymic reaction. Transport proteins within membranes act as enzymes, catalysing solute transport by lowering the activation energy for transport. As with ordinary enzymes, the reaction may be coupled to the hydrolysis of ATP or some other high-energy molecule or it may proceed (energetically) downhill. The analogy with enzymes is especially useful to calculate affinity of the transport protein for its substrate. If we plot the rate of transport versus concentration of substrate being transported, a hyperbolic curve is often obtained. Analysis of this Michaelis–Menten curve (see Case Study 4.1) reveals an affinity constant (K_m) and the maximum transport rate at saturating concentrations (equivalent to V_{max}).

The affinities of both passive and active transport vary widely between different transport systems in plants. Values of K_m can be in the range of micromoles per litre, or less, in which case we refer to the transport system as having a high affinity for its substrate (Figure 4.9a). Alternatively, K_m may be tens of millimoles per litre in which case it would be referred to as a low-affinity transporter (Figure 4.9b). There is no strict discrimination between active and passive transport systems on the basis of their affinity for substrates. Passive transporters can have high affinities and active transporters low affinities. Some transport systems do not saturate even if hundreds of millimoles of substrate per litre are present. This has often been attributed to transport through a pore in the membrane or a channel but there are many examples of channel-mediated transport that saturate at a few millimoles per litre and there is a Ca^{2+} channel from plant plasma membrane that has a K_m in the micromolar range. Finally plants often have multiple transporters for the same solute, providing a range of affinities. This allows transport to proceed

efficiently over a wide range of external concentrations, as would be encountered by a root in soil.

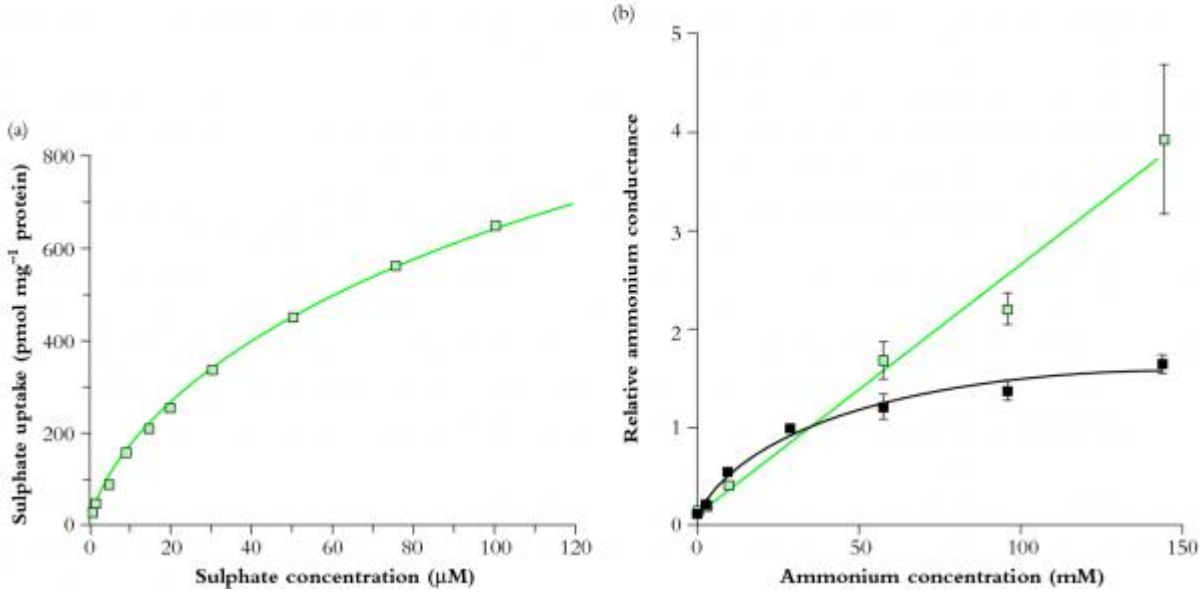

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Figure 4.9 Transport systems with high and low affinities for transported ions. (a) Sulphate uptake into membrane vesicles by a sulphate/ proton cotransporter was measured as a function of external sulphate concentration. This transport system has a K_m of 65 μM. (b) Ammonium (NH_4^+) transport through two classes of channel permeable to NH_4^+ was measured as a function of external NH_4^+ concentration. One channel did not show saturation (straight line) while the other did (K_m = 20 mM) ((a) From Hawkesford *et al.*1993; (b) from Tyerman *et al.* 1995; reproduced with permission of Nature)

4.1.3 Membrane proteins -catalysts for transport

Some proteins involved in solute transport through plant membranes have been identified by patch clamping and, in a few cases, their genes cloned. Ion compartmentation tells us that many more await discovery in higher plants.

(a) Passive transport systems

Proteins involved in passive transport facilitate non-energised flow of solutes and water down their respective energy gradients. Movement of a solute or water through these transport proteins is not coupled to movement of any other solute or to release of free energy from a metabolite. The proteins that facilitate passive transport are diverse; some are specific for particular ions and allow high transport rates per protein molecule (ion channels), some are specific for water (water channels or aquaporins) and some are specific for neutral solutes and may have slower transport rates per protein molecule.

Water channels or aquaporins

Some plant and animal membranse have much higher permeability to water than can be explained by diffusion rates through a lipid bilayer. Futhermore, the activation energy for diffusion of water across a plant membrane is lower than would be expected across a li[id bilayer, where water has to overcome the high-energy barrier of partitioning into a very hydrophobic oily layer (Figure 4.3). Some reports put the activation energy for water flow across membranes as low as the value for free diffusion of water. In other words, water enters the membrane about as readily as it diffuses through a solution. This suggests that water is moving across the membrane through a pathway other than the lipid, perhaps some kind of water pore or water channel. Since the discovery of water channel proteins in animal cell membranes, molecular biologists have also discovered that similar proteins exist in plants.

The approach has been to inject genetic material from plants into *Xenopus* oocytes (a particular type of frog's egg). Either cDNA arising from screens of cDNA libraries can be injected into the *Xenopus*nucleus or poly $(A)^+$-RNA injected into the oocyte cytoplasm where it is translated. The *Xenopus* oocyte is particularly useful because it is large, enabling observations of cell response to foreign proteins. It is one of several expression systems along with *Chara* (giant algal cells) and yeast cells. Plant water transport proteins expressed in the oocyte plasma membrane result in physiological changes; for example, the oocyte swells rapidly when the external osmotic pressure in the bathing medium is lowered (Figure 4.10a). Provided that the increase in water permeability is not a con-sequence of some other change or a side effect of other types of transport, it can be concluded that the protein catalyses transport of water across membranes. In plants, this protein could be located in the tonoplast, plasma membrane or endo-membranes. The first plant aquaporin gTIP (tonoplast integral protein) that was discovered occurs in the tonoplast and prob-ably accounts for its high water permeability. Mercuric chloride inhibits water channels, as shown by using the pressure probe in intact cells (Figure 4.10b, c).

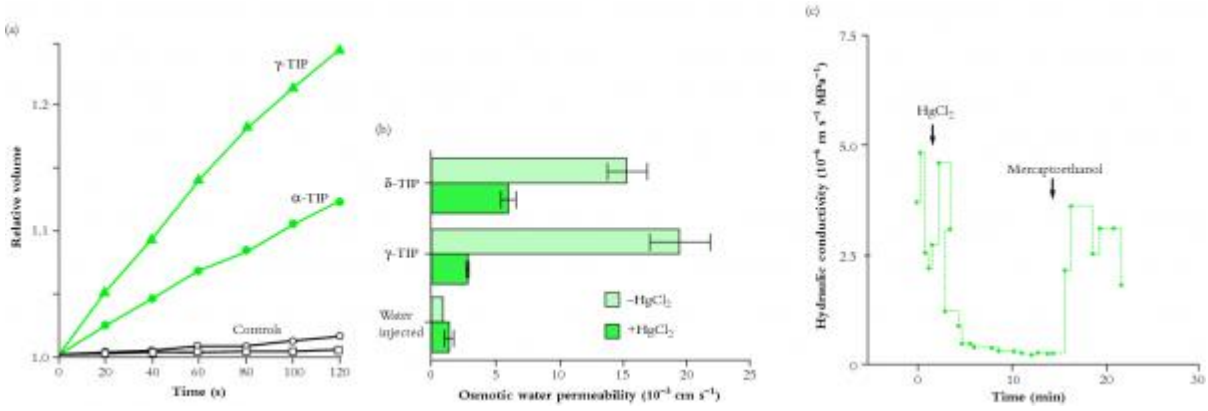

Figure 4.10 Evidence for presence of aquaporins (water channels) in plant membranes. (a) Rate of change in volume of *Xenopus* oocytes after lowering osmotic pressure of the external medium. Oocytes express foreign membrane proteins (Section 4.1.3(a)). In this case two tonoplast integral proteins (TIP proteins) were expressed in *Xenopus* membranes, causing a marked increase in water permeability of the oocyte and rapid swelling. 'Controls' were oocytes without foreign proteins and water—injected oocytes. (b) Sensitivity of two TIP proteins to mercuric chloride ($HgCl_2$), a general inhibitor of aquaporins in animal membranes. Osmotic water permeabilities peaked at *c.* 20×10^{-3} cm s^{-1}. (c) Inhibitory effect of $HgCl_2$ on hydraulic conductivity of the freshwater alga *Chara corallina*. Inhibition is reversed by applying mercaptoethanol to block the action of $HgCl_2$. Under mercury inhibition, the activation energy of water flow increases markedly indicating that water flow is now restricted to ditfusional flow across the lipid bilayer, that is, aquaporins are blocked. Hydraulic conductivity peaked at *c.* 5×10^{-6} m s^{-1} MPa^{-1} ((a) From Maurel et al. 1995; (b) from Daniels et al. 1996; (c) from Schutz and Tyerman 1997; reproduced with permission of (a) EMBO Journal, (b) Plant Cell, (c) Journal of Experimental Botany)

Why are there water channels in membranes when the lipid itself is already so permeable to water (Figure 4.2)? There are several rationales for the presence of water channels in plant membranes. One is that specialised transport proteins permit control of water flow. That is, a water channel protein may be turned on and off, for example by phosphorylation, while water permeability of the lipid is essentially constant. In animal cells, specifically kidney, water channels are controlled by the antidiuretic hormone. It remains to be seen if plant hormones could also influence the function of water channels.

A second rationale for the presence of water channels is to balance water flow and prevent bottlenecks. In roots, water channels are most abundant in the endodermis and inner stele where water flow across membranes is rapid. Aquaporins appear to be present at all points along roots but the possibility that they are more strongly expressed in root apices, where water flow can be rapid, remains open (Figure 3.28).

Ion channels

Following the advent of the patch clamp technique (Section 4.2.3) an explosion has occurred in identification of ion channels in animal and plant cell membranes. The plasma membrane of one plant cell can have as many as four distinct K^+ channels, two types of Cl^- channel and a Ca^{2+} channel. Why are there several channel types for one ion? The answer appears to lie in: (1) the nature of control exerted on the channel and (2) the capacity of large fluxes through ion channels (> 10^6 ions per second) to alter membrane potential rapidly. Ion movements in cells must be regulated tightly over a wide concentration range because of the many processes that are influenced by ion activities (Section 4.1.1).

Channels open and close randomly in time under the control of gating factors. The probability that a channel is open (P_{open} = time open/total time) is governed by various factors including: (1) membrane voltage; (2) binding of a ligand and (3)

membrane tension. Gating of ion channels by membrane tension is likely to be instrumental in the control of *P*. Voltage gating is particularly interesting since ion channels have a strong capacity to alter voltage, therefore influencing their own control factor. This can lead to feedback effects so that transient swings in membrane voltage are often explained by voltage-gated ion channels. When an ion channel opens, it tends to drive the membrane voltage towards the equilibrium potential for whichever ion permeates that channel; gating achieved by this voltage change can close the channel and damp further redistribution of charge.

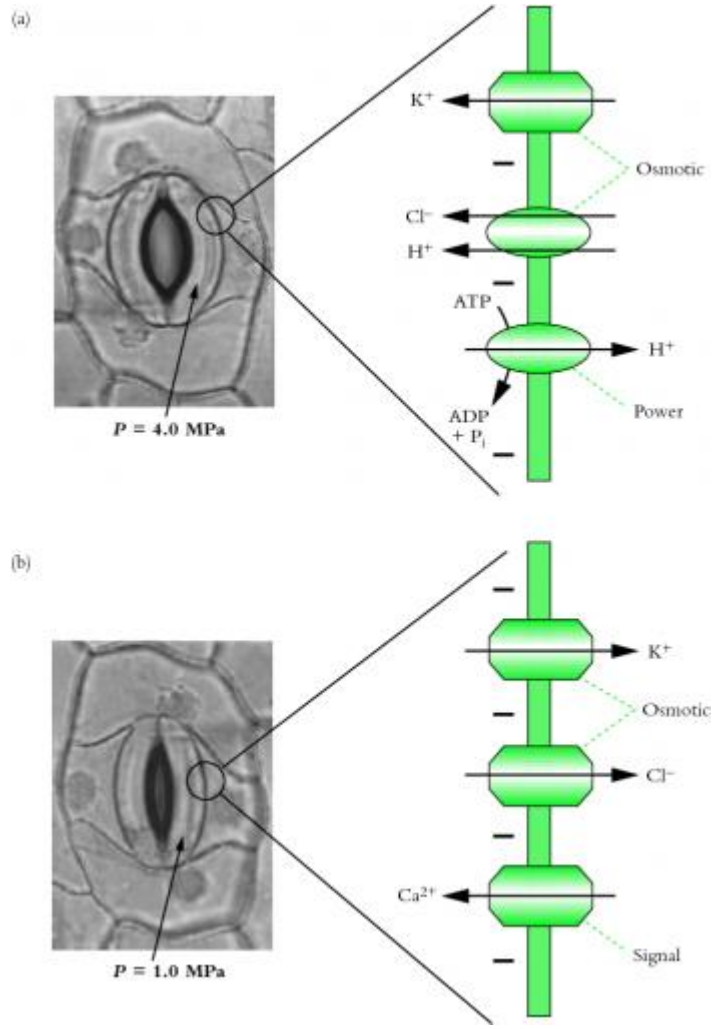

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Figure 4.11 Transport systems in plasma membranes that are essential for volume change in guard cells driven by net flux of potassium chloride (KCl). (a) An open stoma, in which the proton pump generates a large negative membrane potential. Blue light and red light stimulate proton pumping in guard cells. Negative potential drives K^+ influx through a K^+ inward-rectifier channel. The pH gradient developed by proton pumping drives Cl^--proton symport that must exist on the plasma membrane, although it has yet to be demonstrated. (b) A closing stoma where Ca^{2+} influx is a signal that initiates closure. Influx of Ca^{2+} depolarises the membrane (makes it less negative). An anion channel turns on, allowing Cl^- to escape, further depolarising the membrane to the point that membrane potential becomes more positive than the Nernst potential for K^+. The K^+ outward-rectifier channel then opens, allowing $K^+$to leave the cell. Overall, a small

amount of Ca^{2+} enters, followed by efflux of equal amounts of K^+ and Cl^-, causing an osmotically significant change.

Voltage gating can lead to ion channels behaving like nutrient valves. For example, a K^+ channel that only opens when the membrane voltage is made more negative than –120 mV will tend to let only K^+ move inwards, since the Nernst equilibrium potential for K^+ (E_K) is generally less negative than this. Rectification (i.e. only letting current pass in one direction) is a characteristic of many ion channels. This channel is called a K^+ inward-rectifier and is thought to be responsible for K^+ uptake from external solutions containing higher than 1 mM K^+ (producing an inwardly directed electrochemical gradient of K^+). It is present in root hair cells and stomatal guard cells (Figure 4.11(a) and Section 4.2.6). Other channels such as the K^+ outward-rectifier are also sen-sitive to concentrations of K^+ on both sides of the membrane. Combined with voltage gating, this channel becomes sensitive to E_K so that it will only participate in K^+ flow out of cells. The K^+ outward-rectifier is probably responsible for the rapid release of K^+ when guard cells lose osmotic solutes and stomata close (Figure 4.11b). It may also control release of K^+into xylem vessels.

Ligand-gated channels in plants are often either Ca^{2+}-permeable channels or anion-permeable channels. Anion channels in the guard cell plasma membrane are gated by external malate and auxin and seem to require nucleotides (e.g. ATP) on the cytoplasmic side. They are probably involved in depolarising the guard cell which is necessary to drive efflux of K^+ (Figure 4.11b) Anion channels in general are particularly effective at strongly depolarising the membrane since the resting potential is negative while the equilibrium potential for Cl^- is usually positive except in very saline conditions.

Channels are involved in more than transfer of nutrients such as K^+ and NH_4^+. They can also catalyse Ca^{2+} release into the cytoplasm, an important event in signalling. For example, Ca^{2+} channels located on the tonoplast have been shown to be gated by inositol 1,4,5-trisphosphate and another by cyclic ADP-ribose. These molecules are signal transducers in animal cells and are likely to be also involved in stimulus-response coupling in plant cells (Section 9.3). In plant cells, vacuoles contain high concentrations of Ca^{2+} (*c*. 10 mM) so that when Ca^{2+} channels in the tonoplast open, there is a strong gradient for Ca^{2+} to flow into the cytoplasm, where it is scarce. A significant increase in cytosolic Ca^{2+} concentration results. Enzymes in the cytoplasm that are Ca^{2+}-dependent, such as Ca^{2+}-dependent protein kinases, then modify other enzymes by phosphorylating them in a signal transduction cascade.

