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Preface

Plant cells go through distinct phases of development. They are generally formed in meristems and undergo expansion before differentiating and acquiring their final functions. In this sequence, cell expansion is the process that mainly contributes to the cell's size and hence to the plant's size and morphology. Furthermore, it allows the plant to rapidly adapt to changes in the environment and to respond to several hormone signals.

Cell expansion primarily occurs by the uptake of water in the cytoplasm and vacuole of the plant cell. This process is driven by osmotic forces generated by accumulation of solutes by several classes of (transporter) proteins. This causes the vacuole to expand and to exert a pressure against the cell wall. In order to enlarge, the cell wall has to yield to the stress imposed by the turgor pressure. Several families of proteins and enzymes, as well as the composition and architecture of the cell wall itself, render the wall stiff and tough but at the same time modifiable for a drastic increase in surface area. The direction of cell expansion is hereby governed by cytoskeletal elements in the cytoplasm as well as by load-bearing elements in the cell wall. The process of cell expansion is thus a complex process brought about by activities at different levels in both the symplast and apoplast.

This book addresses the involvement of the different actors in plant cell expansion and its control by integrating the up-to-date views of cell biologists, biochemists, physiologists, molecular biologists, biophysicists and microscopists. The combination of these different views, resulting from different experimental techniques and methodologies (explained in distinct boxes), gives a timely summary on what is currently known and believed to occur during the cell expansion process.

August 2006 Jean-Pierre Verbelen & Kris Vissenberg

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Cell Expansion: Past, Present and Perspectives

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Plant size and organ size are dependent both on cell division and cell expansion (Lyndon 1990). Cell division is the process whereby one cell divides into two daughter cells; expansion is the growth in volume beyond the size of the mother cell before mitosis.

Both cell division and cell expansion were correctly defined in the 19th century on the basis of careful microscopic observations. Wilhelm Hofmeister (1867) demonstrated that the nucleus of a mother cell divides and that one half of the contents of the mother cell collects around each of the two daughter nuclei when a new cell wall forms between the daughter nuclei. Julius Sachs (1882) on the other hand clearly depicted the changes in appearance of parenchyma cells during cell expansion in a growing root, with reference to the volume increase of the central vacuole. He further emphasized cell turgor and water uptake as instrumental in causing expansion. He also pointed to the fact that during expansion the existing cell wall was stretched and thinned, but that new material was added keeping wall thickness rather constant (Fig. 1).

In plant organs the peak activities of both events are separated in time or space, a fact also known since the 19th century, as elegantly described and depicted by Sachs (1874). His figures of growing seedling roots gained an immediate popularity and were copied in Strasburger's famous handbook (Strasburger et al. 1894). They remained there as reference illustrations at least up to the 30th edition, published in 1971 (Strasburger et al. 1971).

Since that period of fundamental discoveries the process of mitosis and cytokinesis has been explored intensively and, during the last decades especially, the picture of both aspects has become extremely detailed. It has turned out that the mitotic machinery and its control do resemble that of animal systems but that they are plant-specific and very elaborate (for reviews see Dewitte et al. 2003; De Veylder et al. 2003). The formation of the cell plate, the new cell wall separating the newly formed daughter cells, turns out to be a highly complex cellular activity implying a precise orchestration of cytoskeleton activity, and synthesis and transport of wall components (Otegui and Staehelin 2000a,b; Otegui et al. 2001, and references therein).

Cell expansion has no equivalent in animal systems and progress in the understanding of the process was slow. As mentioned above, from the be-