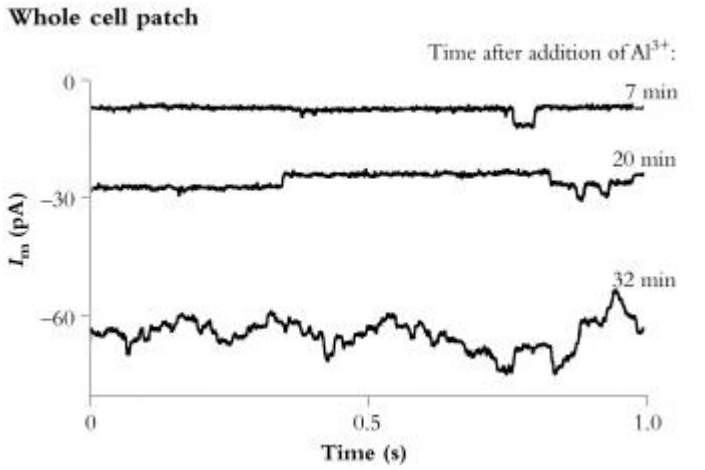

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Figure 4.12 Patch clamp experiments on protoplasts isolated from the root tip of wheat. When aluminium (Al^{3+}) is added, an anion channel opens, as seen from traces of current (I_m) made over 32 min. The single channel openings can be observed (horizontal lines) in the whole-cell configuration (From Ryan et al. 1997; reproduced with permission of PNAS)

Another interesting role of ion channels in roots is to modify the concentration of ions in the surrounding solution. One example is an aluminium (Al^{3+}) tolerance mechanism in wheat roots. Iron–aluminium complexes are a basic ingredient of clay, Al^{3+} being released from these complexes when soil becomes acidic (Section 16.5). Even very low ionised Al^{3+} concentrations (less than 10 µM) are toxic to root tip cells. In some tolerant varieties of wheat, and other species, organic anions (mainly malate in wheat) are released from root tips when tip cells register the presence of free Al^{3+}. Malate and citrate bind very strongly to Al^{3+}, thereby protecting the root membranes from contact with Al^{3+}. Significantly, there is an anion channel in root tip cells that is not evident in mature root tissues and this channel is specifically opened even at low concentrations of Al^{3+} (but not other trivalent cations) (Figure 4.12). Features of the anion channel measured in patch clamp experiments match many of the characteristics of malate exudation from intact root tips exposed to Al^{3+}. This channel may therefore be one of the gene products that confers tolerance in wheat. However, questions remain as to how the presence of Al^{3+} turns on the channel; are there specific receptors for Al^{3+} in membranes closely linked with the channel or do Al^{3+} receptors control the channels via a signal cascade?

Several genes that encode ion channel proteins have now been cloned and studied in expression systems such as the *Xenopus* oocyte. Not surprisingly these genes show some homology with animal channel counterparts and in some instances the plant genes were found by using probes based on this sequence homology (e.g. the voltage-dependent Cl^- channel from tobacco). In the case of *KAT1*, which appears to be primarily expressed in guard cells, there is homology with the *SHAKER* genes of *Drosophila*. However, in *Drosophila* these channel-encoding genes code for outward-rectifier channels, posing interesting questions as to what components of the protein cause the channel to have an opposite voltage

dependency in plants. Using site-directed mutagenesis so that amino acid sequence can be altered in the protein, and studying the altered properties of the channel in an expression
system, it will be possible to determine the regions of the protein responsible for selectivity and voltage sensitivity. It may even be possible to alter the properties of channels so that they become more selective for particular ions.

(b) Some key active transporters

Plasma-membrane-associated H^+-ATPases are crucial to plant cell function but are low-abundance proteins representing at most 1% of plasma membrane protein and 0.03% of total cellular protein. These proteins play a primary role in cells by pumping protons and thereby generating a protonmotive force which drives secondary active transport. Plasma membrane H^+-ATPase has a phosphorylated intermediate during the catalytic cycle which places it in the class of P-type ATPases: this class includes a variety of cation pumps such as the Ca^{2+} pump. Na^+–K^+ ATPases, which are the primary active transport systems in most animal cells, are also P-type ATPases and share some homology with plant and fungal H^+-ATPases. The H^+-ATPase protein has a molecular weight of 100 kD and is thought to function as a monomer *in vivo*. In *Arabidopsis thaliana* there are at least 10 separate genes coding for different isoforms of H^+-ATPase, probably corresponding to different cell and tissue types and to different developmental stages. For example, a H^+-ATPase responsible for energising sucrose transport has been identified in developing legume seeds (Figure 5.32). Variations in affinities of ATPases for ATP might reflect functional requirements of different isoforms.

Fine control of H^+-ATPase activity is achieved by a variety of factors, commensurate with the protein's pivotal role in plasma membrane transport. Cytoplasmic pH is closely regulated in plant cells and the H^+ pump has a central role in achieving this homeostasis. Plant hormones such as auxins stimulate H^+-ATPases and the resulting acidification of cell walls is thought to be a primary step in cell wall loosening to allow expansion growth. Auxin also seems to increase H^+-ATPase incorporation into membranes, probably by activating genes involved in synthesis or incorporation of protein into membranes. Fungal elicitors and toxins (e.g. fusicoccin) also stimulate H^+-ATPase and these have been used in many studies on regulation of the pump. Specific receptors for these molecules on the plasma membrane appear to set off a signalling response capable of stimulating H^+ pumping. Secondary messenger pathways in the cytoplasm and plasma membrane involving G-proteins and protein phosphorylation/dephosphorylation are involved in signal amplification, probably through a Ca^{2+}-dependent cascade. In this case, control of the pump resides partly in an autoinhibitory domain on the carboxy terminus of the protein because trypsin digestion of this domain (or engineering coding errors into the gene) results in increased activity.

The plasma membrane H^+-ATPase has been purified and incorporated into an artificial lipid bilayer. This has allowed coupling between ATP hydrolysis and pumping of H^+ to be measured electrically under conditions where the energy supply and gradient can be rigorously controlled. Such experi-ments on the isolated pump as opposed to *in vivo* studies where other transport systems interfere should yield unprecedented detail of the pump mechanism particularly if it is combined with site-directed mutagenesis to alter some key amino acids in the protein.

Various types of sugar, amino acid and peptide and amine transporters have been identified in plants, originally via classic biochemical and biophysical techniques and more recently by molecular approaches. Various amino acid transporters have been identified in plants and several genes have been cloned. These genes are not all from one gene family and the transporters they encode show differences in specificity to amino acids. The sugar transporters are vitally important for redistribution of assimilates to non-green parts of the plant and to developing seeds. The mechanism of long-distance transport of assimilates in the phloem is ultimately driven by plasma membrane H^+-ATPase, developing voltage and H^+ gradients capable of energising symport or antiport of sugars (Section 5.6). One of the sucrose transporter genes is expressed in phloem, either in the companion cell plasma membrane or sieve tube membranes. Another sucrose transporter gene is expressed during seed development. Specialised plant tissues are likely to have assimilate demands which differ from those cells surrounding them and therefore we might expect further progress in the identification of tissue-specific transporters. Vascular tissues, which exhibit substantial cell to cell variations in assimilate fluxes, are candidates for such an analysis.

CASE STUDY 4.1 The power of biological pumps

R. L. Bieleski

Early studies on transport processes in plants explored the general features of mineral nutrient uptake. When a pump works against a gradient, energy has to be used; and so it came as no surprise that anything which blocked energy-generating pathways (poisons such as cyanide, azide or dinitrophenol) or slowed metabolism (cold temperature, anaerobiosis) interfered with uptake. Once ions were inside cells, they did not diffuse readily back into the surrounding medium, even when energy supply was virtually halted by these methods; that is, the pumps had non-return valves. Though ions did not move back into the medium, they were readily transferred to other cells and eventually to the body of the plant. Our ability to explore pump behaviour took a great leap forward once radiotracers became available to study the mechanisms involved. Over the last 50 years or so, our

concepts of ‘how pumps work’ have developed and changed, and Australasian scientists have been strong contributors to the learning process. In the early 1950s, a group in the CSIRO led by R. N. Robertson pioneered the use of concepts and methods taken from physics to attack this biological problem, and in so doing helped found the discipline of biophysics. Based on their use of carrot slices and the chloride (Cl^-) ion as a model, they interpreted uptake as an essentially ‘electrical’ phenomenon, with ion movement being directly coupled with flow of electrons through the respiratory cytochrome pathway.

Various observations soon began accumulating which called for a rethink. In a nutshell, pumps operated on too many dif-ferent molecules and had too many biological properties to be run in that way. For example, when working in Robertson’s laboratory, I showed that glucose and sucrose movement into sugar cane slices also satisfied the criteria of pump-driven processes (e.g. with selectivity and movement against a concen-tration gradient), like Cl^- transport, yet the sugar molecule was non-ionic and should have been inaccessible to electrical systems. A possible option was that sucrose was made into a charged form (e.g. by phosphorylation), but other evidence was pushing us more towards modelling uptake on the behaviour of enzymes.

Anaylsis of pumps with an enzyme analogy

Why was an individual pump so selective, with methyl glucose behaving very differently from glucose, with nitrite and nitrate having totally separate uptake systems, and with orthophosphate (HPO_4^{2-}) appearing to share its pump with arsenate ($HAsO_4^{2-}$) but not with sulphate (SO_4^{2-})? The enzyme-like characteristics of potassium (K^+) uptake were demonstrated by E. Epstein (University of California) using barley roots (Figure 4.16). This enzyme analogy was fruitful because uptake rates plotted against concentration fitted a rectangular hyperbola allowing a ‘double-reciprocal’ or ‘Lineweaver–Burke’ analysis of uptake. For a single hyperbola, a plot of (1/rate) against (1/concentration) gives a straight line which reveals V_{max} (‘capacity’ of the pump, its maximum rate of uptake) and K_m (the sucking power or ‘affinity’ of the pump for its supply material). Competition between K^+ and Rb^+ is evident as competitive inhibition of Rb^+ uptake in response to additions of K^+ (Figure 1), a phenomenon first identified in enzyme kinetics. Even uptake of sugars into sugar cane closely followed this enzyme-like pattern, with a K_m (250 µM for sucrose) of the same order as that being found for various cations. We still use these kinetic terms today and though kinetic parameters are mathematical abstractions as they stand, they took on more meaning when it was realised that K_m often resembles the concentration of that ion found in typical soil solutions, while V_{max} is comparable to the rate of supply needed by the plant to support its maximum growth rate.

Like enzymes, pumps can change their activity in response to presence or absence of substrates. Section 4.2.7(a) describes how this occurs for K^+ and orthophosphate

which allosterically regulate their carriers according to nutrient demand. Nitrogen uptake illustrates how the full range of pump activities is coordinated to optimise supply of such an important inorganic resource. Ammonium (NH_4^+), the favoured nitrogen source in plants, is taken up by a constitutive transport system (i.e. uptake capacity is always present). If NH_4^+ fulfils the nitrogen demand of a plant, a nitrate (NO_3^-) pump is not needed so NO_3^--transporting capability drops to a low level. If this plant is then supplied NO_3^- and not NH_4^+, NO_3^--carrier activity is rapidly re-formed (*induced*) to sustain nitrogen uptake. Nitrite (NO_2^-), a third but less desirable nitrogen source, can induce formation of an NO_2^--specific pump. Such inducible pumps are sometimes called *permeases*.

These 'electrical' and 'enzyme' concepts have begun to merge. Uptake is now seen as powered by the respiration path but with proton flow rather than electron flow providing the driving force, while solute entry itself is controlled and given specificity by enzyme-like transporter proteins subject to the same sort of expression controls as other gene products. These proteins have a central hydrophobic portion which sits in the lipoidal cell membrane, with two hydrophilic ends that inter-face with the aqueous apoplasm and cytoplasm at the outer and inner membrane surfaces respectively. According to one model, 5 to 12 of these proteins join in a ring to make a tube with a central channel crossing the membrane, through which transported molecules pass. The energy required to push materials against a concentration gradient is met by an ATP-driven proton pump which shifts protons (one H^+ per ATP hydrolysed) from inside to outside the membrane; then as H^+ returns across the membrane, its inward movement is tightly coupled with that of the material being pumped, so providing the necessary thrust in a symport or coport process.

Pumping orthophosphate

My own research interest has centred around two particular pumps, one for uncharged sugars (particularly sucrose) and one for the anion orthophosphate. They represent the extremes of what is required from a pumping system. Of all the solutes in plant cells, sugars are typically present in greatest concen-tration, and have to be moved from cell to cell in the greatest amount — in enzyme terms, the sugar pumps have to have a very high V_{max}. In contrast, an orthophosphate pump must achieve a 10 000-fold concentration of a scarce resource, similar to that between the atmosphere and a high vacuum chamber of a freeze-drier. This requires a 'high-affinity' pump (a very low K_m). Because of its extreme nature, orthophosphate shows some transport phenomena particularly well when used as a model system. For example, beetroot slices that have been pretreated by aerating in 1 mM $CaSO_4$ at room temperature for 24 h can demonstrate some basic ortho-phosphate transport features in a three-hour laboratory session.

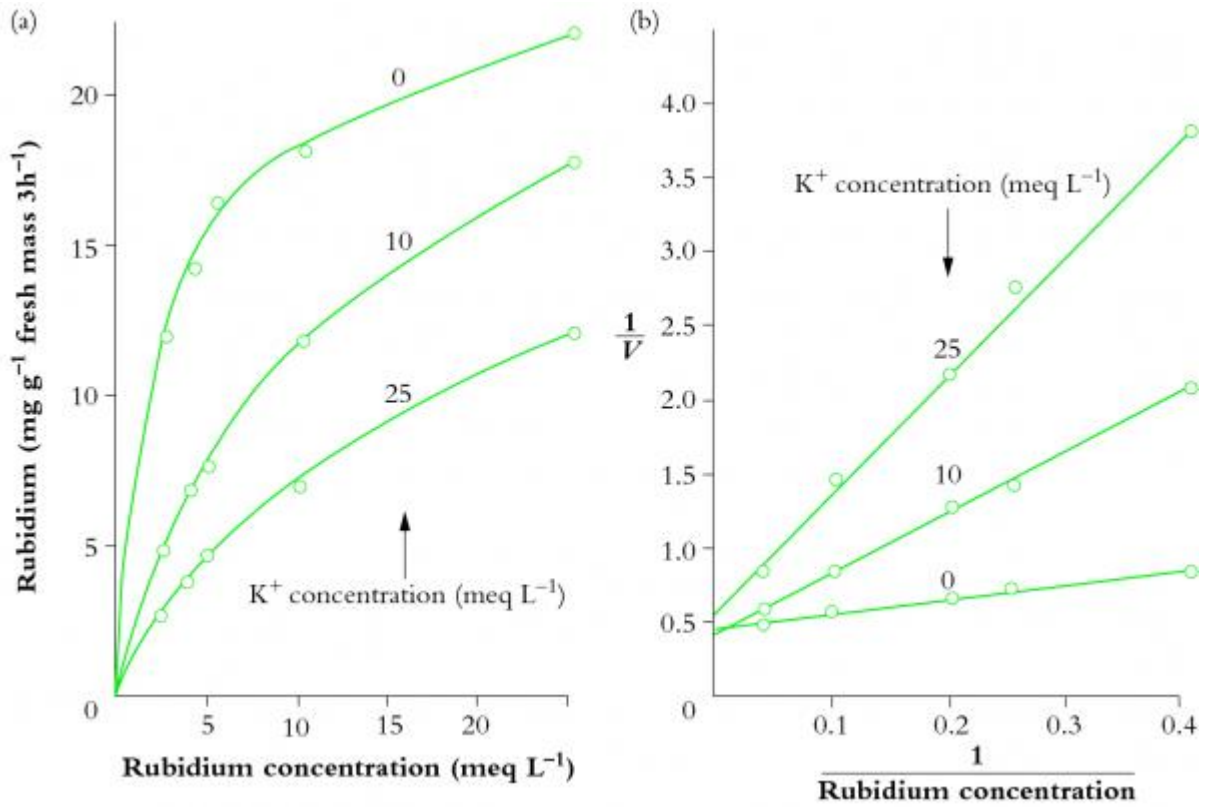

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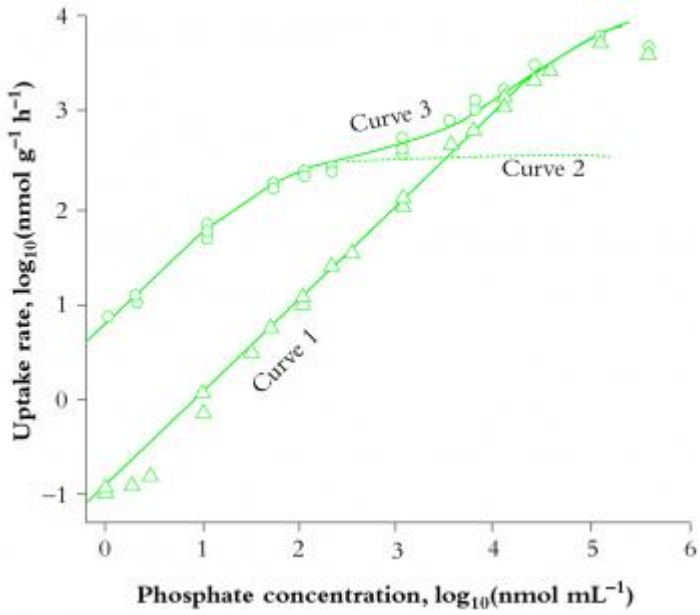
Figure 1 Effect of rubidium concentration on rubidium uptake rate into barley roots, expressed in a standard plot (a), and as a double-reciprocal plot (b). In the double-reciprocal plot, the intercept on the y-axis gives ($1/V_{max}$), and the intercept on the x-axis gives ($-1/K_m$).The three lines illustrate the competitive inhibition of rubidium uptake by potassium (Epstein and Hagen, 1952; reproduced with permission ofthe American Society of Plant Physiologists)



[16]

Figure 2 Coexistence of low-affinity and high-affinity orthophosphate pumps in celery vascular bundles. Freshly excised bundles have only one pump. Curve 1 is the calculated line for a K_m of

75 mM and a V_{max} of 9500 nmol g^{-1} fresh mass h^{-1}, and it gives an excellent fit with the experimental points (Δ) for that tissue. When the excised bundles are aerated for 24 h in 0.1 mM $CaSO_4$ (aged), they develop a second orthophosphate pump which adds on to the first. It has the constants K_m = 58 µM and Vmax = 355 nmol g^{-1} h^{-1}, and is shown by curve 2. Total orthophosphate uptake in aged bundles (O) is the sum of uptake by the two pumps (curve 1 + curve 2 = curve 3) (Bieleski 1966; reproduced with permission of the American Society of Plant Physiologists)

Interpretation of orthophosphate transport has, however, presented one problem, in that the uptake rate/concentration curve often fails to conform to the simple 'enzyme' relation-ship of a rectangular hyperbola (Figure 1). Various inter-pretations have been made, but the most common is one I have been partly responsible for developing, in which we see the relationship arising from the joint operation of *two* enzyme-like orthophosphate pumps having distinct kinetic characteristics (Figure 2). We call these the 'high-affinity' and 'low-affinity' systems, where the 'high-affinity' system (the one that is good at scavenging phosphate from the environment) has a very low K_m, around 2–5 µM orthophosphate, and the 'low-affinity' system has a K_m of 300–800 µM orthophosphate. Leading on from this, there has been a debate about whether the two systems coexist in the plasma membrane (the view of E. Epstein) or whether we are seeing the contrasting behaviour of two different membranes in series (plasma membrane and tonoplast; G. Laties) or different cell types within the experi-mental material (M. Pitman and others). Subsequent research findings support all three concepts as companions rather than competitors. The original two-system patterns were probably the product of two carriers located in the plasma membrane. However, patch clamping now provides evidence that the tonoplast as well as the plasma membrane has a very effective phosphate transporter (Section 4.2). Similarly, studies by molecular biologists, particularly with *Arabidopsis* mutants as a model, confirm the coexistence of separately coded 'low-affinity, and 'high-affinity' orthophosphate uptake systems; but they also imply additional orthophosphate transport systems concerned with unloading and redistribution of phosphate around the plant, and which are expressed in specific tissues. In my view, the joint presence of a 'low-affinity' and 'high-affinity' system allows plants to cope with an extremely wide range of orthophosphate concentrations. Though the soil con--cen-tration encountered by the *plasma membrane* of root cells is around 1–5 µM, the concentrations inside the cell confronting the *tonoplast* are around 5000 µM. Furthermore, if a cell unloads its orthophosphate or dies, and releases its solutes into the fluids of the intercellular system (the *apoplasm*), it could potentially expose the plasma membrane of adjacent cells to equally high orthophosphate concentrations. Having two systems adapted for different ends of the concentration range may give cells a better ability to manage orthophosphate uptake and redistribution than by using one system alone. Isolation of phosphate transport mutants should help a great deal in unravelling questions about the interplay of the two systems.

In summary, a plant controls orthophosphate entry by managing two inward pump systems which adjust in response to external supply (Section 4.2.7(a)). However, situations may arise where there are major and rapid changes in demand for orthophosphate inside the plant under conditions where the external supply has not changed (for example, resulting from a period of darkness or a sudden drop in nitrogen supply). If a plant were able to modulate orthophosphate loss actively through the efflux mechanism as well as orthophosphate uptake itself, a much more effective total control of net orthophosphate uptake would be achieved. Efflux may be a plant's safety valve for orthophosphate. There is plenty of territory here for future research, but it is my guess that efflux will turn out to be part of the mechanism for maintaining the orthophosphate balance of the plant (homeostasis), with cytoplasmic orthophosphate concentration being the signaller and controller of its operation.

The concept of active, outwardly facing pumps pushing things out of a cell into the outside world has been undervalued. People interested in phloem transport (particularly of sugars) have been half-way there in talking of 'unloading', where move-ment is from the cell into the apoplasm around it. Mostly, the possible involvement of pumps in such 'unloading' is not discussed because the concentration gradient favours passive (diffusive) movement anyway. However, as the ortho-phosphate story shows, pumps may sometimes be needed to provide control of flow rather than to overcome a gradient. It is something of an irony that the most visible and common example of sugar unloading we have, the secretion of nectar by a flower nectary, almost certainly has to involve an outward-facing pump, in that the final 'sink' concentration is extremely high, sometimes exceeding 250 mg sugar mL^{-1} (about 1 molar), so that secretion must be against a concentration gradient.

Most of this case study has been about orthophosphate pumps, with their ability to overcome steep gradients. But in finishing, it is worth looking at the *mass* of material a sugar transporter must move. A tissue which is actively transporting sucrose (e.g. excised phloem tissue obtained as stripped celery vascular bundles) can move up to 2 mg sucrose g^{-1} fresh mass h^{-1}. That corresponds to 3 ¥ 10^8 molecules of sugar per cell per second, when each molecule has to be handled individually by a transporter assembly! Each transporter assembly is about 30 Å across, so that even if 10% of the membrane surface were occupied by sucrose transporter sites, the transport rate would be about one molecule of sucrose per transporter site per second. Powerful pumps indeed!

References

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Epstein, E. and Hagen, C.E. (1952). ‘A kinetic study of the absorption of alkali cations by barley roots’, *Plant Physiology*, **27**, 457–474.

4.2 Regulation of nutrient ion exchange

4.2.1 Compartments, channels and transporters

Transport phenomena at the membrane level give an insight into how cells acquire and compartmentalise resources. Elec-trical and chemical gradients across membranes, transport of solutes through membrane proteins and factors controlling these proteins all contribute to precise control of individual solute levels within cells. Little in water and solute transport is left to chance. Even passive diffusion of molecules across mem-branes is influenced by channels whose specificity, frequency and control responses are all encoded genetically. Tight regulation of transport at the cell and organelle level are often dependent on metabolic conversions to optimise resource use in whole organisms. For example, malate delivered to the symbiosomes of N_2-fixing nodules is metabolised to provide carbon skeletons and energy while ammonium (NH_4^+) leaves the symbiosomes through an NH_4^+ channel, providing a nitrogen source for the plant (Whitehead *et al.* 1995).

Membranes also participate in control by providing the boundaries of compartments from which solutes can be sequestered or withdrawn — vacuoles are a common example of this kind of compartment. For instance, vacuoles act both as repositories for toxic ions and internal stores of calcium (Ca^{2+}) for secondary messenger signalling (Section 4.1.3(a)).

4.2.2 Transport in cells and tissues

Net transport through a membrane is normally a balance between inflow and outflow across that membrane. So, the rate of solute absorption by a cell, tissue or organ can be controlled by changes in either the amount going in (influx), the amount coming out (efflux) or a combination of both. Over long periods we observe the net sum of these fluxes but a closer look at membrane physiology shows us that flux in each direction might be under independent control(s). Some examples include gating factors (Section 4.1.3(a)) and high-energy compounds (e.g. PP_i) in ion pumps (Section 4.1.2(d)). In this way, each subcellular compartment has characteristic membrane properties which determine the direction and rate of ion flux through that compartment. Resource use by whole tissues is optimised through this fine control of subcellular compartmentalisation and long-distance transport, both contributing to a plant’s capacity to withstand variations in

resource supply. For example, orthophosphate concentration in the cytoplasm is maintained at steady levels by ortho-phosphate transport across the tonoplast, with vacuoles acting as an orthophosphate reservoir during phosphorus deficiency (Lee *et al*. 1990). At the whole-plant level, adequate phosphorus reaches young, growing tissues by long-distance transport in the phloem.

Single cells do not function as isolated entities therefore solute accumulation by whole tissues cannot be predicted solely from the transport properties of its component cells. Gradients in solute concentration can develop across bulky tissues because ions diffuse only slowly through apoplasmic spaces. The proposal that ion uptake capacity at the root epidermis exceeds that in deeper cell layers is consistent with coordination of influx mechanisms and bulk root structure. In many cases we struggle to provide even a broad outline of what happens. This section aims to synthesise a picture of where transport processes fit into whole-tissue behaviour. Examples of how experimentation has led to an understanding of the regulation of nutrient fluxes are given in the hope that this might stimulate the interest of a reader in joining the search. New reasons for optimism come from two powerful techniques, patch clamp methods and genetic manipulation.

4.2.3 Patch clamping

Patch clamp analysis involves sealing very fine glass capillaries, with tip diameters of about 1 µm, to the surface of protoplast or vacuole membranes. Using a brief (a few milliseconds) pulse of 1 V, it is possible to rupture the small area of membrane sealed across the orifice at a capillary tip, leaving a protoplast or vacuole like a balloon stuck on the end of a straw (Figure 4.13a). Solution within the capillary diffuses into the membrane-bound space, replacing the cytoplasm or vacuolar contents. Data from this preparation provide infor-mation on the whole membrane surface and it is called the ‘whole-cell’ configuration. Alternatively, a small patch of mem-brane sealed across the tip can be retained after tearing it away from the protoplast, which is discarded. The inside surface of the plasma membrane then faces the solution in the experimental chamber which can be changed at will. This configuration is called an ‘inside-out’ patch. (It is also possible to get ‘outside-out’ patches — Figure 4.14.)

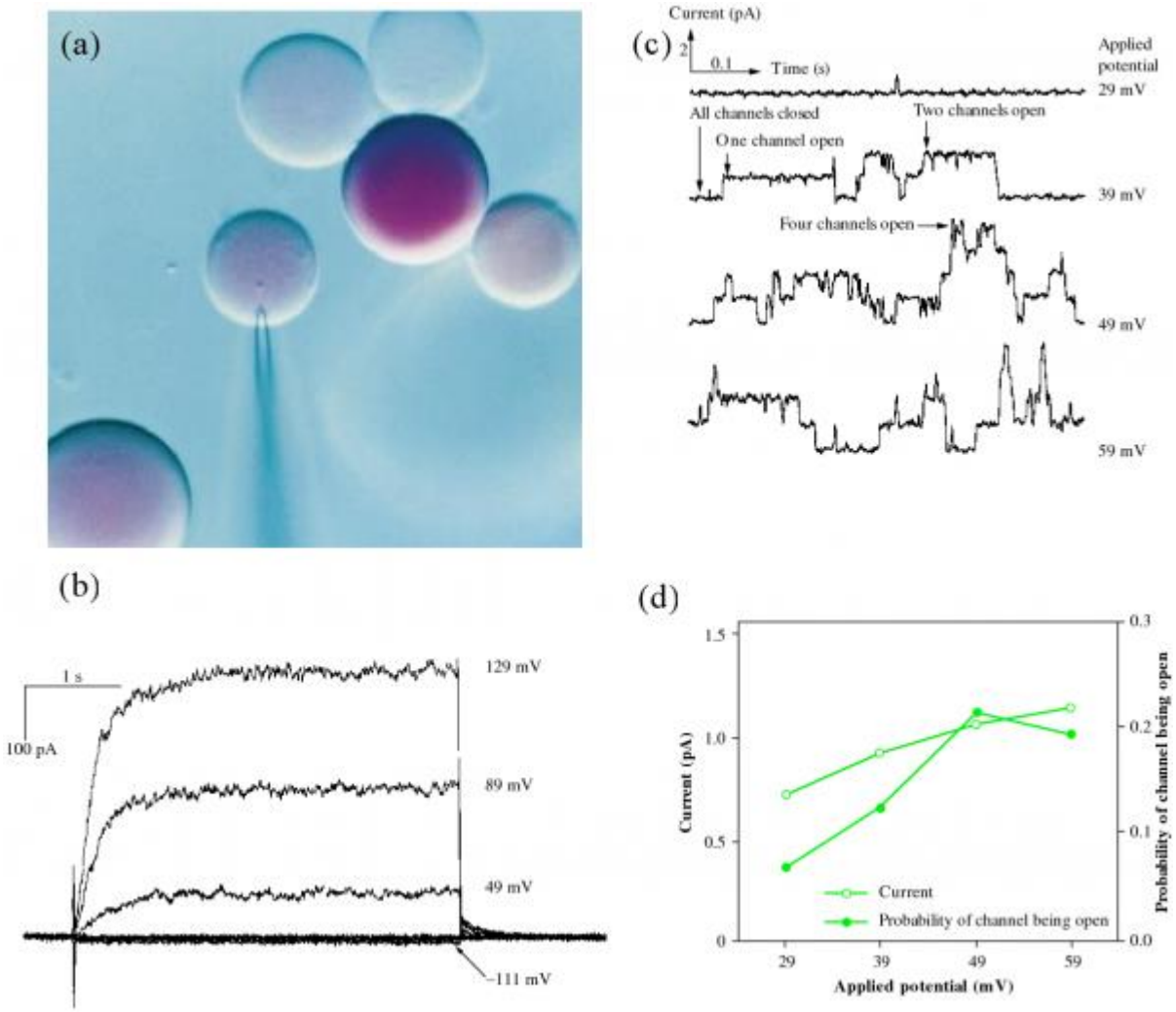

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Figure 4.13 Patch clamp recordings of orthophosphate channels in vacuole membranes. (a) A photomicrograph of a vacuole (*c.* 40 µm diameter) isolated from beetroot, sealed to the end of a glass microcapillary (Figure 4.14). The capillary is filled with a salt solution which diffuses into the vacuole. (b) Currents that flowed across the vacuole membrane when pulses of voltage ranging from -111 to +129 mV were applied. Data from these and other voltages suggest that the currents were due to flow of both K^+ and orthophosphate ions. (c) Currents flowing through individual ion channels in an 'outside—out' patch (Figure 4.14). The individual steps in current represent the opening or closing of a single channel measured at 29, 39, 49 and 59 mV. There are at least four channel molecules in this patch of membrane. (d) Increasing applied voltage from 29 to 59 mV raises electrical current through a single channel (O) and probability of channel opening (•). Because current extrapolates to zero at a negative voltage close to the Nernst potential for orthophosphate, it is likely that orthophosphate carries current through these channels. Decreasing probability of opening as potentials decrease towards zero suggests that these channels only function at positive potentials.

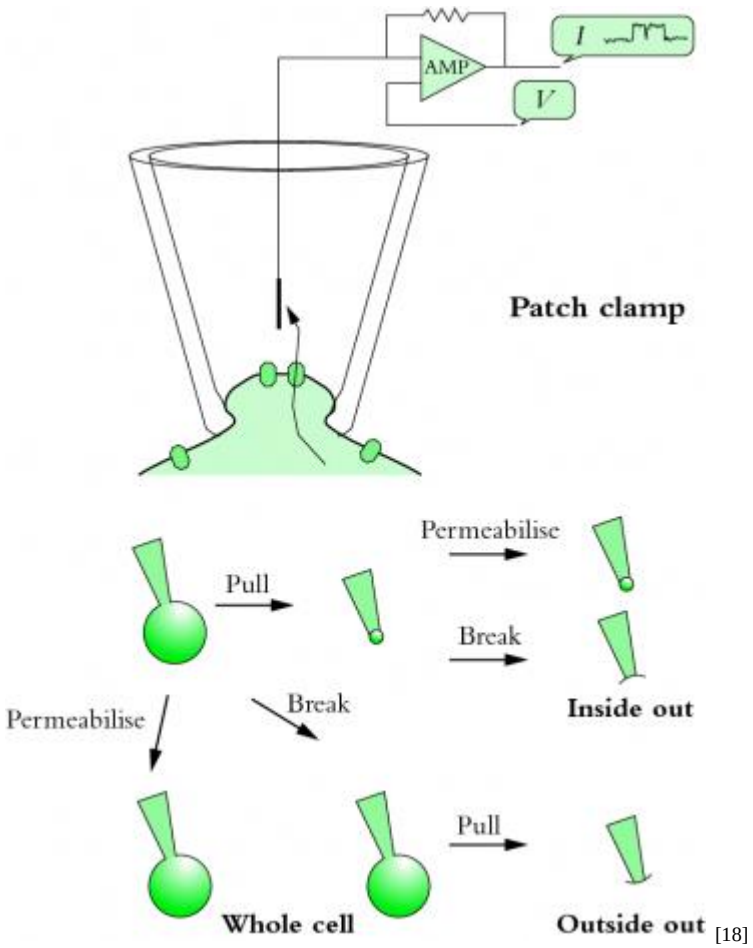

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Figure 4.14 Patch clamping is used to measure tiny electrical currents across membranes. A patch of membrane is sealed against the rim of the tip of a glass microcapillary such that all charge passes through the membrane. Keeping voltage (V) constant (clamped) across the patch will reveal whether ion channels are present. Opening and closing of channels is indicated when square steps are seen in current (I) across the patch after amplification (AMP). Useful configurations include: whole cell (e.g. current flow across an entire tonoplast); inside out (capillary solution in contact with outer face of membrane); and outside out (capillary solution in contact with inner face of membrane)

Ions are charged so their fluxes can then be studied by the flow of electric current that accompanies ion movement. Voltages are applied to simulate the potentials developed by electrogenic pumps and other processes. Because the area of a membrane patch is so small, a millionth of a square millimetre, it usually contains only very few, may be one to ten, ion channels. Consequently the opening and closing of even a single ion channel molecule significantly alters the flow of current across the patch. These single channel events can be seen on-line as they happen. Ogden and Stanfield (1994) give a good description of these techniques.

Controlling the composition of solutions on either side of the membrane is both a great strength and a weakness of patch clamp experiments. By setting the conditions on both sides of the membrane we can obtain incisive information about the function of a number of components involved in solute movement. However,

we can only mimic the chemical composition of the cytoplasm, with its thousands of con-stituents, and therefore suffer the limitations of normal *in vitro* experimentation. The cytoplasm contains many molecules which affect ion transport; until these are identified, they will probably not be used in bathing media. So while we might have definitive information for the conditions of our experi-ments, we must still make estimates and extrapolations to speculate about what happens in cells that are not disturbed by the experimental procedures we use. This does not negate the great contribution that patch clamp methods are making to our knowledge, instead it emphasises that there is still a lot of work to be done.

4.2.4 Patch clamping: a window on ion regulation

Figure 4.13 illustrates a patch clamp experiment. It summarises a study of an ion channel which transports K^+ ions across the tonoplast separating the vacuole from the cytoplasm of beetroot. Figure 4.13(a) is a photomicrograph of the whole vacuole configuration, where the vacuole is attached to the end of a microcapillary and the bit of membrane covering the orifice at the tip has been ruptured. The microcapillary is filled with 50 mM potassium orthophosphate (KH_2PO_4) and so the vacuole also contains this solution. The pattern of current traces obtained during a sequence of pulses at voltages ranging from –111 to +129 mV is shown in Figure 4.13(b). When the cytoplasmic side (i.e. bathing solution) is positive relative to the vacuole (+49 to +129 mV), a flow of current develops slowly over one or two seconds in response to the square wave pulse. At negative voltages (–111 mV) there is very little current. This is a case of voltage gating (Section 4.1.3(a)).

Single channels can be studied too using small patches of membrane. Figure 4.13(c) shows data from four channels in an 'outside-out' patch of membrane isolated from a beetroot vacuole. Opening and closing of individual channels are seen as abrupt step-like changes in current. Traces for a range of voltages show that as the voltage gets more positive the height of the current steps increases. A plot of the height of current steps against voltage (Figure 4.13d) can be extrapolated back to the *x*-axis, showing the point where the diffusion gradient for the ions moving through these channels is balanced by the voltage applied (*reversal potential*). Calculation of the Nernst potentials (Equation 4.7) shows that this voltage is close to the diffusion potential for orthophosphate but at least 50 mV too negative for K^+ions. Figure 4.13(c) also shows that as voltage increases, the channel is open more frequently and for longer. A careful analysis of each transition permits us to calculate the probability of a channel being open (P_{open}) and to plot it against voltage. Figure 4.13(d) shows that at voltages of less than +100 mV the chances of a channel being open are at most 20%. Consequently, these experiments have not only identified a channel in the tonoplast from beetroot

which allows orthophosphate to cross from vacuole to cytoplasm but also indicate how flow is achieved. Voltage is critical. When the energy gradient for orthophosphate flow out of the vacuole is favourable (*cytoplasm positive relative to vacuole*), orthophosphate channels are most likely to open and let this flux proceed. While strongly negative voltages could drive orthophosphate the other way, the channels close and consequently it is effectively a one-way transport. We also know that the channel's
initial response to voltage is rather sluggish.