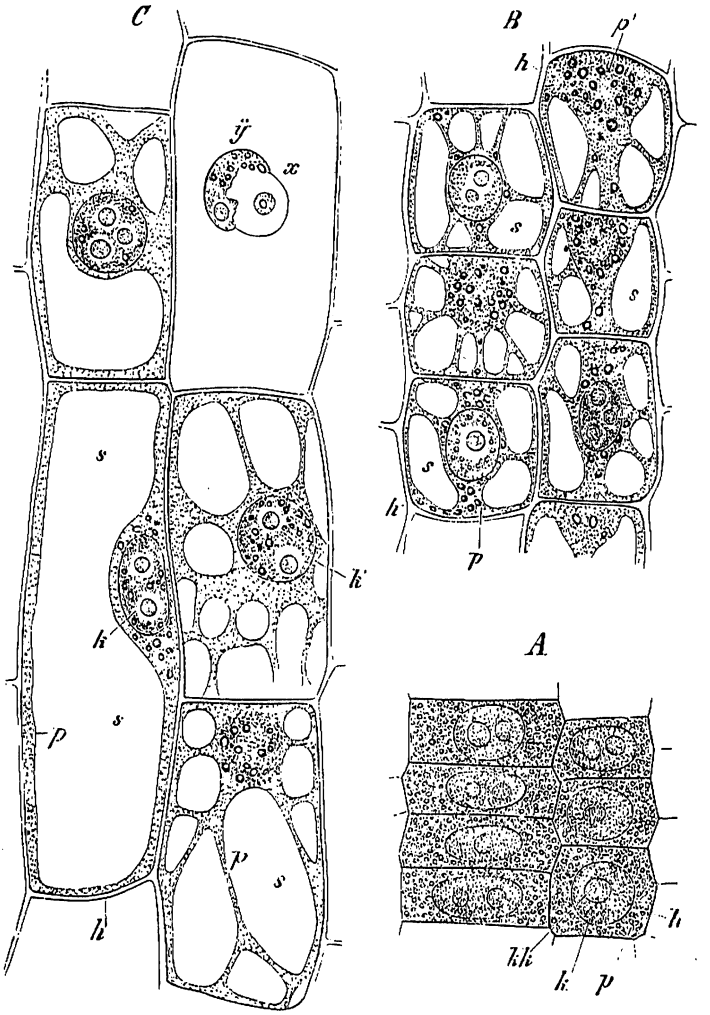

Fig. 352. Parenchymzellen aus der mittleren Schicht der Wurzelrinde von Fritillaria imperialis; Längsschnitte, nach 550maliger Vergrößerung. *A* dicht über der Wurzelspitze liegende, sehr junge Zellen, noch ohne Zellsaft; *B* die gleichnamigen Zellen etwa 2 Millimeter über der Wurzelspitze, der Zellsaft *s* bildet im Protoplasma *p* einzelne Tropfen, zwischen denen Protoplasmawände liegen; *C* die gleichnamigen Zellen etwa 7—8 Millimeter über der Wurzelspitze; die beiden Zellen rechts unten sind von der Vorderfläche gesehen, die große Zelle links unten im optischen Durchschnitt gesehen; die Zelle rechts oben durch den Schnitt geöffnet; der Zellkern lässt unter dem Einfluss des eindringenden Wassers eine eigenthümliche Quellungserscheinung wahrnehmen (*x y*).

Fig. 1 Parenchyma cells from the cortex of the root of *Fritillaria imperialis* in a longitudinal section of fresh material. *A* cells immediately above the root tip without vacuoles. *B* cells about 2 mm above the root tip with small developing vacuoles. *C* cells 7–8 mm away from the root tip with large vacuoles

ginning botanists knew that during cell expansion it was mainly the vacuole that grew considerably in volume and also that the existing cell wall became thinner as it was stretched but "reinforced" by addition of new wall material. A crucial step for the understanding of the physiology behind expansion was made by the discovery that auxin affects elongation and its control (Went and Thimann 1937). Most of the research, however, only refined the existing descriptive knowledge (Avery and Burkholder 1936; Erickson and Sax 1956). Interest within the scientific community was indeed very moderate, as witnessed by the limited attention to cell expansion in notorious handbooks (Esau 1960; Clowes and Juniper 1968; Wareing and Philips 1973; Fahn 1974; Bidwell 1979).

A reliable view on the state of the art in the early 1960s can be found in the *Encyclopedia of Plant Physiology, vol XIV* on growth and growth substances (Ruhland 1961). It clearly depicts the nascent interest in the process of cell expansion. Cell expansion receives little attention in the anatomy chapter (one sentence) but is treated in detail in the chapters "Cell expansion and metabolism (Ziegler H)", "Physics of cell elongation (Burström H)" and "The growth of the cell wall (Preston RD)". These chapters contain detailed information on in vitro extensibility of cell wall preparations and on changes in cell wall composition (cellulose, hemicelluloses, pectin and proteins) in elongating coleoptiles and hypocotyls.

Around that time, the attempts to understand cell expansion shifted into a new gear. On the theoretical side, Lockhart (1965) summarized a lot of experimental data on wall extensibility in a formula that was readily comprehensible for the whole scientific community and that continued life as the "Lockhart equation":

$$r = \Phi(P - Y)$$

where r is growth rate, Φ is extensibility of the cell wall, P is turgor pressure (i.e. the source of cell wall stress) and Y is yield threshold (i.e. the minimum pressure required for growth).

This simple equation clearly states that the rate of cell expansion is a product of the imbalance between turgor pressure and the mechanical properties of the cell wall, emphasizing that the principal players are thus to be found in the symplast as well as in the apoplast.