4.2.5 Turgor pressure controlling ion flow

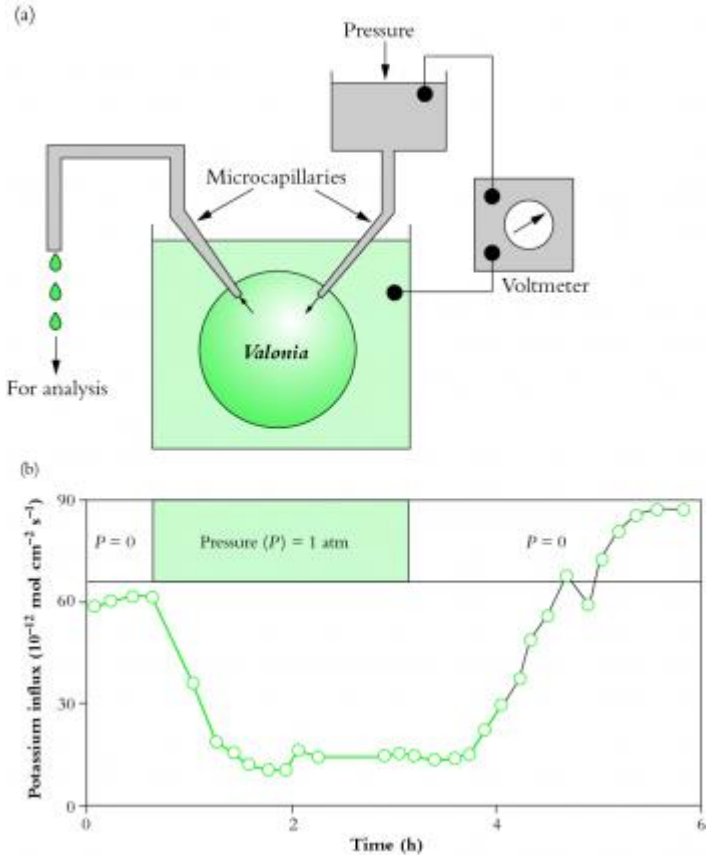

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Figure 4.15 (a) Microcapillaries can be used hydrostatically to increase the turgor pressure in large cells of the alga *Valonia*. Solute flux into cells was estimated from solution flowing out of the cell through one microcapillary while pressure was applied through another. (b) A decrease in K^+ influx as a pressure of 1 atmosphere was applied to cell contents, followed by an increase in influx as pressure was released.

In some large-celled algae, such as *Valonia ventricosa*, turgor pressure (*P*) appears to control solute uptake to achieve *P* regulation. As *P*decreases, solute uptake increases in what appears to be *turgor regulation*. To demonstrate this, two fine capillaries (Figure 4.15a) were inserted into *Valonia* cells (Gutknecht 1968). The capillaries allowed *P* to be controlled and for cells to be perfused with solutions containing radio-active tracers. When *P* was increased by even modest amounts,

there was a dramatic decrease in the net flux of K^+ into the cell (Figure 4.15b). This was reversed when the pressure difference was removed. Both influx and efflux were affected although influx was only affected at low *P*. Efflux was almost directly proportional to *P* over the range studied. How does the cell 'measure' *P*? It has been suggested that it is done by specialised areas of membrane that are compressed as *P* increases. Initial experiments to test this involved compressing the mem-brane with brief pulses of voltages that are much higher than plant membranes normally experience. While this makes those of us who are scientifically conservative a little uncomfortable about such results, subsequently experiments demonstrate the presence of pressure-sensitive ion channels. These channels open under the influence of pressure to allow ions to flow across the membrane and may provide an explanation for the effect of *P* on ion efflux. Pressure-sensitive efflux appears to play a role in assimilate unloading from legume seed coats (Section 5.6.3(c)) and there is definitive evidence for mechano-sensitive channels in higher plants.

4.2.6 Transport proteins underlying the dual mechanism of potassium uptake

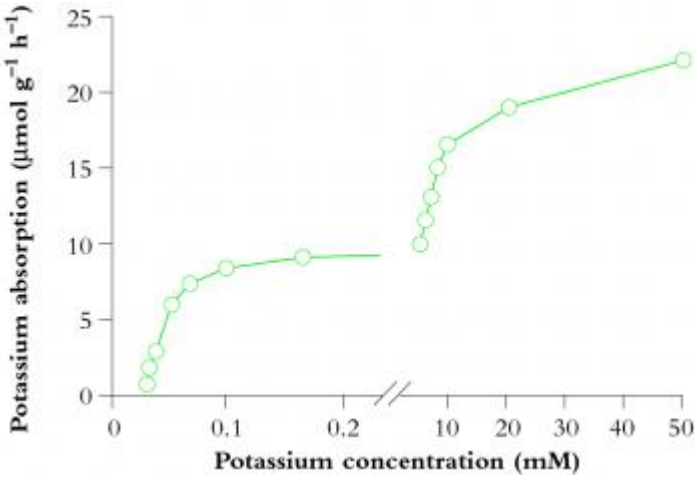

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Figure 4.16 Dual isotherm of potassium influx into barley roots showing high- and low-affinity uptake systems operating at low and high (K^+) concentration ranges, respectively (Based on Epstein 1976)

In many cases, more than one membrane transport mechanism contributes to active influx of a given ion. When roots absorb ions, there appear to be at least two influx mechanisms for each species of ion; K^+ is a particularly well studied example (Epstein 1976). The principal evidence for a dual mechanism comes from the relationship between K^+ concentration and the rate of K^+ absorption (i.e. kinetics of K^+ absorption). In practice this is done by measuring how much of a radioactive tracer for K^+ is absorbed in a standard length of time from solutions at a range of concentrations. Plotting absorption against concentration gives a curve which does

not fit the mathematical function predicted for a single membrane trans-porter. However, proposing that there are two transporters with different affinities for K^+ explains the data better. The exact figures depend on the plant species studied and experimental conditions but typically the 'high-affinity' phase of uptake (Mechanism I) operates at half its maximum rate (V_{max}) at a concentration (K_m) of about 0.02 mM whereas K_m for the 'low-affinity' phase (Mechanism II) is around 1000 times higher at 20 mM (Figure 4.16). The 'high-affinity' phase operates at external concentrations below about 0.5 mM; above this concentration, 'low-affinity' uptake dominates influx.

In addition to the difference in K_m, there are other differ-ences between the two uptake systems. 'High-affinity' K^+ uptake is insensitive to (1) presence of Na^+ and (2) which counter-ion accompanies K^+(e.g. Cl^-, SO_4^{2-}). These ions can have a marked effect on 'low-affinity' uptake. Calcium ions can stimulate 'high-affinity' but they inhibit 'low-affinity' uptake. The membrane proteins which give rise to these uptake phases are now being identified by molecular cloning and patch clamping. The following is an account of the proteins which are thought to catalyse 'high-affinity' and 'low-affinity' K^+transport.

A 'high-affinity' K^+ transporter from wheat roots has been cloned (reviewed by Maathuis and Sanders 1996) and the mem-brane protein involved named HKT1. Patch clamp ex-periments on protoplasts from *Arabidopsis thaliana* roots confirm that this high-affinity K^+ transporter is an H^+/K^+ symporter. This advance in our understanding was made possible by expressing a gene encoding HKT1 in *Xenopus*oocytes (Section 4.1.3(a)) and showing that they could absorb Rb^+ ions (as a tracer for K^+) with similar kinetic properties to roots (a K_m of about 0.029 mM and selectivity for K^+ over Na^+, Rb^+, Cs^+ and NH_4^+). Patch clamp experiments were then performed on these transformed oocytes, and roots of *Arabidopsis thaliana,* to solve a question about the high-affinity K^+ transporter which has long been debated — exactly how is it energised? By studying the effects of pH on the current associated with K^+ influx it was shown that HKT1 is a symporter which carries one H^+ ion with each K^+ ion. Energy is derived from the proton gradient set up by H^+-ATPase in the plasma membrane pumping H^+ ions into the apoplasm (Section 4.1.3(b)). As H^+ ions return to the cytoplasm down this energy gradient via the symporter, the energy released drives K^+ influx. In wheat, the expression of *HKT1* is localised to cortical cells of roots and cells bordering the vascular tissues in leaves. Both cell types are important in absorbing nutrients, from soils in one case and leaf xylem in the other.

The low-affinity K^+ transporter is thought to be a K^+ channel that only allows influx (an inward-rectifying channel). The low-affinity K^+ transporter resembles an inward-rectifying K^+ channel in that it has (1) similar responses to K^+ concen-tration (K_m = 4 mM) and (2) similar ion selectivities. This K^+ channel is voltage dependent and only open at membrane potentials more negative than about –100 mV. When the electro-genic H^+-ATPase across the plasma membrane is operating,

the membrane potential is normally in excess of –150 mV. Somewhat analogous to the orthophosphate channel discussed in Section 4.2.4, the electrical potential not only provides energy for K^+ transport but also the condition necessary to open the channel. The gene *KAT1*, which has been isolated from *Arabidopsis*, encodes a protein which is an inward-rectifying K^+ channel and has these properties (Section 4.1.3(a)). In this way, a channel and a strong gradient in electrical potential are able to sustain high rates of K^+ influx.

4.2.7 Regulation of carrier proteins

(a) Effects of nutrition on V_{max} and K_m

Molecular biology is improving our understanding of ion transport as genes encoding membrane proteins are cloned. Transport of K^+ is better understood at a molecular level than that of other plant nutrients. Additionally, a mycorrhizal ortho-phosphate transporter is described in Section 3.5. The greatest challenges lie in elucidating ways in which ion carriers are regulated (Glass 1983; Michelet *et al.* 1994). The following account of the plasticity of uptake systems in plants demonstrates that ion transporter activity is tightly controlled within membranes.

Absorption of a particular ion is affected by the amount of that ionic species already present in the tissue — as con-centration increases, influx decreases in a logical feedback. The effects are specific. For example, roots which are deficient in sulphur absorb sulphate ions about 20 times faster than non-deficient roots. However, the rate of orthophosphate absorption is not increased in sulphur-deficient tissue (Hawkesford and Belcher 1991). Slower rates of absorption when luxury amounts of an ion are present are due to repression of 'high-affinity' transporters. Even though 'high-affinity' transporters are 'repressed' when nutrient requirements are low, 'low-affinity' transporters are 'constitutive', that is, present and functioning regardless of the amount of the ion in the tissue. However, repression or activation of 'high-affinity' transporters is not the sole basis of regulation of ion uptake. In a study of 'high-affinity' K^+ absorption by ryegrass and white clover roots, K_m and V_{max} changed with K^+ status of tissues (Figure 4.17); higher internal K^+ concentrations reduced the affinity of carrier proteins for K^+ and slowed the maximum rate of K^+ absorption. In addition to the site directly involved in transporting ions, transporters must have other sites which bind substrate ions. These other sites are situated on a cytoplasmic domain of the protein and influence ion transport, but only in an indirect 'allosteric' way. When there is an increase in the cytoplasmic concentration of the ion, a higher proportion of these binding sites is occupied by ions. A process-based model of ion transport predicts four binding sites for this 'high-affinity' K^+ transporter (Glass 1976). Binding causes a subtle change in tertiary structure of the transporter, lowering its affinity for transporting ions and reducing the rate of transport. In this way, evolution of the protein has

given the plant control over its internal ion status. The details of this allosteric binding are still not defined.

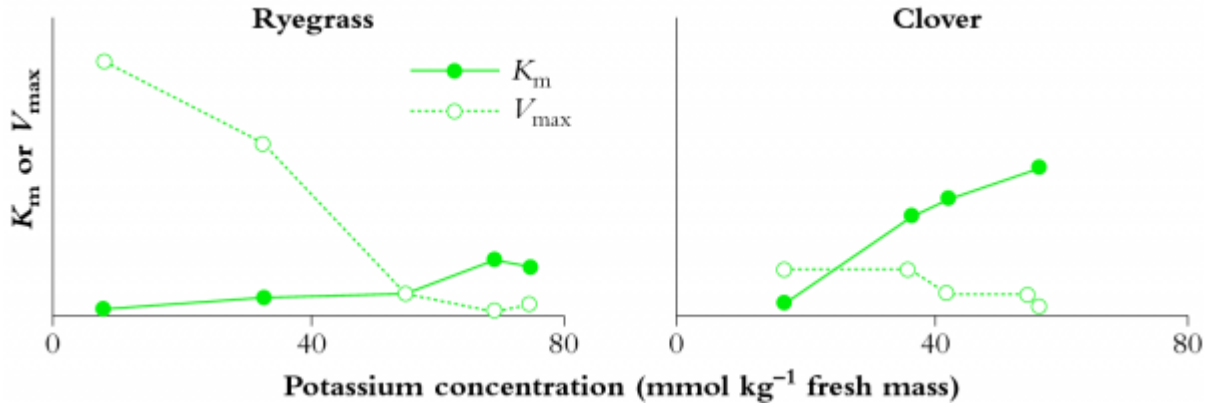

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Figure 4.17 Potassium (K^+) absorption was measured as uptake of radioactive Rb^+ (a tracer) by roots of plants which had been grown in different levels of K^+. In ryegrass, K^+ deficiency elevated Vmax without much effect on Km whereas in clover, K^+ deficiency elevated affinity for K^+ (lower Km) without much effect on maximum uptake rate (V_{max})

Orthophosphate uptake by *Arabidopsis* seedlings is regulated differently. In this case, K_m of the 'high-affinity' transporter does not change but the rate of absorption increases three-fold when plants are phosphorus deficient. For the 'low-affinity' mechanism, K_m decreases during phosphorus deficiency (i.e. affinity of orthophosphate for the transporter increases) but, rather unexpectedly, V_{max} decreases. Such a response is counter-intuitive but lowering phosphate uptake capacity in plants that are phosphorus deficient might be related to lower growth rates leading to lower nutrient demand. For orthophosphate ions, an allosteric regulation of absorption would not be expected to operate in the same way as it does for K^+. The cytoplasmic concentration of orthophosphate ions is tightly controlled and it only changes when phosphorus deficiency is very severe. Low cytoplasmic concentrations cannot therefore provide the basis for an allosteric feedback on uptake. In the case of orthophosphate, and possibly other ions, there is another factor contributing to the regulation of net absorption — an efflux of orthophosphate from the cells. This efflux is significantly lower in plants that are phosphorus deficient (see Case study 4.1).

(b) Specificity of ion carrier proteins

Ion transporters can show very high levels of specificity. Ions which are very similar chemically to the transported ion may not be transported at all. For example, the 'high-affinity' K^+ transporter recognises K^+ but not Na^+ ions even though the two are chemically very similar (they are adjacent alkali metals in the Periodic Table). However, the same transporter absorbs Rb^+ with very similar kinetics to K^+ absorption; Rb^+ is also an alkali metal but is larger than K^+. When

K^+ and Rb^+ ions are both present, they compete for the transporter according to their relative concentrations (see Figure 1 in Case study 4.1). Transport appears to require binding of ions to a recognition site on the transporter, conferring specificity. Other examples of specificities are transporters which absorb Zn^{2+} and Cu^{2+} but not Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+} or Co^{2+} and one which trans-ports Cl^- and Br^- but not F^- or I^-. The ions NO_3^- and ClO_3^- are also absorbed by the same transporter. In some experi-ments, good use has been made of these specificities. The radioactive isotope of rubidium, $^{86}Rb^+$, is widely used as a tracer for K^+ because the K^+ isotope has an inconveniently short half-life.
Similarly $^{36}ClO_3^-$ is used as tracer for $NO_3^-$for which there is no useful radioactive form.

4.2.8 Nutrient transport through plants

The membrane transport phenomena discussed above give rise to coordinated transport of nutrients into growing cells where they are used. This transport might be from roots to shoots or from mature leaves to growth zones. In either case, nutrient transfer is a complex process (Sutcliffe 1976) where active and passive processes as well as bidirectional flow combine to optimise delivery of each ionic species. Coordination of these processes is especially evident in long-distance transport, where fluxes of nutrients, water and photoassimilates depend upon membrane properties of vascular tissues (Chapter 5).

The pathway of nutrient transport from root surfaces to shoot meristems illustrates the importance of membranes in ion movement. Ion uptake into root cells (Section 3.6) requires participation of transport proteins in root cell membranes, such as the dual mechanism of K^+ import (Section 4.2.6). Selective uptake of calcium is probably achieved by influx through Ca^{2+} channels. Further adjustment of internal ion levels might ensue through efflux (e.g. of orthophosphate) and modification of the V_{max} and K_m of carrier proteins.

Ions taken up by cortical cells and transported through the symplasm to xylem parenchyma cells are probably unloaded into mature xylem vessels via ion-specific channels (de Boer and Wegner 1997). Release into immature xylem elements could involve energy-dependent carriers and ion channels. Uptake of ions into shoot cells from dilute xylem sap is catalysed by energy-dependent carrier proteins; how many of these proteins are shoot specific remains to be determined. Ion transfer into growing cells is via the phloem, as transpiration from enclosed meristems is generally small, precluding passive inflow of xylem sap. Once the nutrient- and photoassimilate-rich phloem sap reaches meristems, the final selection of solutes occurs across the membranes of expanding cells. Solute uptake in these cells is regulated by demand (expansion rate) and mediated by many intracellular factors. Turgor-activated channels might play a part in maintaining solute balance of growing cells. Moreover, the tight feedback control on K^+ influx

identified in roots is probably indicative of similar feedback processes in growing shoot cells. The result is a selective, dynamic process of resource delivery to growing cells, providing solutes for osmotic balance and biosynthesis.

4.3 Cell enlargement

4.3.1 Water relations

Sections 4.1 and 4.2 dealt with acquisition of ions by plant cells, emphasising control of ion fluxes and input of energy to maintain electrochemical gradients. Pumps concentrate selected solutes within cells to form a sap in which metabolic reactions can occur. Pumps also raise osmotic pressure (Π) within cells above that of the external solution, setting up a gradient in free energy that draws water across plasma mem-branes into cells. Rising concentrations of common solutes such as sucrose, potassium ions or nitrate ions inside (or outside) plant cells produce approximately equivalent rises in Π.

As water enters a plant cell the cell swells, causing the plasma membrane to exert force on the adjacent cell wall. When tension develops within this wall, an opposing wall pressure raises the energy of water within the cell until it equals that of water outside. Water ceases to cross the plasma membrane, hydraulic equilibrium is reached and the cell has developed a turgor pressure (P) equal to $\Pi_{inside} - \Pi_{outside}$.

The energy of water is therefore an entity described by two variables, P and Π. It is defined experimentally by the term water potential (Ψ) according to the relationship:

$$\Psi = P - \Pi \quad (4.9)$$

This equation holds only when no water is flowing across the plasma membrane, an assumption that is not applicable to cell walls during expansion. However, it is widely used to link solute acquisition with hydrostatic pressure, thereby capturing the concept of osmotically driven growth.