Since then, detailed data were gathered on the composition and the interaction of the primary cell wall and its then-known components: cellulose, hemicelluloses, pectins and proteins. Cellulose was found to be synthesized by cellulose synthases (Arioli et al. 1998) that are organized in cellulose synthase complexes (Kimura et al. 1999). Fluorescent labelling of these rosettes pointed to the role of the cytoskeleton in the orientation of the cellulose microfibrils in the wall (Paredez et al. 2006). The acid growth theory was substantiated by the discovery of expansins (McQueen-Mason et al. 1992), while many other proteins and processes with putative roles in cell wall loosening

were described (Cosgrove 2005). Mechanisms emerged that counteract the loosening of the cell wall and so arrest cell expansion (Cooper and Varner 1984). Aquaporins were described as universal facilitators of water transport through vacuolar membranes (Crispeels and Maurel 1994). The mode of action of auxins and of the other plant growth regulators became much clearer (e.g. Weijers and Jurgens 2004). These are the scene and the actors that make the content of this volume. Most of the recently published reviews focus on or are limited to the cell wall. As stated above, the Lockhart equation indicates that both apoplastic and symplastic players are involved in cell elongation. This volume therefore combines actual state-of-the-art papers on the different aspects of the cell's biology involved in expansion and its control. Nuclear ploidy is often related to cell expansion (Nagl 1979). As this is only the case in about half of the plant species, endoreduplication does not seem fundamental for expansion. It will therefore not be treated. It also needs to be mentioned that cell expansion includes diffuse expansion (in most cells) and tip growth (in certain specific cells). The latter method of cell growth will not be treated as it has been covered by Rui Malhó in another volume of this series (Malhó 2006).

During cell expansion, the cell wall clearly is a centre of activity. Up to now, however, an adequate model of the cell wall structure and how this structure permits both an increase in surface and the incorporation of new wall material still remains elusive. Using wall microscopy, selective extraction of components followed by structural analysis and in situ spectroscopic approaches, several artificial models have been proposed. Cosgrove (2000) mentions and discusses three models that differ only in the types of interaction and spacing of the different components. These specific associations and locations of the components need to be further elaborated to fully understand the mechanism of cell wall enlargement.

At the onset, during, and at the end of cell expansion, undoubtedly different sets of genes and proteins are expressed and active/inactive. Several of these crucial genes and proteins are starting to emerge, but the complete picture is far from clear. The combination of the knowledge on the three-dimensional architecture (e.g. of the *Arabidopsis* root, which is well-described) and cell type-specific expression profiling as performed by Birnbaum et al. (2005) could eventually provide the complete transcriptome of single cells in the root apex. This information could then provide all of the changes in gene expression that occur when a cell switches from a meristematic to an expanding cell or when a cell responds to environmental and hormonal stimuli. Similar proteomic approaches could give complementary information on protein involvement in the cell's crucial developmental processes and switches.

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Solute and Water Relations of Growing Plant Cells

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Abstract Cell expansion requires the continuous uptake of water into cells, which in turn is driven through osmotic forces generated by accumulation of solutes. Herein, we assess the significance of water and solute transport across cell membranes as a rate-limiting step during cell expansion. Two membranes are considered, the tonoplast, which separates the largest intracellular storage compartment (vacuole) from the portion of the protoplasts where most enzymatic reactions take place (cytoplasm), and the plasma membrane, which constitutes the site of exchange between protoplasts and apoplast (cell wall). Most of the solutes that generate the bulk of osmolality are heterogeneously distributed between cells, tissues and cell compartments, and this heterogeneity must be taken into consideration in studies on growth. Because of differences in transmembrane potential at the plasma membrane (significantly negative) and tonoplast (close to zero), ion channels and transporters are likely to make different contributions to solute transport across these two membranes. The osmotic permeability of the tonoplast exceeds that of the plasma membrane by a factor of 100. This aids cell-internal osmotic equilibration and renders the plasma membrane rate-limiting for water uptake into cells or transcellular water transport. Candidate aquaporins, ion channels and transporters which could mediate solute and water transport specifically into growing cells are reviewed in this work.

1 Introduction

1.1 Growth

Growth requires the co-ordination of many processes and has to be adjusted to environmental constraints. Since growth is a prerequisite for any organism to reach its full potential, it is intricately linked to development. The definition of growth varies, depending on which variables are used as the reference system, yet there are some aspects of growth which do not vary: (i) growth is irreversible; (ii) growth of multicellular organisms is due to expansion of (some of) its individual units—cells; and (iii) as cells grow, they pass through

well-defined developmental stages. In addition, in multi-cellular organisms, growth is often restricted to specialized regions, "growth zones". Although growth requires the production of new cells and expansion of these cells, it is the latter that is responsible for the bulk of size increase, particularly in plants, which have, on average, larger cells compared to animals.

The above has several implications for the study of growth. A particular process must be unidirectional, when integrated over the entire growth period. For example, water moves in and out of a growing cell, but at the end of the growth period, cell water content and wall extension have increased irreversibly. Growth must also be studied at the level of the individual cell, since turgor pressure, the mechanical force driving wall extension, is defined at cell level. A range of tissues has to be analyzed since organ expansion requires the coordinated expansion of cells of different tissues. A cell goes through various developmental stages as it passes through the growth zone and it is likely that the molecular cause of growth limitation changes with development. For example, as cells elongate and mature, the cellular ratio of vacuole:cytosol changes. The two compartments differ in solute relations and this impacts on solute requirements of cells. Since cells are growing in specialized regions in which they act as sinks for energy, water, carbon and solutes, their demands have to be met—possibly in competition with other growing regions—by those regions which provide these resources. The question is not so much "what limits growth?" but "which factor limits growth at a particular developmental stage of a cell?", "which particular tissue or which cell type within a tissue limits expansion of the organ (Peters and Tomos 1996)?" and "where does the limitation originate, within the plant or externally?" If we are to manipulate the growth of cells and yield of plants, we need to identify molecular targets.

1.2 Walls, Water and Solutes

From the biophysical point of view, the three main factors potentially limiting cell expansion are cell wall, water and solutes (Fricke 2002a). Wall mass per cell increases during expansion and cell wall polymers must give in—"yield"—to cell turgor pressure. Water must flow into cells to increase volume and maintain turgor, while solutes are needed to generate the osmotic force driving water uptake into cells. Since water cannot be pumped actively into cells, the only way to increase cell water content is by generating a downhill gradient in chemical potential of water, i.e. the water potential, through osmotic forces across membranes.

The idea that cell wall properties differ between growing cells (yielding walls) and non-growing cells (non-yielding walls) or that environmental stressors affect growth through alteration of wall properties has been supported by numerous studies (e.g. Cramer 1992; for review, see Cosgrove 1993;

Hsiao and Xu 2000). More recently, these changes have been related to specific proteins (expansins, McQueen-Mason et al. 2006, in this volume), enzymes (xyloglucan endotransglycosylase/hydrolase (XTH, Nishitani and Vissenberg 2006, in this volume); peroxidase) and wall components (Cosgrove et al. 2002; de Souza and MacAdam 1998; Fry 1998; Huang et al. 2000; Reidy et al. 2001; Ruan et al. 2001; Thompson et al. 1997; Schunmann et al. 1997; Palmer and Davies 1996; Rose et al. 2002; Yokoyama et al. 2004). It is not known how, at the molecular level, changes in wall properties are modulated or wall-modifying proteins are regulated (De Cnodder et al. 2007, in this volume). In contrast, although water and solutes have received less attention, more is known about the regulation at the molecular level of candidate transporters and channels (Chaumont et al. 2005; Cherel 2004; Luu and Maurel 2005; Tornroth-Horsefield et al. 2006; Tournaire-Roux et al. 2003; Very and Sentenac 2002). This provides an ideal basis for identifying molecular mechanisms through which cell expansion is controlled.

Aquaporins are channels facilitating the movement of water and/or small neutral solutes across biological membranes. They have been found in a wide range of organisms and account for a considerable portion of membrane protein (Chaumont et al. 2005; Johansson et al. 2000; Maurel et al. 2002; Schaffner 1998). Classification of aquaporins occurs according to their subcellular location (TIPs, tonoplast intrinsic proteins, PIPs, plasma membrane intrinsic proteins), their organ of discovery (NIPs, nodule, or NOD26-like intrinsic proteins) or their molecular size (SIPs, small basic intrinsic proteins). Most aquaporins transport primarily water and increase the osmotic water permeability coefficient of the membrane (P_f) several-fold when expressed in *Xenopus* oocytes. Other aquaporins are less specific and also transport solutes such as glycerol, urea, boron, hydrogen peroxide or, as recently suggested, ammonia, carbon dioxide and silicon (Biela et al. 1999; Dordas et al. 2000; Gerbeau et al. 1999; Hanba et al. 2004; Henzler and Steudle 2000; Loque et al. 2005; Ma et al. 2006; Uehlein et al. 2003). It has also been suggested that aquaporins fulfil less of a transport role but can function either as osmo- and turgor sensors (Hill et al. 2004) or as markers for targeting vesicles to the central vacuole (Ma et al. 2004).