4.3.2 Cell wall expansion

Table 4.2 Gross composition of cell walls of oat coleoptiles, maize cob and pine sapwood, expressed as percentages of wall dry weight. Protein concentrations were not recorded in pine sapwood but were probably below 2%. Note high lignin levels in wood and maize, conferring structural strength

Component	Oat coleoptile[a]	Maize cob[b]	Pine sapwood[b]
Cellulose	25	38	54
Non-cellulosic polysaccharide	51	0.5	8.8
Pectic substances	0.3	0.5	1.0
Polyuronide hemicelluloses	—	42	3.0
Lignin	—	17	26
Cuticular substances	4.2	—	—
Proteins	9.5	3.2	—
Ash	—	1.4	—

(After[a] Bishop *et al.* (1958); [b]Siegel (1962))

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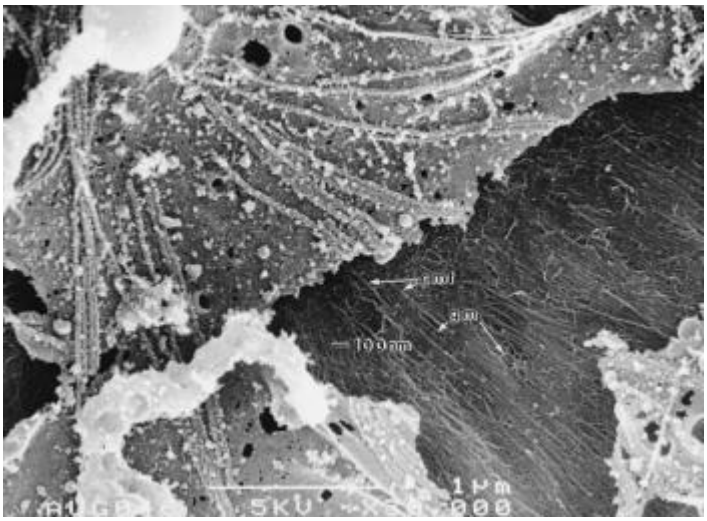

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Figure 4.18 High resolution scanning electron micrograph of the primary cell wall of onion (*Allium cepa* L.). The root has been saponin treated then freeze—fractured to reveal the inner face of a cell wall. Cellulose microfibrils (cmf) and gel matrix components (gm) that cross-link these microfibrils are arrowed, revealing most microfibrils lying at a similar angle within the wall. The upper left corner of the image shows a remaining fragment of plasma membrane, with microtubules running diagonally across the membrane (Courtesy of P. Vesk)

Inflow of water results in cell expansion (growth) as the prim-ary cell wall stretches to accommodate water uptake. Cell walls are, however, not infinitely extensible; directionally controlled cell expansion under pressure sets plant cells apart from animal cells. So, an increase in plant cell volume is achieved through coordination of many events: cell walls yield to *P*, solute and water fluxes are initiated, membranes surrounding the vacuoles and cytoplasm expand and new wall and membrane components are synthesised. Cell wall yielding is of special significance in plant growth because all plant cells are encased in a matrix of wall polymers (Table 4.2) which resists expansion sufficiently to generate pressures within the cell contents but yields sufficiently to allow cell expansion in growth zones (Figure 4.18; see also Case study 4.2). Expansion of plant cells is intriguing

because wall yielding, an extracellular process, is so exquisitely coordinated by events within the cell. So subtle is this coordination that different walls of a single cell generally have different extension rates, even though each wall is subject to the same *P*. The cytoskeleton is a central player in co-ordination of wall expansion; intracellular microtubular arrays influence orientation of cellulose microfibrils in the wall. Microfibrils do not stretch longitudinally so growth can only proceed normal to the microfibril axes (Figure 4.19). In this way, cytological events help shape cells.

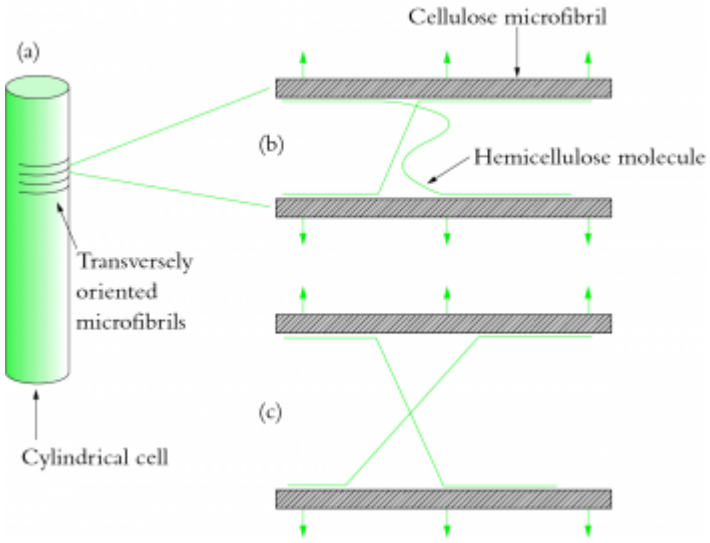

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Figure 4.19 Model of cell wall expansion. (a) Cellulose microfibrils oriented normal to the axis of a cylindrical cell undergoing longitudinal elongation. A typical epidermal cell would grow with this strong polarity. (b) Microfibrils (hatched) are shown in parallel arrays, joined by a loose and a tight hemicellulose polymer which are each hydrogen bonded to the microfibril. Arrows denote P-driven separation of microfibrils. (c) After a period of growth, increasing numbers of hemicellulosic polymers develop tension as the cell extends, giving rise to wall pressure. Note the lengthening of the distance spanned by the polymers through time. New wall polymers are normally secreted from cells to maintain wall mass (From Passioura and Fry 1992; reproduced with permission of Journal of Experimental Botany)

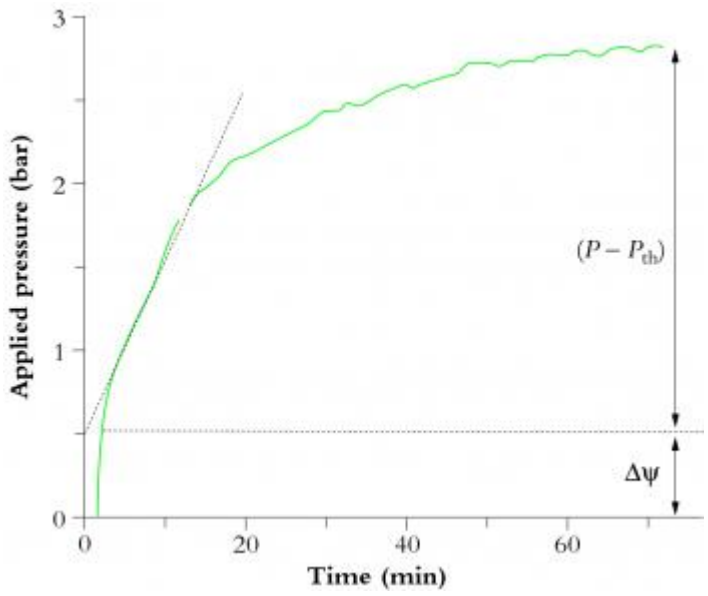

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Figure 4.20 Stress relaxation in a slowly growing pea epicotyl measured with the pressure-block technique. The atmospheric pressure which must be applied to a pea seedling to block growth increases as tissues 'relax'.The initial rate of relaxation (dashed line) is used to calculate applied pressure required to overcome ΔΨ which is set up by steady-state tissue expansion (prior to pressure application). Subsequent relaxation (after 2-4 min) reveals the applied pressure required to counter effective pressure generated within cells to drive growth ($P - P_{th}$) (From Cosgrove 1993; reproduced with permission of The Plant Cell))

Cell wall yielding and water uptake occur simultaneously in a growing cell but they can be uncoupled from one another, giving an appreciation of the hierarchy in which they occur. An ingenious set of experiments outlined below indicates that the yielding of cell walls to *P*, often termed wall relaxation, actually predisposes a cell to water influx and volume expansion. Normally, the restoration of *P* follows as active solute accumu-lation (e.g. through ion pumps) raises intracellular osmotic pressure (P). That is, changes to cell walls appear to precede water uptake. Two lines of work by Cosgrove and colleagues at Pennsylvania State University have led to this conclusion. In one case pea epicotyls (young shoots) were kept in a humid atmosphere but cut off from a continuous water supply in order to prevent water uptake. Cells of the growing zone relaxed (*P* decreased) whereas those of the non-growing zone did not relax. This could be detected because no water was available to restore *P*. This suggests that cell wall relaxation occurs even in the absence of water uptake: a consequent loss of *P* would normally be overcome by uptake of xylem sap or soil moisture if it was available. A second series of experiments involved the 'pressure block' technique in which gas pressure was used to block growth by annulling cell *P*. Experimentally, this can be done by sealing stems of intact plants into a gas-tight chamber and increasing the pressure of the atmosphere around the tissues. A plot of pressure applied versus growth (Figure 4.20) shows that the applied pressure required to block growth increased over time, suggesting that cell walls relaxed steadily through time even though growth had ceased. Again, this is evidence for a *relaxation of cell walls* leading to

growth rather than an uptake of water driving wall expansion and growth. To translate this into a cellular context, cell wall yielding to pressure exerted by the plasma membrane is more likely to be the primary event in growth than osmotically driven influx of water. In practice, these events seem to occur simultaneously and might be considered as partners which sustain cell growth.

4.3.3 Application of water relations equations to growing cells

While we have made a case for wall relaxation as a primary influence on cell expansion, there is also a requirement for cells to maintain sufficient *P* in order to keep deforming walls and sustain growth. This is illustrated by the 'pressure-block' experiments (Figure 4.20) in which increasing pressures are required to block growth by lowering *P*. Under con-ditions of adequate water and solute supply, yielding charac-teristics of the cell wall (wall rheology) are major determinants of growth. However, in drought, severely drying winds or dehydrating, saline soils, water deficits might be severe enough to cause complete loss of *P*. It is therefore important to relate variables which play a part in cell expansion, such as cell wall properties and water flow, to Equation 4.9.

Long-distance vascular transport in plants is driven by pressure gradients which arise either osmotically (such as in the roots of guttating plants) or hydraulically (transpiring canopies). Water enters the cells of growing tissues at a rate determined by local Y gradients and resistances. Hence, *P*, P, hydraulic conductivity (L_p) of the cell wall and plasma mem-brane and wall rheology will all influence cell growth rate. While some of these variables are difficult to measure on a cellular scale, reasonable theoretical models have been devel-oped to describe how they relate to each other. The analysis to follow mostly relates to water flow into individual growing cells. However, the analogy to whole tissues is often apparent and will sometimes be considered where the two have principles in common.

How readily water flows through cells and tissues is encapsulated in the term hydraulic conductivity (displacement of water per unit of pressure and time). Hydraulic con-ductivity is especially important when water travels over long distances, such as across a stem, and where there are barriers to water flow, such as suberised layers of roots and tyloses in trees. The equation for water uptake (increase in cell volume) can be expressed as:

$$\frac{dV}{dt} = A\,L_p\,(\Delta\Psi) = L(\Delta\Psi) = L(\Delta\Pi - \Delta P) \qquad (4.10)$$

where $\frac{dV}{dt}$ is the rate of cell expansion ($m^3\ s^{-1}$), L_p is specific hydraulic conductivity ($m\ Pa^{-1}\ s^{-1}$), A is membrane surface area (m^2), L is hydraulic conductance ($m^3\ Pa^{-1}\ s^{-1}$) and $\Delta\Psi$ is the gradient in water potential (Pa) generated by osmotic ($\Delta\Pi$) and turgor (ΔP) pressure gradients across membranes. Therefore, the rate of water uptake into cells is dependent on L_p and the inward gradient in Ψ. However, the delivery of water into growing cells through theri walls and membranes is only part of the growth process; we must also incorporate the characteristics of irreversible cell wall expansion into an analysis of growth, thus recognising the pivotal role of wall relaxation in growth.

Lockhart (1965) described cell expansion with a form of the following equation for *P*-driven growth:

$$\frac{dV}{dt} = \phi(P - P_{th}) \qquad (4.11)$$

where φ is the wall yielding coefficient, sometimes referred to as wall extensibility ($m^3\ Pa^{-1}\ s^{-1}$) and P_{th} is threshold turgor pressure or yield threshold (m^3 Pa). This equation and variations on it have become a paradigm for cell and tissue growth.

Equations 4.10 and 4.11 both provide plausible views of growth but raise the question of whether supply of water for cell expansion or irreversible yielding of cell walls is the key control step in growth. Experiments of Cosgrove cited above illustrate that relaxation of cell walls occurs independently of water inflow. It is generally considered that entry of water into rapidly growing cells is not a limiting factor for cell expansion. That is, Equation 4.11, which has no hydraulic term, is very relevant to growing plant cells. However, in some circumstances water flow might limit growth rate, particularly in tissues where the source of water (e.g. xylem vessels) is far removed from the cells controlling growth (e.g. epidermal cells). In droughted plants, too, water flow might limit expansion of new cells.

An equation encompassing this interplay between entry of water and wall relaxation would therefore be useful for analysing growth experiments. To achieve this, Equations 4.10 and 4.11 can be combined to form an equation describing water uptake and cell wall relaxation in a single cell in solution as follows:

$$\frac{dV}{dt} = \frac{\phi A L_p(\Delta\Pi - P_{th})}{\phi + AL_p} = \frac{\phi L(P - P_{th})}{\phi + L} \qquad (4.12)$$

Although rather complex, this equation marries the dual require-ments for water acquisition and irreversible wall expansion in growing cells. Accurate estimates for the variables can be made in single cells with the aid of a cell pressure probe.

Heterogeneity within multicellular tissues reduces the accuracy of measurements, especially of conductivity. Equation 4.12 reinforces the point that any analysis of growth must focus on whether L exceeds φ substantially enough to reduce the right side of Equation 4.12 to Equation 4.11.

By postulating that Equation 4.11 provides a model for cell expansion, an appraisal can be made of the relative importance of cell wall properties (φ and P_{th}) and turgor (P) in determining growth rates. Equation 4.11 can be expressed as a linear relationship between dV/dt and P. However, viewing it as an equation for a straight line suggests that the parameters φ and P_{th} are rather less variable than we now believe them to be; the Lockhart equation is better viewed as a model linking three variables (φ, P_{th} and P) which can modulate growth in response to developmental or environmental cues.

4.3.4 Cell wall properties: determinants of growth rate

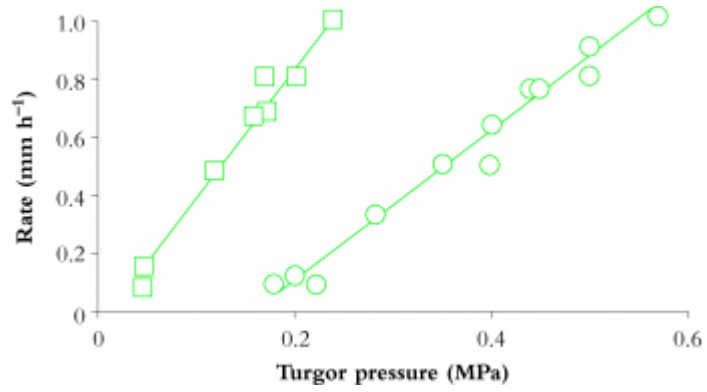

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Figure 4.21 Turgor pressure measured in soybean leaves elongating at different rates imposed by water deficits. Plants were grown in chambers (circles) and outdoors (squares). Note that the linear relationship between growth and *P* holds in both environments in spite of shifts in slope and intercept, denoting changes in φ and P_{th} respectively

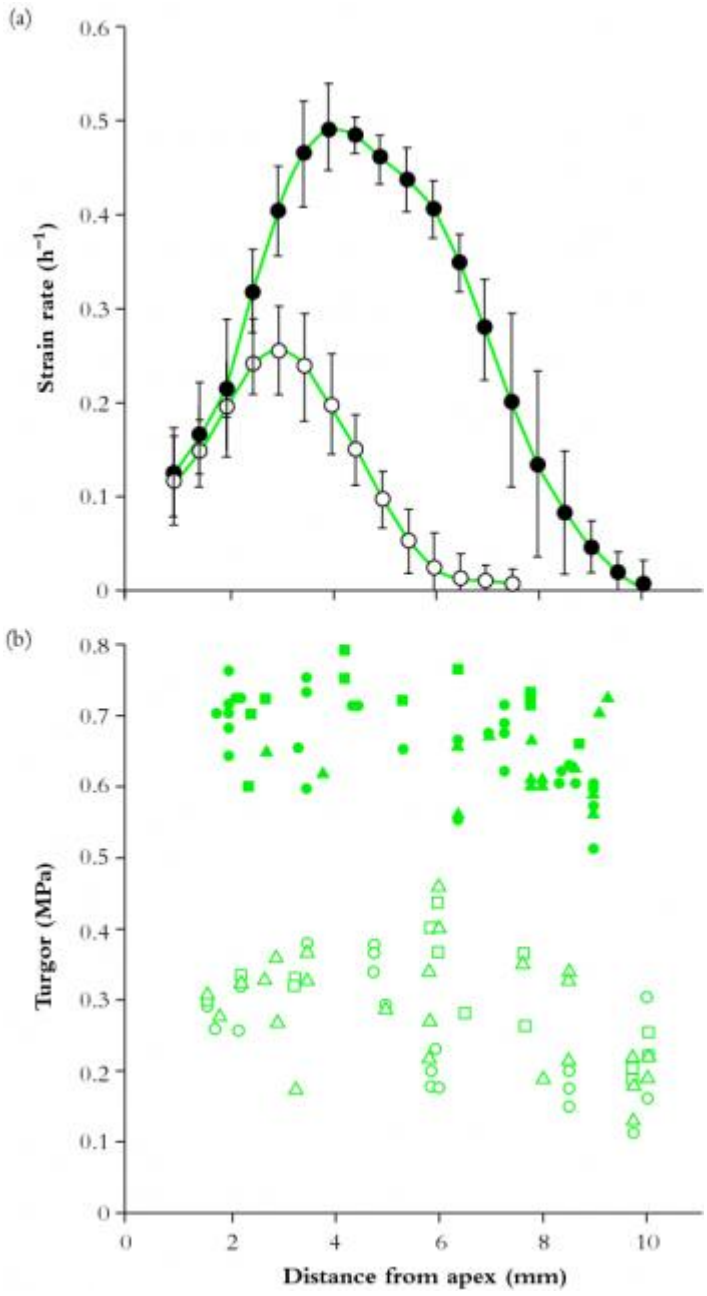

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Figure 4.22 Spatial distribution of (a) elongation rates and (b) turgor pressures along apical zones of maize roots grown either in well-watered (Ψ = -0.02 MPa; filled circles) or rather dry (Ψ = -1.6 MPa; open circles) vermiculite. Note that drought only depressed growth at positions more than 2 mm from the apex but *P* was lower at all positions on droughted roots. (From Spollen and Sharp 1991; reproduced with permission ofthe American Society of Plant Physiologists)

Investigating the relationship of cell and tissue growth to *P* gives some indication of the importance of φ and P_{th}, in growth regulation. Led by the idea that the primary determinant of growth is *P*, many experimenters have set out to show that growth and *P* are correlated in multicellular tissues. Occasionally strong correlations are found but more often not. First, the Lockhart analysis is not designed for multi-cellular tissues which have specialised growth zones, poorly defined cell geometries or strong gradients in water activity. Moreover, during the

long periods taken to assess growth rates, water-stressed tissues might develop higher Π, leading to recovery in *P*. Simultaneous changes in cell wall rheology can be induced at low P, raising growth rates above the low values predicted if φ and P_{th}, were constant. Overall, short-term perturbations to growth of single cells and simple tissues are most likely to reveal the subtle role played by wall rheology in growth.

Bunce (1977) showed that drought—induced variations in *P* in soybean leaves correlated with variations in growth rate (Figure 4.21). Furthermore, a comparison between plants grown in chambers and outdoors reveals a shift in the relationship between leaf elongation and *P*. Specifically, leaf growth of plants grown outdoors was halved by a 0.1 MPa drop in *P* while chamber—grown plants experienced only a 30% drop over the same range. Putting aside the heterogeneity within a growing soybean leaf, Equation 4.11 suggests that both φ and P_{th} have changed in response to plant growth conditions. Cell walls of plants growing outside yielded more readily to *P* (lower P_{th}) and then extended faster than the cell walls of plants in chambers (greater φ). In bean leaves exposed to light to initiate rapid growth (van Volkenburg and Cleland 1986), φ and P_{th} changed over time but long—term growth effects were more closely related to φ than $P - P_{th}$. Short-term fluctuations in growth were ascribed to changes in $P - P_{th}$, implying subtle roles for the two variables of the Lockhart equation in whole plants.

In roots, too, cell wall properties participate in growth regulation (Figure 4.22a and b). Drought—induced water deficits in maize roots lowered *P* in apices to about 0.3 MPa, almost 0.4 MPa below that of well—watered roots (Figure 4.22b) (Spollen and Sharp 1991). Overall growth rates of the two sets of roots in wet and dry conditions were 2.8 and 1.0 mm h^{-1}, respectively. In broad terms, this might be thought to represent a case of lowered *P* inhibiting root growth but local rates of elongation along the root axis (Figure 4.22a) show that the distribution of growth is also affected by drought. Figures 4.22(a) and (b) together demonstrate that even in roots with a restricted water supply and low P, tissues less than 2 mm from the apex of the roots always elongated at the same rate. Reduced water supply to the roots therefore induced a change in either φ or P_{th} of the most apical cells, allowing these distal cells to continue elongating unabated in dry conditions.

CASE STUDY 4.2 A perspective on plants: significance of cell walls

B. E. S. Gunning

Cell walls determine most of the fundamental features of the Plant Kingdom.

More than one billion years ago certain key evolutionary events set the cells that were to become the progenitors of plants apart from the other primordial organisms. What were these defining attributes and what part did they play in founding the Plant Kingdom?

Some would say that photosynthesis was the key to plant evolution. It arose first in prokaryotes and later passed to eukaryotes. Certainly it was essential, but was it alone sufficient to trigger evolution towards the Plant Kingdom? The theme of this case study is that the full potential of photosynthesis could not be realised by the progenitors of plants until they had evolved a suitable cellular environment, of which a vital component is a *cell wall*. Photosynthesis still occurs in unwalled, evolutionary dead-ends like *Euglena*, reinforcing the view that a truly seminal cellular state was only achieved when photo-synthesis in a eukaryotic cell was combined with a cell wall. Consider now how cell walls confer unique features on plant cell organisation and function, and how they underpin the entire lifestyle and marvellous diversity of plants.

Cell walls: strength through osmotic regulation

Why did the first eukaryotic, photosynthetic, walled cells have such distinctive evolutionary potential? Probably all life was aquatic at the time and regulating water and solute balance (osmotic regulation) was critical for survival. The earliest cells were almost certainly in osmotic balance with the fluid in which they lived. However, as cell metabolism became more complex, internal solute concentrations rose. When solute concentrations in the surrounding medium dropped, water would tend to diffuse from the external medium (high water potential) into cells along water potential gradients (Section 4.3). Cells which do not have walls, like *Amoeba*, deal with osmotic imbalance by expelling water, otherwise they would burst (Figure 1). A cell wall containing strong but flexible micro-fibrils (Figure 4.18) offered the progenitors of the Plant Kingdom an alternative solution. Hydrostatic pressure created by water flow into cells is opposed by the mechanical strength of cell walls (Section 4.3), generating wall pressure. In living cells, this neatly balanced cell turgidity is maintained by control of osmotic processes, mechanisms for turgor sensing and organisation of cell wall composition.

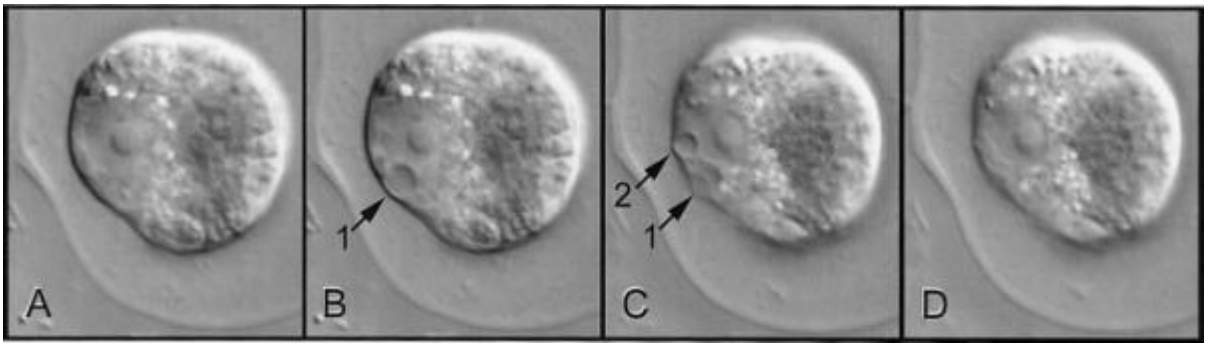

Figure 1 Contractile vacuoles filling and emptying in a *Chlamydomonas* cell. Four pictures were taken about two minutes apart to illustrate the filling and emptying cycle of two contractile vacuoles in the cell. (A) The two contractile vacuoles are empty and invisible at this magnification. (B) One vacuole has filled (arrow). (C) This vacuole is emptying and the second vacuole is filling. (D) Both vacuoles have emptied again. Part of the boundary of the gelatinous envelope that surrounds the cell is visible in each picture (Micrographs courtesy B.E.S. Gunning)

Most plants adjust the osmotic properties of their cells so that they can live in a turgid state regardless of the water potential in their environment. This brings great advantages for plants. The balance of forces in a turgid cell generates more than just water balance. It confers rigidity and mechanical strength, as witnessed by comparing a wilted leaf with a turgid leaf. Another way to gain strength is, of course, to synthesise thick cell walls. However, organs composed mainly of thin-walled cells, like leaves, can support themselves if they are turgid. They therefore do not need to synthesise the large amount of wall material that would be required if strength relied solely on wall rigidity. This is especially important in growing regions of plants where cells must extend plastically (Section 4.3). Primary cell walls also confer enough strength on tissues for them to hold their shape and form — for example, enough to let a root tip penetrate through soil. In general, as cells mature the plastic properties of their walls give way to increasing rigidity.

Here, then, at the dawn of the Plant Kingdom, was a new form of osmotic regulation with many inherent evolutionary possibilities. It proved to be a springboard for the appearance of other novel features of plant cell structure and function.

Cell walls, vacuoles and cytoskeleton: partners in production of large cells

Many distinguishing features of plant cells relate to cell walls. Vacuoles, for example, are found in the vast majority of plant cells, but seldom in animals. They probably evolved from an original digestive (lysosomal) compartment, indeed they still have some digestive roles in plant cells. Now one of their main roles is to store osmotically active solutes, thus partnering cell walls in maintaining turgor. In so doing they have a huge impact on the architecture and size of plants. Their presence permits economical production of large cells in which a small amount of biosynthetically expensive cytoplasm is distributed as a thin film over a large surface area between the wall and vacuole(s). Large, turgid, vacuolate, walled cells are in turn economical building blocks for increasing body size. In fact some 90% of all increase in volume during plant growth comes from an enlarging vacuolar compartment and concomitant stretching of cell walls. This process transforms small, densely cytoplasmic, meristematic cells into mature, vacuolate cells.

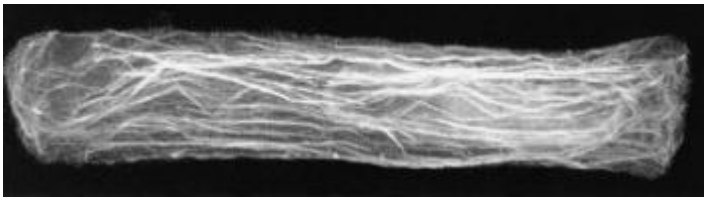

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Figure 2 Microfilaments of actin. In this elongating cell from a wheat root tip, strands of actin ramify through the cytoplasm, mostly running along the length of the cell. They are stained here with a fluorescent antibody and viewed by confocal microscopy. The cell nucleus is just visible, lying in the right-hand end of the cell. Actin protein polymerises into microfilaments, and these often aggregate into bundles such as those imaged here. Polarity of the actin molecules determines the direction of cytoplasmic streaming along the microfilaments (Micrograph courtesy B.E.S. Gunning)

[30]

Figure 3 Microtubules have many roles during the cell division cycle. The following stages can be seen in cells from a wheat root tip stained with fluorescent-labelled antibody to the protein tubulin (from left to right): (i) pre-(or post-) division, when microtubules lie transverse to the long axis of a cortical cell, just under the plasma membrane. Microtubules govern congruent deposition of cellulose in the growing cell wall; (ii) the cell has become committed to divide and is establishing the future site and plane of division by laying down a dense band of microtubules (the 'pre—prophase band') that passes right around the cell; (iii) the metaphase stage of mitosis, with chromosomes lined up on the equator of the division figure, connected to poles of the mitotic spindle by bundles of microtubules that ultimately separate daughter chromosomes; (iv-v) early and later stages of development of the 'phragmoplast', an apparatus of microtubules and actin in which a new cell wall is initiated between the daughter nuclei; (vi) division almost complete, with just a few remnants of the phragmoplast visible and two daughter cells almost separated, although their cortical microtubules are not yet recognised; (vii) daughter cells have formed new arrays of cortical microtubules, similar to those of stage (i). See Figure 10.10 for further perspectives (Based on Gunning and Steer 1996)

Walls and vacuoles together give the opportunity to make big cells and hence big plants, but this potential can be realised only if an associated metabolic problem is overcome. Thousands of biochemical reactions are needed to support life. For them to proceed fast enough the interacting molecules must collide sufficiently

frequently. Simple diffusion in the confined volume of small cells allows them to do this — one of the advantages of being small. Frequency of collisions drops off greatly if the colliding molecules have to diffuse over longer distances, or are present in dilute solutions, as might happen in cells that have taken advantage of walls and vacuoles to enlarge dramatically. One way in which this potential physical limitation on life processes is alleviated in present-day plant cells is that a cytoskeletal system stirs and mixes the cytoplasm. The process is visible in most large walled cells and is fascinating to watch. Like stirring reactants in a beaker, it helps to overcome diffusion barriers.

Actin and tubulin are ubiquitous components of the cyto-skeleton of plants and animals. Actin molecules are the units of 'microfilaments' which provide tracks for cytoplasmic streaming (Figure 2). To achieve streaming, actin acts in concert with myosin (another cytoskeletal component), proteins and ATP as an energy source for mixing. Molecules of tubulin are polymerised to make 'microtubules', which have multifarious roles in living cells (Figure 3). Special roles related to cell wall development are discussed below.

Cell walls have a unique biosynthetic apparatus linked to unique cell morphogenesis

Plant cell walls comprise two phases: microfibrils (mainly of cellulose, the world's most abundant biopolymer) are embedded in a gel matrix of other polysaccharides and some very specialised proteins (Figure 4.18; Table 4.2; Section 4.3). Two distinct sets of biosynthetic apparatus generate the micro-fibrils and the matrix components, bringing further unique features to plant cell organisation.

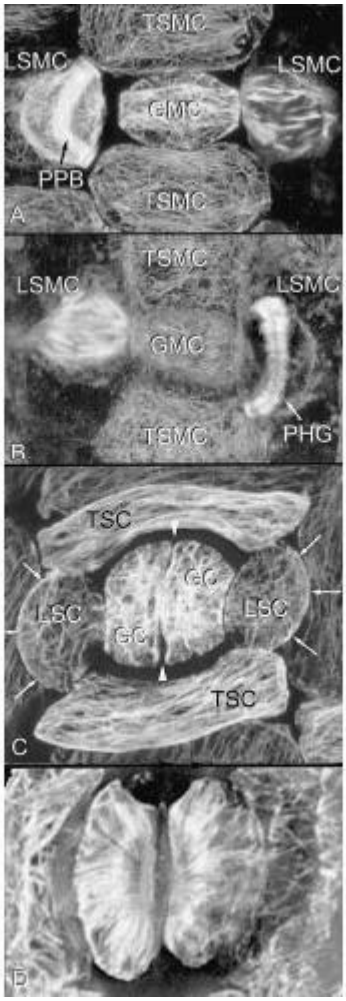

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Figure 4 The basic types of microtubule array can vary greatly in specialised cells and tissues. Developing stomata show many complexities, including asymmetrical cell divisions and formation of cell walls with unusual microfibril reinforcement. Four stages of formation of *Tradescantia* stomata are shown here, using microtubule staining. In (A) the central guard mother cell (GMC) is surrounded by terininal and lateral subsidiary mother cells (TSMC and LSMC); here the LSMC on the left has a curved pre—prophase band (PPB) which predicts the shape of the future wall of the subsidiary cell (arrows in C).The LSMC on the right is in mitosis. (B) This shows a later stage, with the LSMC on the left in mitosis and that on the right with a curved phragmoplast (PHG), also predicting the shape and position of the future wall. (C) Divisions are complete. Arrows show the walls that were formed successively under the influence of the pre—prophase band and phragmoplast. The guard mother cell seen in (A) and (B) has now divided longitudinally (between arrowheads) to form two guard cells (GC) in the stomata. All four subsidiary cells (two TSC and two LSC) have now been formed. During differentiation of guard cells, microtubules in the cell cortex radiate from the future pore (D). Cellulose microfibrils are deposited in this orientation, creating a cell wall that can respond to turgor changes in such a way that the stomatal pore can be opened and closed (Micrographs courtesy A. Cleary)

One biosynthetic apparatus consists of cellulose-producing enzyme complexes in the plasma membrane. They often work under the guidance of an array of microtubules that lies at the inner face of the plasma membrane and directs growing cellulose chains into specific orientations. This is a vital regulatory system

because the strength of cell walls depends on the orientation of its microfibrils. The microtubule cytoskeleton lying beneath the plasma membrane is a tool by which cells control the local directional strength of their walls. Microtubule arrays indirectly determine the shape that a cell assumes when it is stretched by turgor (Figure 4). There is nothing like this combination of membrane-based synthesis and guidance by cytoskeletal microtubules in animal cells. In plants it is a major mechanism of cell shaping and lies at the heart of much of plant morphogenesis.

The second biosynthetic apparatus for wall production does have a counterpart in animals, but the flavour is different, thanks again to the wall itself. All eukaryotic cells have an elaborate system of membranes in which certain proteins are made, modified and secreted. Most of the proteins secreted by animal cells are glycoproteins, that is, proteins with carbo-hydrate side-chains attached to them. A special region of the membrane system, the Golgi apparatus, adds these side-chains. Plants also make glycoproteins, but the great bulk of their Golgi activity is given over to manufacturing cell wall matrix polysaccharides. This differing biosynthetic emphasis might account for differences in organisation of the Golgi apparatus in plants and animals. In animal cells, Golgi bodies are usually central, near the nucleus, whereas in plant cells they are widely dispersed in multifunctional ‘Golgi-stacks’ of membranes. After the wall matrix materials have been made, vesicles containing them are delivered from Golgi stacks to particular regions of the cell surface and thence to growing cell walls. Especially in large cells, this intracellular movement depends once again upon the actin-based cytoplasmic streaming system that evolved in walled cells.