Before the discovery of water channels, it was assumed that cells have little control over the regulation of diffusional flow of water through membranes. Since then, several studies have shown that facilitated movement of water through aquaporins accounts for most of the transmembrane flow of water in plant cells. Whereas membrane water diffusion is characterized by equal osmotic water permeability (P_f) and diffusional water permeability (P_d) coefficients, and a high Arrhenius activation energy (E_a), facilitated water transport through pores has a P_f higher than P_d, a low E_a and, in addition, is blocked by mercurial compounds indicative of the proteinaceous nature of the pore (Maurel 1997). According to the composite transport model of water flow through tissues, water moves along three major pathways: along

the apoplast (wall space), along the symplast (through plasmodesmata) or through membranes (including passage through aquaporins) (Steudle and Peterson 1998). Notably, the driving force for water movement differs between apoplast (hydrostatic gradients) and transmembrane flow (osmotic gradients); the driving force for water movement through plasmodesmata may be either. The hydraulic conductivity of the apoplastic path is at least one order of magnitude higher than the hydraulic conductivity of the transmembrane path (for review, see Steudle and Peterson 1998).

The likelihood that hydraulic properties of tissues limit growth and that aquaporins are involved very much depends on the main paths along which water moves from a plant internal source (xylem, phloem) to peripheral tissues (e.g. mesophyll, epidermis). For example, in maize roots, the growing tip region is supplied with water mostly from phloem via the symplasmic path, whereas more distal regions are supplied via the transmembrane path (Hukin et al. 2002). Alteration of aquaporin activity in the tip region should have little effect on growth. In contrast, in growing hypocotyl tissue of soybean, a large number of small-volume xylem parenchyma cells exists, through which water has to pass as it moves from (inner-lying) xylem to peripheral tissues. Water has to cross many membranes per distance travel as it passes through xylem parenchyma. This creates a hydraulic bottleneck and significant growth-induced water potential gradients (Boyer 1985; Nonami et al. 1997). In the growing grass leaf, xylem parenchyma or mestome and parenchymatous bundle sheath cells may fulfil similar functions (Boyer and Silk 2004; Fricke 2002a). Over-expression or increase in activity of aquaporins in these tissues should overcome some of the hydraulic limitation in growth—provided growth is limited hydraulically in the first place!

What surprises most about existing work on biophysical limitation of growth is the scarcity of studies on the solute aspect, in particular on solute transport properties specific to growing tissues (for review, see Van Volkenburgh 1999).

In the following sections, we will look in more detail at the potential roles which water and solute transport play during cell elongation. We will focus on two membranes, the tonoplast, separating vacuole from cytoplasm, and the plasma membrane, forming the interface between protoplasm and wall space. Since solute requirements of vacuole and cytosol and of different leaf tissues and cell types differ, it is possible that one particular solute, for example, Ca, limits growth in one cell type but not in another. Therefore, we will briefly review the distribution of solutes between cell compartments, cells and tissues, particularly for leaves. For water, the situation is easier since it is present in the same chemical form in each compartment. However, the number of different aquaporins per plant species is considerable (Chaumont et al. 2001; Johanson et al. 2001; Sakurai et al. 2005), probably reflecting tissue- and cell-specific regulation of water transport through membranes.

2
Solutes

2.1
Solutes—Continuous Deposition During Growth

Theory predicts that as cells expand and cellular contents are diluted, solutes must be deposited (Silk and Erickson 1979) continuously and at high rates to maintain the osmotic force driving water uptake into cells. If solute accumulation did not occur in parallel to cell expansion, cell osmolality and turgor would continue to decrease until close to zero. For example, a grass leaf epidermal cell can elongate to 50 times or more its original size as it passes through the elongation zone (Schnyder et al. 1990). Without solute uptake, an initial osmolality of 300–400 mosmol kg^{-1} (Fricke 2004a) and turgor of around 0.5 MPa (Fricke 2002b) would be "diluted" half-way through the elongation zone to 12–16 mosmol kg^{-1} and 0.02 MPa, respectively. This would be insufficient to expand a wall and drive water uptake in an apoplastic environment which has either a significant tension or solute potential.

There exist few studies on grass leaves, and some on roots, where osmolality in the elongation zone has actually been determined at the level of the cell (see Technical Box) (Pritchard 1996; Fricke 1997, 2002a; Fricke and Peters 2002; Martre et al. 1999). These studies show that cell osmolality changes little along the elongation zone. In maize roots, cells expand in volume by as much as 50% h^{-1} (Pritchard 1994) and this means that cell solute contents must also increase by about 50% h^{-1}—a considerable task for a cell which has a total solute concentration of 250–350 mM. In grass leaves, cells elongate at relative rates as high as 12–20% h^{-1} and have total solute concentrations in the range 300–400 mosmol kg^{-1}. Estimated solute flux rates per cell surface are in the upper region of values for plant cells (although considerably lower than rates for guard cells which require fast movement of solutes across membranes for functioning; reviewed in Fricke and Flowers 1998).

In barley leaves, turgor increases after cells have exited the elongation zone, while osmolality stays the same (Fricke 1997). This suggests that net uptake of solutes by cells is linked to elongation, regardless of whether solute uptake regulates growth or vice versa, whereas turgor remains at a certain level during growth due to continuous yielding of the wall and rises, once wall properties are modified (stiffened).

2.2
Two Major Cellular Solute Compartments: Vacuole and Cytosol

Most solutes entering a growing plant cell are destined to the large central vacuole. When a new cell is produced through division and commences elongation, it contains a number of smaller-sized vacuoles ("vacuon"). As a cell

elongates to reach its mature size, a large central vacuole forms by fusion of vacuon in an autophagic process (for review, see Marty 1997). This central vacuole can account for almost 100% of the cell's volume (epidermis; ca. 99%) or "only" about 60% (mesophyll). The other major cellular compartment to which solutes are destined is the cytosol/cytoplasm (for simplicity, we do not distinguish here between these two terms but use "cytosol" throughout), which comprises around 40% of the cell's volume in fully-expanded mesophyll cells but only about 1% of the cell's volume in the epidermis. Cytosol and vacuole are separated by one membrane, the tonoplast. Membranes cannot expand by more than 3% in surface area (Wolfe and Steponkus 1981) and, therefore, vacuole and cytosol must be iso-osmotic, their total osmotically active solute concentrations must match each other (if not, hydrostatic pressure differences would develop which would distort or even rupture the tonoplast). Fortunately—to a plant cell, not to those studying it! —requirements for particular solutes differ between vacuole and cytosol. Cytosolic solute concentrations have to be in tune with specific metabolic demands and maintained within a narrow range, whereas vacuolar solutes fulfil less specific functions and concentrations vary more. Leigh once termed this pointedly "the selective cytosol and the promiscuous vacuole" (Leigh and Wyn Jones 1986).

The best-studied solutes are K, in particular, and those ions (Na, Cl, heavy metals) that are linked to specific environmental stresses (e.g. salinity). Cytosolic K is crucial for many metabolic processes such as protein biosynthesis and enzyme activation. As a result cytoplasmic K concentrations are maintained at 60–80 mM whereas vacuolar concentrations can exceed 300 mM, particularly in the epidermis (Cuin et al. 2003; Fricke et al. 1996; Walker et al. 1996), or decrease close to zero (Fricke et al. 1996). The metabolic function of K in the cytosol cannot be replaced by any other solute, but its function as a major vacuolar osmolyte can be replaced by cations such as Na and Ca (Box and Schachtman 2000).

In leaf epidermal cells of barley, vacuolar Ca concentration exceeds cytosolic Ca concentration by a factor 10^5 to 10^6 (Fricke et al. 1995) and in salinized plants, vacuolar Na and Cl can exceed 500 mM, while cytosolic concentrations are below 100 mM. On the one hand, the vacuole serves as a buffer and exchange site of solutes for the cytosol; on the other hand, the vacuole represents the potentially largest hazard to the cytosol.

Cell elongation affects the cellular ratio of cytosol:vacuole. Vacuolar volume increases manifold, whereas cytosolic volume increases little or may not increase at all (root hairs). Leaf epidermal cells of grasses elongate to 50 times or more their original volume. A cell which commences elongation and has a cytosol : vacuole ratio of 1 : 1, may only increase vacuolar volume and finish with a ratio of 1 : 49 – 99—a ratio observed in mature epidermal cells (1–2% cytosol; 98–99% vacuole). A mature mesophyll cell contains about 40% cytosol. However, mesophyll cells are by factor 10 – 100 smaller than epidermal

cells (grasses), and the increase in total amount of cytosol per cell during elongation will be small. If it was not for the conflicting demands on solutes of vacuole and cytosol, a growing cell may not have to take up any solute for the cytosol. The real challenge for maintaining solute homeostasis in the cytosol during cell expansion is the vacuole. Being an infinitely larger osmotic sink which requires and stores solutes as it expands, the vacuole drains solutes from the cytosol and threatens to flood the cytosol with those solutes that are stored at much higher concentrations.