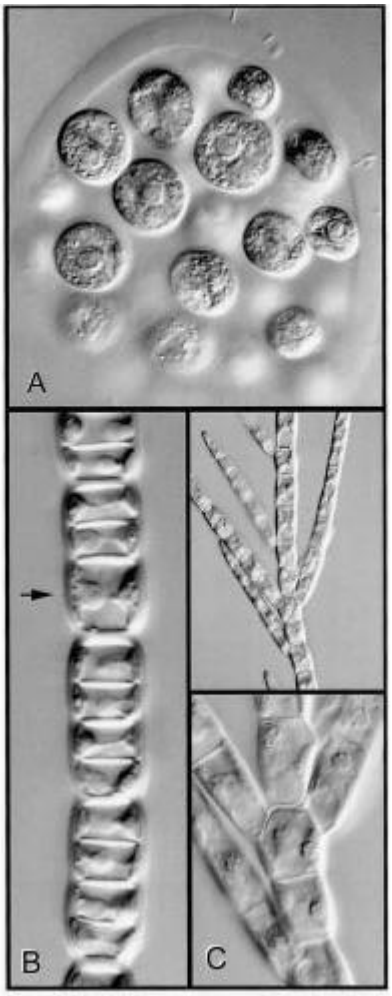

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Figure 5 Cell walls, planes of cell division and the form of a plant body are illustrated in genera of green algae. (A) Colony of *Eudorina*. The constituent cells are embedded in a gelatinous matrix. At the end of cell division daughter cells separate from one another. (B) This shows what happens when division is always in one plane. Sharing of new cross-walls by daughter cells causes them to adhere to one another. The arrow indicates a cell that was about to divide. (C) A vital new feature — the ability to change the plane of division generates branching systems of adherent cells (*Stigeoclonium*, low and high magnification views) (Micrographs courtesy B.E.S. Gunning)

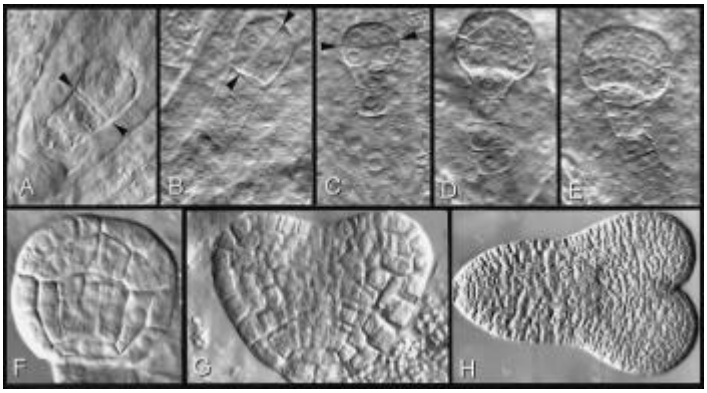

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Figure 6 Cell walls, planes of cell division, and form of the plant body illustrated through embryogenesis in a higher plant. Embryo formation in *Arabidopsis* provides an example of highly

regulated planes of cell division during formation of a specifically shaped plant body. Arrowheads in (A) to (C) show successive planes of division in very young pro—embyos. Subsequent divisions (D-H) build up a heart-shaped embryo with surface and inner walls and embyonic root and cotyledons. (D) The complete suspensor filament as well as the globular pro—embryo. In (A) to (E) the embryos are embedded in endosperm tissue in the embryo sac; in (F) to (H) they have been isolated from their embryo sacs (Based on Gunning and Steer 1996)

Enlargement of existing cells is but one component of plant growth and shaping. Production of new cells by cell division is the other. Although cell division is universal in plants and animals — indeed the regulatory genes are very similar in both kingdoms — the cell wall again imparts a uniquely botanical flavour. Major differences appear towards the end of cell division, after mitosis, when separate daughter cells are formed. In animals newly formed cells are flexible and can migrate and adjust their position in the body. Cell walls prevent such adjustments in plants so plants have to place their new cells with enough precision to make multicellular tissues in which component cells lie in functional arrange-ments. Figures 5 and 6 illustrate the importance of planes of division in plant development, taking examples from very simple algae (Figure 5) and embryo formation in a flowering plant (Figure 6).

Two elaborate cytoskeletal devices place new cell walls accurately (Figures 3 and 4). The first is a preparation for cell division. The cytoskeleton of the parent cell establishes the site and plane of division even before the nucleus undergoes mitosis. This cytoskeletal apparatus (pre-prophase band) is not found in present-day algae (although many algae can still control the plane of division in their cells) and may have arisen after the algal stage of plant evolution. A second cytoskeletal structure initiates the actual fabrication of new cell walls. It is initiated between daughter nuclei and grows outwards to join the parental walls at a predetermined site. This apparatus, termed a phragmoplast, did evolve in advanced algae and occurs in the ancestors of higher plants. Neither of these cytoskeletal devices for establishing and implementing precise sites and planes of cell division occurs in animals.

The cell wall: constraints and opportunities in nutrition

Cell wall properties have implications for plant nutrition. The close-knit fabric of cell walls sieves out all but very small nutrient molecules. This rules out a feeding mechanism that was probably common in early life forms — engulfing particles of food in loops of plasma membrane and internalising them for digestion. The first walled cells had to adapt their nutritional habits leading to at least two evolutionary outcomes. Present-day fungi subsist on external food sources by secreting enzymes that digest macromolecules sufficiently to allow the products to pass through cell walls. Roots of higher plants also secrete extracellular enzymes such as phosphatases which liberate inorganic phosphate. Higher plants also entered into

an intra-cellular symbiosis with photosynthetic organisms, which then served as internal sources of organic carbon compounds. This led to green plants, whose present-day chloroplasts are held to be much-modified descendants of originally free-
living photo-autotrophs (Section 1.2). Symbiotic association between walled hosts and photosynthetic partners laid the foundations for a magnificent diversity of plant life, mentioned at the start of this case study.

Cell walls circumscribe pathways of transport within plants

Another adaptation of cell walls allowed early colonists of the land to develop division of labour between roots and shoots and rise to the airy heights of fields and forests. External cuticle layers, which reduce loss of water to the atmosphere, let cells of aerial parts survive provided that water could be delivered from plant organs in contact with external sources, mostly roots. Numerous other adaptations of wall structure occur, some related to mechanical strength or protection, and many to transport of water and nutrients (Sections 5.1 and 5.2).

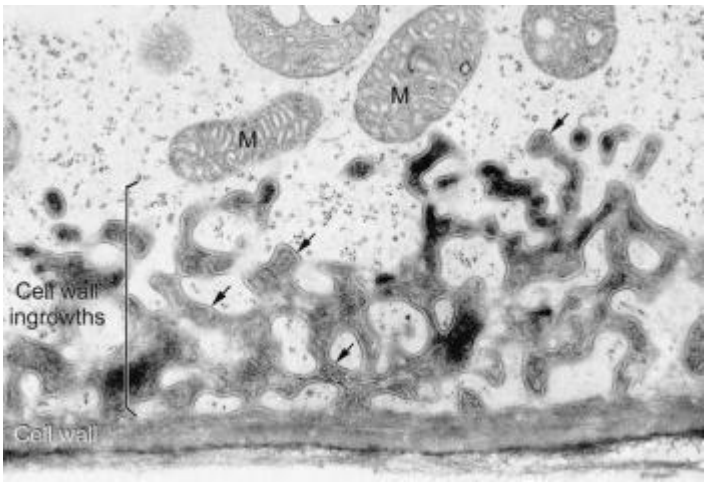

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Figure 7 Transfer cell wall. Elaboration of a cell wall into projections that are lined by plasma membrane (arrows), thus providing an enhanced surface area for exchange of many different types of solutes. Transfer cells develop in various plant tissues involved in transport. Mitochondria (M) are usually found in the vicinity of the wall labyrinths (A. Browning and B.E.S. Gunning, freeze-substituted transfer cell in the haustorium of a *Funaria* sporophyte, based on Gunning and Steer 1996)

The molecular construction and small pore sizes of cell walls limit the size of molecules that can be transported around plant bodies. One pathway of transport consists of the interconnected lattice of cell walls themselves — the ‘apoplasm’. Impregnation of the wall matrix with hydrophobic substances creates apoplasmic barriers in some strategic locations (Section 3.6.4); in other locations the apoplasm is open and permeable. In ‘transfer cells’, fingers of wall protrude into the cytoplasm and provide an unusually large surface area for transport across the

adjacent plasma membrane (Figure 7). Many sites of intensive absorption or secretion possess this wall adaptation.

From the very early evolution of multicellular plants, fine cylindrical extensions of cytoplasm — plasmodesmata — have pierced the wall between adjacent cells (Section 10.1.2). By passing through cell walls and the middle lamella, plasmodesmata form a transcellular commune of living cell contents known as the 'symplasm'. Through this important cell to cell transport pathway, bounded by a continuous plasma membrane system, tissues evade some of the transport constraints imposed by cell walls.

Division of labour into roots, stems, leaves, meristems and other organs depends upon mass transport of metabolic products. Because solutes transported around plants must sometimes traverse cell walls, there can be no equivalent of the bloodstream of animals, which delivers macromolecules in a mass flow. Only small, wall-permeable molecules (e.g. sucrose and amino acids) are suited for mass transport in plants. In turn, the need to import and export these small molecules determines the nature of many biochemical pathways and physiological systems in plants.

Cell walls: a sensory and signalling system

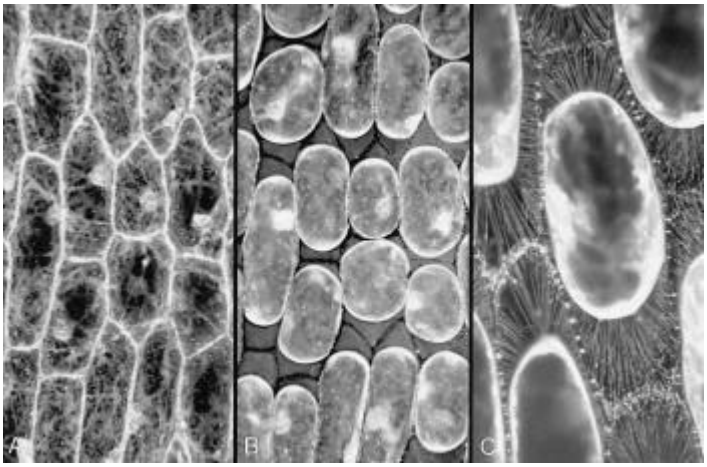

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Figure 8 Connections between the plasma membrane and cell wall. (A) Some cells in an onion bulb scale leaf epidermis, stained with the fluorescent dye DIOC(6) and viewed by confocal microscopy. Cytoplasm is seen as pale strands at the cell surface, traversing the large vacuole and often passing to the nucleus. Cell boundaries are bright because the surface cytoplasm (especially endoplasmic reticulum) is intensely fluorescent. (B) Precisely the same field of view after plasmolysis in 0.6 M sucrose. The cell walls are now visible as dark lines between the shrunken protoplasts, which still show brightly fluorescent surfaces. (C) A reconstruction of many planes of focus at a higher magnification to show some of the hundreds of stretched strands of plasma membrane that connect the protoplasts to the cell wall. These strands form because molecules in the plasma membrane (and peripheral cytoplasm) remain tethered to the wall during plasmolysis. The plasma membrane therefore becomes pulled out into very fine strands when the protoplasts shrink (A and B are based on Gunning and Steer 1996; C, micrograph courtesy B.E.S. Gunning)

Plasma membranes and cell walls meet at a very special interface: a living cell abuts a non-living but chemically active external covering. Here the cell perceives much about the outside world. Innumerable connections between the inner face of the wall and the outer face of the plasma membrane are revealed by plasmolysis (Figure 8). These fine strands are indicative of molecules that link walls to plasma membranes. In some places links extend even further, connecting the wall through the plasma membrane to strands of endoplasmic reticulum and perhaps to elements of the cytoskeleton. These strands are ideally located to transmit physical signals arising from mechanical disturbance at the wall–membrane interface. Wall pressure in expanding, turgid cells might be communicated via these strands to activate membrane processes such as mechanosensitive channels.

Classical plant biology points to roles for a linked wall–membrane sensory apparatus. Charles Darwin showed that bean roots are 100 times more sensitive to touch than human touch receptors. Stimuli that he himself could not perceive alter root growth patterns. Plants respond dramatically to touch and to stretching and compression of cell surfaces. ‘Wind pruning’ of trees is a familiar example of a large-scale effect. Specialised touch receptors occur in tendrils and insectivorous plants. They also trigger mechanical pollination mechanisms. All such stimuli are perceived at the outer face of a cell wall, whence signals pass to and are transduced in the underlying cytoplasm.

Another class of wall-mediated sensing deals with chemical rather than physical stimuli. Plant cells detect certain short chains of sugar residues (oligosaccharides), derived from enzymatic hydrolysis of cell wall polysaccharides, with extraordinary sensitivity and specificity. This gives plants early warning of attack by pathogens, which normally have to digest their way through the cell wall as they begin their infection, liberating oligosaccharide signal molecules as they penetrate. Some of the plant’s own hormonal signalling system probably also uses oligosaccharides, independent of pathogen attack. In other words, the wall contains messages built in to its molecular construction, ready to trigger growth or defence responses when released. More than most other phenomena, this illustrates the subtlety with which the cell wall is integrated into the life of plants.

Cell walls: chemical and functional diversity

Many constituent molecules give rise to hundreds of different wall polymers with diverse functions. Classical staining reactions to identify wall components are giving way to new approaches to wall function. Molecular probes such as antibodies and separation techniques give deeper insights into the great diversity and specificity of wall composition. Polysaccharides, for example, can be extraordinarily complex with wide-ranging variation in constituent sugar units, branching patterns, sequences and substituents. They can thus be extraordinarily specific in signalling and recognition systems.

Part of the chemical diversity of cell walls is related to functional diversity of cell walls in varied roles such as skeletal support, waterproofing, deterring herbivores, sustaining tension in the transpiration stream, protection of specialised cells like pollen grains from desiccation, and so on. Increasingly, however, very subtle chemical modifications of walls are viewed as ways in which cells can recognise each other and their positions in tissues and organs. There is now an appreciation of the role of chemical signalling as a guide to cell fate in plants, analogous to cell signalling systems in animals.

Cell walls: consequences of a sedentary lifestyle

Cell walls impose a sedentary lifestyle on plants. With few exceptions, mobility of plants is limited to local movements of plant parts, explorations of the environment by growth of individuals and colonisation by reproductive units. Inevitable outcomes of being rooted to the spot include intense neigh-bourhood competition below ground for water and nutrients and above ground for light, adaptations to varied environ-ments, subtle environmental sensing mechanisms, amazingly diverse chemical, physical and sacrificial defence strategies, breeding systems that employ mobile organisms to disperse propagules, and a type of cell wall that protects the only really mobile cell category, pollen grains, during their aerial journey. Events at all levels in plant biology are influenced by this sedentary lifestyle. That the habit comes from the evolutionary decision to regulate osmotic properties by means of a cell wall is not always explicit, but the underlying fact is there!

Cell walls: a focus of current research

Strictly, cell walls are not alive but to dismiss them as an inert and uninteresting box around cells could not be further from the truth. Cell walls are the major determinant of plant form and function, whether viewed at the level of individual cells, whole-plant physiology or characteristics of the Plant Kingdom.

Not surprisingly, cell walls are one of the main foci of modern research in plant science. Their chemical complexity demands new techniques for separation, purification and analysis of their constituents, as well as studies of how the molecules interact and cross-link in the intact wall. Such knowledge is needed to understand how cells grow and recognise each other. Advanced computing is being added to biochemistry and biophysics in efforts to unravel the ‘micro-engineering’ properties of walls, necessary for looking at larger aspects of growth, for instance in shoot meristems where sheets of cells stretch, deform and grow out into leaf primordia. Increasingly the powerful methods of molecular genetics are being brought to bear. Already many mutants have been isolated with specific deficiencies in wall components, leading in turn to abnormal behaviours in growth, development and physiology. The research spectrum stretches from basic science to practical applications, the latter stemming from uses of cell walls in fibre, paper,

fabrication, fuel and chemical industries. As usual, for the practical applications to prosper, plant scientists must learn much more about the basic biology — we must 'first know the nature of things'.

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4.3.5 How rapidly do cell walls respond to changes in P?

Precise changes in *P* can be achieved by varying the concentration of slowly penetrating solutes (e.g. mannitol) in the bathing medium. Imposing an increase in $\Pi_{outside}$ (= $\Psi_{outside}$) induces an abrupt, almost equivalent decrease in *P*. Changes in wall rheology often follow rapidly, in the order of minutes and may be faster. In maize roots, Frensch and Hsiao (1994) used this approach to show that loss of *P*was compensated for by resetting of P_{th} to a lower level, helping to hold $P - P_{th}$ constant and maintain growth rate. Speculation on how cell wall properties are rapidly modified appears in Section 4.3.6. The lower *P* in roots subjected to lower $\Psi_{outside}$ was temporary; recovery of *P* by up to 0.3 MPa followed over the next 30 min. Cells deep within the root recovered first, suggesting that sugars unloading from the stele to re-establish *P* act in concert with changes in cell walls to maintain root growth under drying conditions.

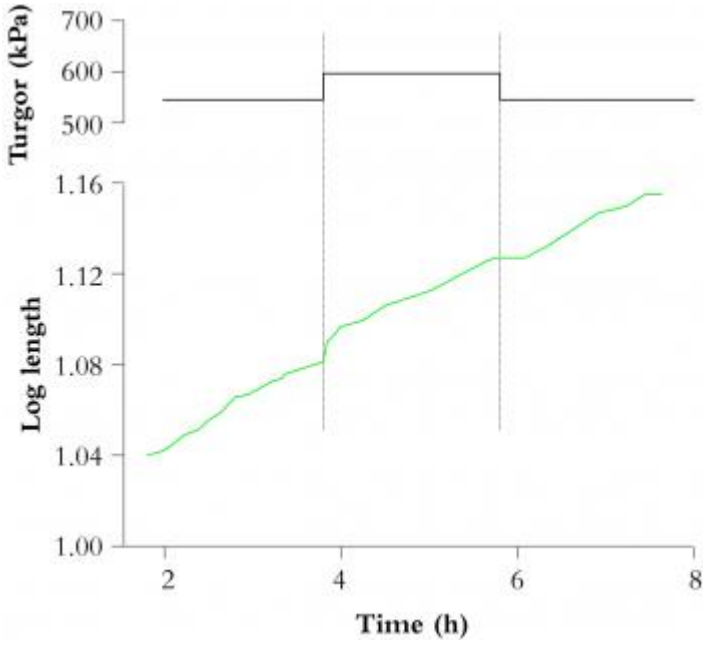

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Figure 4.23 Effect of a perturbation in *P* on elongation of giant algal cells (*Nitella*). A 50 kPa increase in *P* caused a transient (within minutes) increase in growth followed by a return to initial

elongation rate. Sustained high *P* did not further affect elongation of cells (From Green et al. 1971; reproduced with permission of the American Society of Plant Physiologists)

Cells of the giant alga *Nitella* have provided the most convincing evidence for wall properties of single cells responding to *P* (Green *et al*. 1971). Again, *P* was manipulated by making rapid changes in $\Pi_{outside}$. When a 50 kPa increase in *P* was induced by placing the cell in hypotonic solution (Figure 4.23), the elongation rate rose rapidly (within minutes) but quickly settled to the same rate as the cells in the original solution. That is, cell elongation was independent of *P* within 10–15 min of the initial perturbation. Clearly cell walls were responding to the altered water status of the cells, becoming either less extensible (lower φ) or yielding less readily to internal pressure (higher P_{th}).

Later experiments have been performed on barley seedlings in which roots were pressurised in order to elevate artificially shoot *P*. In this case, a *sustained* rise in shoot *P* induced by pressurising the roots and xylem sap caused a *temporary* jump in leaf elongation rates but a return to existing rates was, as with *Nitella*, rapid. Again, the cell wall properties (φ and P_{th}) enunciated in the Lockhart equation responded to the water status of the growing cell.

Growth can also be manipulated by photoinhibiting pea seedlings with blue and red light (Kigel and Cosgrove 1991). No change in water supply was imposed and yet reduced cell wall relaxation was apparent through either lowering of φ (blue light) or increases in P_{th} (red light). It appears that cell wall properties induce changes in growth rate, regardless of whether growth is perturbed by light treatments or hydraulic effects. Substantial effort is now directed towards defining molecular events which start and stop growth.