2.3 Vacuolar Solutes: Few and Heterogeneous

Plant cells accumulate a range of solutes in the vacuole to generate osmolality. Different tissues and different cell types within one tissue accumulate different solutes (Fricke et al. 1994b; Karley et al. 2000a; Leigh and Storey 1993; Leigh and Tomos 1993; Volkov et al. 2004). The best-studied example is the mature grass leaf and the distribution of vacuolar solutes between the two main tissues, epidermis and mesophyll (for information about bundle sheath, see Koroleva et al. 1997). Potassium, nitrate and Na are present at similar concentrations in the epidermis and mesophyll. Depending on plant nutrition, this distribution can change (Fricke et al. 1996). Calcium, at osmotically significant concentrations (> 5–10 mM), is found almost exclusively in the epidermis and is absent from the mesophyll; P distributes the opposite (Fricke et al. 1994a). Chloride concentrations are higher in the epidermis and increase in this tissue in particular in response to salinity (Fricke et al. 1996). Notably, the tissue distribution of vacuolar P and Ca between epidermis and mesophyll is opposite in leguminous species, possibly in dicotyledonous species in general (for a review, see Leigh and Tomos 1993; for the distribution in *Arabidopsis*, see Volkov et al. 2004).

The available data suggest that the epidermal vacuole uses predominantly inorganic ions (K, Na, Cl, Ca, nitrate) for the generation of osmolality, whereas the mesophyll vacuole uses a mixture of both, inorganic and organic (sugars, amino acids) solutes. There are some exceptions concerning absence of organic solutes from epidermal vacuoles. Within the barley leaf epidermis, cells closest to stomatal pores can accumulate large (> 100 mM) concentrations of malic acid. Within the epidermis of *Thellungiella*, a halophytic close relative of *Arabidopsis*, almost 400 mM of S accumulates in epidermal vacuoles (as determined by energy-dispersive X-ray analysis). This represents most likely 200 mM of glucosinolates.

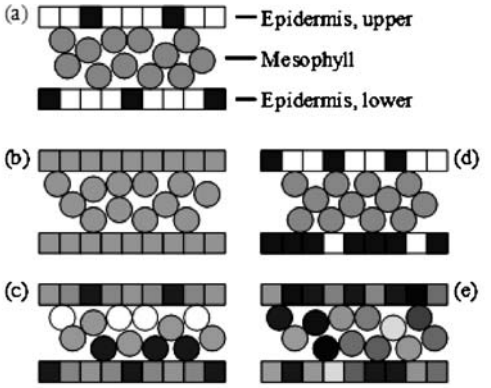
Vacuolar solute concentrations can differ also between cells within one tissue, in a non-random way (Fig. 1). In barley, solutes are distributed unevenly between the adaxial (upper) and abaxial (lower) epidermal layer and between different cell types, particularly within the anatomically more complex adaxial epidermis (Fricke et al. 1994c, 1995).

Fig. 1 **A–C** Compartmentation of solutes between leaf tissues. Differences in solute content or concentration are symbolized by different *shades of grey*. **A** Possible distribution patterns between the two major leaf tissues, epidermis and mesophyll: **a** Solutes are distributed differentially between mesophyll and epidermis. In the epidermis, solutes distribute between cells according to a reduced pattern; in the mesophyll, solutes distribute evenly. **b** Solutes distribute evenly between leaf tissues and there exist no differences in solute concentrations. **c** Solute distribution within the epidermis follows a complex pattern; similarly, within the mesophyll, solute concentrations differ between cells bordering the adaxial or abaxial epidermis or bordering only other mesophyll cells. **d** as in **a**, except that upper (adaxial) and lower (abaxial) epidermis differ in solute concentrations (see also **B**). **e** There are no systematic differences in solute concentrations between tissues and cells but solutes distribute randomly. **B** Nitrate and chloride distribute opposite between the adaxial and abaxial epidermal layer in fully expanded leaves of barley (Fricke et al. 1995). In the adaxial epidermis, nitrate concentrations increase with time (days) and level off at 200–250 mM, while Cl concentrations remain below 100 mM; in the abaxial epidermis, the distribution is opposite. The micrograph shows a cross-section of a barley leaf. **C** Solute concentrations differ in a systematic way between cells of the upper (adaxial) epidermis of barley leaves. The distribution appears to be related to the proximity of cells to stomatal pores or the top of ridges. The micrograph shows a surface view of a double-leaf replica of the upper epidermis of a mature barley leaf ►