4.3.6 Biochemical processes in walls of growing cells

Rapid physical changes in cell walls (Section 4.3.5) have long been ascribed to wall loosening and rigidifying factors. Evidence that these rheological changes occur almost instantaneously, coupled with the abundance of cell wall proteins, have prompted a search for enzymes as wall-modifying factors. In principle, relatively few enzymes could account for quick changes in wall plasticity, such as that observed in *Nitella*, particularly if tension within the wall could be relieved simply by breakage and re-formation of H-bonds between wall polymers. However, it is more likely that many enzymes are required to loosen, rigidify and stabilise the range of wall polymers found in higher plant cell walls. The interplay be-tween f and P_{th} in wall modifications, depending on environ-mental conditions, cell type and ontogeny, also points to a high level of molecular complexity underlying wall rheology.

Early experiments on acid-induced wall growth (Section 4.1.3(b)) raised the possibility that protons contribute to rapid wall loosening. Specifically, H^+ could activate cell wall enzymes with an acid pH optimum by lowering wall pH below 5. This view of cell wall loosening is still largely hypothetical because so few components of the wall loosening process, especially enzymes, have been identified. Indications that H^+ concen-trations in cell walls influence growth rates come from the blue light inhibition of cucumber seedling growth — depolarisation of plasma membranes (reduced H^+ extrusion) precedes wall stiffening (Section 4.3.5), raising the possibility that wall alkalinisation inhibits growth. A second line of evidence comes from the expansins (growth-inducing proteins) which are highly active in walls of living cells at pH 4.5 but not at pH 6.8 (Section 4.3.6(b)).

(a) Extension of the cell wall matrix

Ordered assembly of cell walls as they are exocytosed from plasma membranes is critical for processes such as growth and morphogenesis. The three-dimensional structure of walls arises through interaction of several families of molecules: inextensible cellulose microfibrils lie embedded in a carbohydrate-rich matrix of hemicelluloses (e.g. xyloglucans and glucuronoarabinoxylans), pectins, proteins and sometimes lignin (Table 4.2). Further biochemical changes to some of these molecules occur within walls, including cross-linkage reactions leading to polymerisation. Whether the response of walls to abrupt changes in *P* is being considered (as in Section 4.3.5), or steady growth of an elongating tissue, molecular events in cell walls are important.

The first clue to regulation of cell wall relaxation is that growth of most plant cells is not isodiametric (equivalent stretching in all directions). Ethylene application can induce cells to balloon around their girth but most cells elongate according to a strict polarity set down throughout the organ. Cellulose microfibrils in cell walls prior to initiation of growth tend to be transversely oriented (at 90°) to the axis of growth (Figure 4.19). The inextensible nature of cellulose microfibrils is good physical evidence that elongating cells are constrained from increasing in girth and must grow by separation of adjacent microfibrils. This places the focus on the non-cellulosic polymers of the cell wall (the so-called gel matrix) as participants in growth.

(b) Enzymes and wall tension

Species variation in the carbohydrate composition of cell walls suggests that different enzymes are required to act on these substrates in different species. Xyloglucan, a xylose–glucose polymer, is a prime candidate for binding cellulose microfibrils together in dicotyledonous plants where it is abundant. Xyloglucan adheres strongly to cellulose in a strong stoichiometric relationship. An enzyme capable of cleaving and rejoining xyloglucan chains has also been isolated, providing the elements theoretically required for a biochemical model of wall

extensibility. The activity of this enzyme (xyloglucan endotransglycosylase or XET) in young barley seedlings does not peak exactly in the region of maximum leaf growth but a role for XET in growth is suggested by the stimulatory effect of gibberellic acid on both XET activity and growth (Smith *et al*. 1996). XET might have more functional significance in dicotyledons in which xyloglucan is often a very abundant wall polymer. Glucanases isolated from maize cell walls enhance auxin-stimulated growth of maize coleoptiles, antibodies to glucanase blocking the effect, but no further indication of the role of these enzymes in growth has emerged.

Other experiments have demonstrated that a class of cell wall proteins, expansins, are potential agents for catalysing cell wall yielding *in vivo* (McQueen-Mason 1995). Figure 4.24a shows that sharp gradients in growth along the hook of a cucumber hypocotyl are paralleled by a gradient in extension of these tissues when stretched under acid conditions (Figure 4.24b) but not at neutral pH (Figure 4.24c). When tissues were killed by boiling, extension was blocked (Figure 4.24d). Hypocotyl extension requires acid pH and non-denatured proteins.

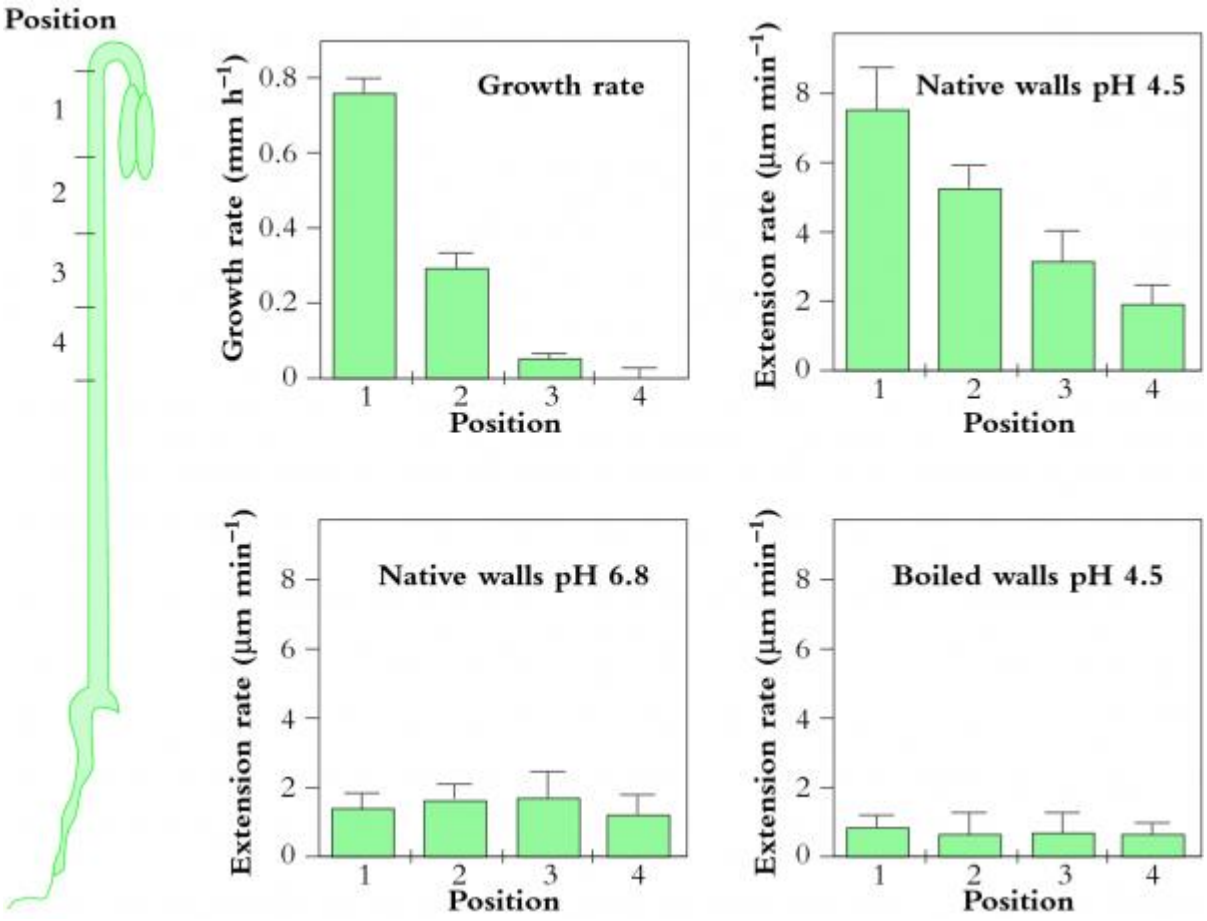

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Figure 4.24 Distribution of growth and wall extension at four positions along a cucumber hypocotyl. (a) Growth rate is most rapid near the hook. (b) Hypocotyl segments were frozen, thawed, abraded and stretched under a 20 g load in an acidic buffer (pH 4.5), revealing most

rapid extension in the fast—growing hook. (c) When measured at pH 6.8, segments extended very little. (d) Segments in which enzymes were denatured by boiling did not extend under the load (From McQueen—Mason 1995; reproduced with permission of Journal of Experimental Botany)

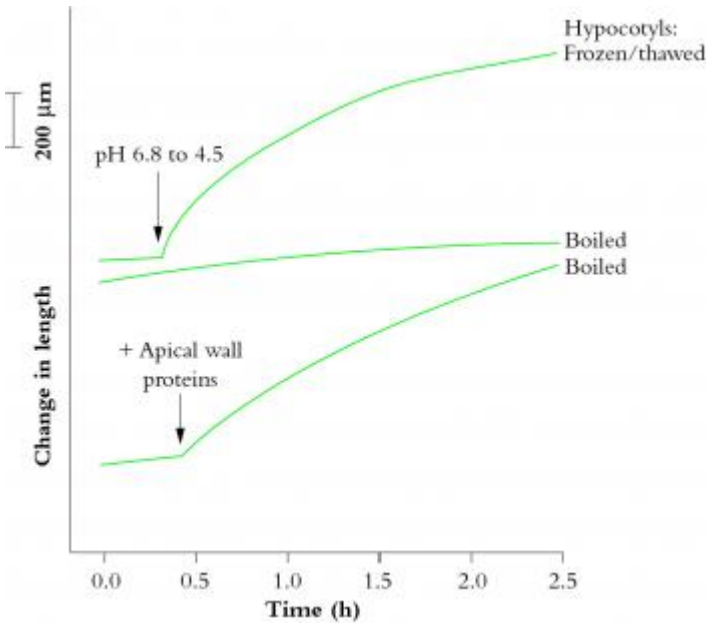

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Figure 4.25 Extension of apical 1 cm segments of abraded cucumber hypocotyls which were either frozen and thawed or boiled (proteins denatured) prior to application of a load. After boiling alone there was negligible extension but addition of a crude preparation of 'apical wall proteins' extracted from rapidly growing cell walls induced hypocotyls to stretch. Hypocotyl segments which were frozen and thawed began to stretch only after transfer from neutral pH (6.8) to acid pH (4.5), demonstrating the dependence of wall extension on acid conditions (From McQueen—Mason et al. 1992; reproduced with permission of Plant Cell)

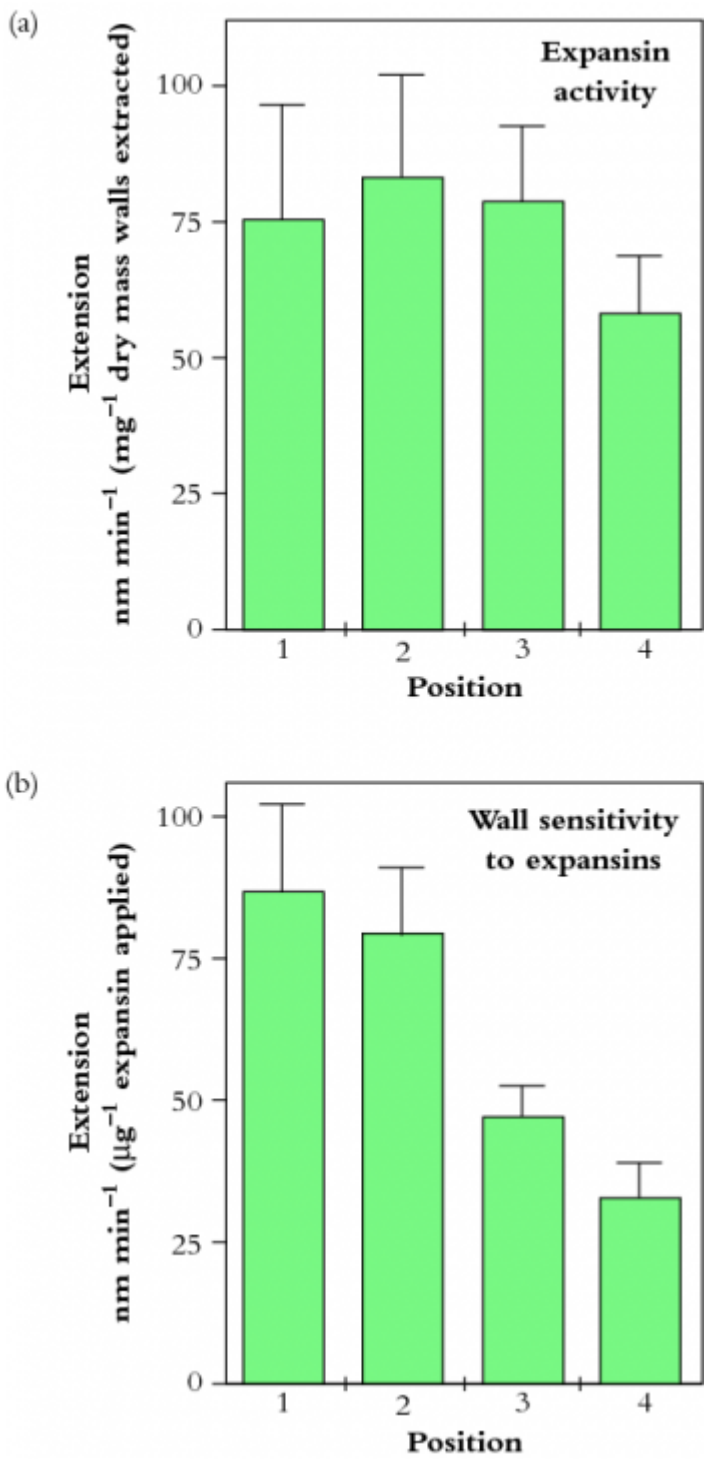

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Figure 4.26 (a) Extension activity assayed in crude preparations containing wall proteins extracted from tissues at four positions along the axis of a cucumber hypocotyl (see Figure 4.25). Protein extracted from these four positions was assayed by adding it to apical hypocotyl segments that had been frozen, thawed, abraded and clamped into an extensometer prior to stretching. Note that even non-growing tissues (position 4) had wall proteins capable of inducing extension. (b) Segments which were boiled to denature wall proteins stretched more on addition of partially purified expansin if they were taken from the growth zone of the hypocotyl, indicating a loss of expansin sensitivity as cells exited the growth zone (From McQueen-Mason et al. 1992; reproduced with permission of Plant Cell)

Two lines of evidence point to expansins having a direct role in cell extension. First, preparations of proteins from growing cell walls caused boiled cell walls to stretch sig--
n-ificantly at a pH of 4.5 but not at a pH of 6.8 (Figure 4.25). Purification of this showed that a 25 kD expansin was capable of catalysing wall extension. Second, while expansin activity does not decline in tissues as they exit the growth zone (Figure 4.26a), *sensitivity* of these tissues to added expansin does decrease (Figure 4.26b). This might be interpreted as a decreasing availability of substrates for expansin to act on in expanded walls. In the case of expansins, it is proposed that the 'substrates' are hydrogen bonds between hemicelluloses and cellulose microfibrils. Expression of expansin-encoding genes, release of the enzyme into cell walls, wall acidification and accessibility to H-bonds might all play a role in determining wall rheology. Expansin applied to meristem surfaces alters the development of leaf initials, suggesting multiple roles for expansin as a wall-loosening factors. Undoubtedly, further enzymes which play a role in wall loosening in other species and tissues await discovery.

What is clear is that the biophysical consequences of instantaneous changes in tension in cell walls (e.g. induced by changes in *P*) will be followed by a phalanx of biochemical events including wall polymer synthesis and altered gene expression. Sustained expansion of plant cell walls cannot be explained simply by inexorable wall hydrolysis; if it were, cell walls would weaken to breaking point during growth. The 'setting' of long-term cell expansion rates is likely to hinge on biochemical events underlying wall relaxation and reinforcement.

(c) Cessation of cell wall expansion

Molecular events leading to cessation of wall expansion are even less well understood than those which initiate growth. A common view is that sufficient tension develops over time in the molecules cross-linking cellulose microfibrils (e.g. xyloglucans) to prevent further wall expansion. Essentially, when a cell has reached its final dimensions its wall is 'locked' into a final, hardened conformation. Molecules with a specific role in growth cessation are thought to be exocytosed into cell walls, providing either substrates for cross-linkage reactions or enzymes catalysing cross-linkage of pre-existing wall polymers. Identification of cross-linkage reactions between
moieties found in the cell wall have led to a search for their presence *in vivo*. For example, ferulic acid residues in grass cell walls can cross-link to produce di-ferulic acid and potentially stiffen walls through formation of a polysaccharide-lignin network. Unfortunately, in rice coleoptiles the abundance of the di-ferulic form bore no relation to growth cessation.

Secondary cell walls generally form after primary walls have ceased to grow but the familiar rigidity of secondary cell walls (e.g. wood) is mostly viewed as distinct from stiffening of primary walls. Lignification of primary walls commences earlier

than once thought and is a possible factor in growth cessation (Müsel *et al*. 1997). Such a response might be controlled through release of peroxide into walls in much the same way as seen in walls subject to fungal attack. Peroxidases are targeted as candidates for the catalysis of these reactions.

The number of non-cellulosic polymers coming under tension as a cell wall expands will rise if there is not a continual release of that tension, probably through enzyme action. Therefore the *degradation* of enzymes responsible for polymer cleavage might also play a role in cessation of growth. However, experiments with expansins are a good reminder that the sensitivity of the wall (making substrate for enzymes) is also an important factor in growth cessation. Understanding rigidification of this complex matrix of polymers demands input from the disciplines of biology, chemistry and physics. Combining established techniques with novel approaches to the study of individual cells (e.g. Fourier-Transform Infra-red microspectroscopy and the cell pressure probe) will bring new insights to the molecular basis of wall expansion.

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[32] http://plantsinaction.science.uq.edu.au/edition1//?q=figure_view/194
[33] http://plantsinaction.science.uq.edu.au/edition1//?q=figure_view/195
[34] http://plantsinaction.science.uq.edu.au/edition1//?q=figure_view/196
[35] http://plantsinaction.science.uq.edu.au/edition1//?q=figure_view/197
[36] http://plantsinaction.science.uq.edu.au/edition1//?q=figure_view/201
[37] http://plantsinaction.science.uq.edu.au/edition1//?q=figure_view/202
[38] http://plantsinaction.science.uq.edu.au/edition1//?q=figure_view/204
[39] http://plantsinaction.science.uq.edu.au/edition1//?q=figure_view/205