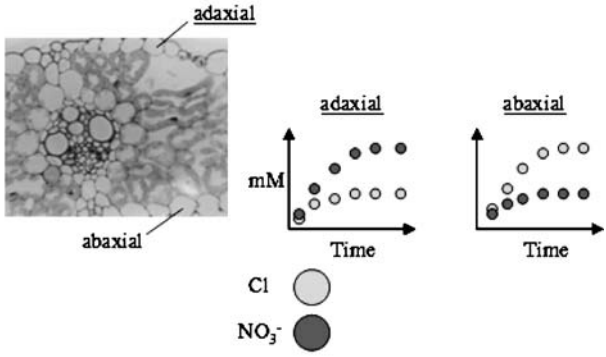
The above data were obtained for mature, transpiring leaf tissue. Growing leaf tissues compartmentalize solutes in a similar way. The main difference is an inability of growing tissue to accumulate large (> 50 mM) concentrations of Ca in epidermal cells (Fricke 2004b). In response to salt, both growing and non-growing tissues of the developing leaf three of barley accumulate Cl and Na, and loose K, but their K : Na ratios are affected differently (Fricke 2004a). Sugars, which are negligible in the epidermis, contribute less than 20% to bulk osmolality in the leaf elongation zone, but may have an important role during osmotic adjustment to salinity and drought in the mesophyll and bundle sheath (Barlow 1986; Delane et al. 1982; Hu and Schmidhalter 1998). When salt is added to growth media and growth stops transiently (for 20–30 minutes), solutes start to accumulate in a strict base-to-tip pattern, aiding first osmotic water uptake and growth in the basal leaf elongation zone before allowing mature tissue to recover turgor and adjust osmotically to the low-water potential environment (Fricke et al. 1994b).

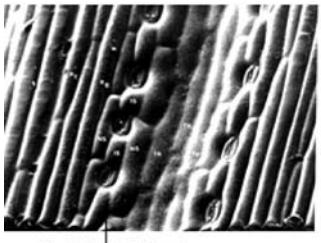
We do not know the molecular mechanisms through which a differential accumulation of solutes between tissues (epidermis versus mesophyll; growing versus mature tissues) is achieved. There exist two principle mechanisms: differential supply of solutes to tissues; or differential transport properties of tissues, at the plasma membrane or tonoplast (for a review, see Karley et al. 2000b; Leigh and Tomos 1993). In the case of Ca, the absence of a transpiration stream that passes radially through the grass leaf elongation zone (which is enclosed in sheaths of older leaves) may explain the abundance of epidermal Ca in emerged compared to growing tissue: Ca is carried with the transpiration stream to the site of evaporation, where it is left behind (see also

(A) Possible distribution patterns of solutes between tissues



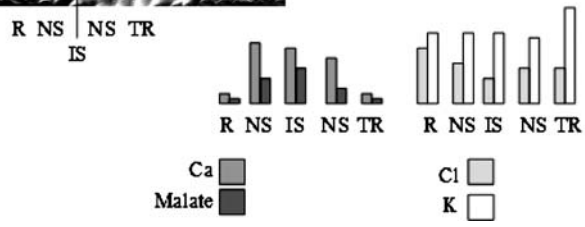
(B) Solutes are differentially distributed between adaxial and abaxial epidermal layer in barley





(C) Solutes are differentially distributed between epidermal cell types

(R, ridge; NS, near-stomatal; IS, inter-stomatal; TR, trough)



Storey and Leigh 2004). In contrast, Karley et al. (2000) using a patch-clamp approach, concluded that differences in Na accumulation between barley leaf epidermal and mesophyll protoplasts could be explained by differences in transport properties of Na at the plasma membrane.

2.4 Solute Transport: Plasma Membrane and Tonoplast

What determines concentrations of solutes in the vacuole, processes at the tonoplast or at the plasma membrane? The truth lies probably somewhere between. The tonoplast is the membrane delineating the vacuole and as such would be expected to exert a prime controlling function. The plasma membrane constitutes the boundary between protoplasm and apoplast and as such controls what enters and exits the cell. The driving force for movement of solutes is provided by metabolic energy stored as a pH gradient between two compartments (e.g. sucrose) and by the electrochemical gradient of a particular solute (e.g. K, Ca). Gradients in pH between cell compartments are established through activity of H^+-pumps (ATPases and pyrophosphatases; Hasegawa et al. 2000). The pH gradient along the apoplast-cytosol-vacuole path is mirror-image like. The apoplastic pH is 5 – 6 and 1 – 2 units smaller (H^+ concentration 10 – 100 times higher) than the cytosolic pH (pH 7.0 – 7.4), but similar to the vacuolar pH. Different mechanisms must operate for solutes such as sucrose to move across the plasma membrane into the cell (up-ward gradient in pH) and to move across the tonoplast from cytosol to vacuole (down-ward gradient in pH). For the movement of ionic solutes such as K and Ca, the membrane potential is paramount.

Membrane potential is a driving force for ion movement and regulates channel activity through voltage-gating. Membrane potential between apoplast and cytosol (more negative) is typically in the range – 120 to – 200 mV, but can be as little as – 70 mV in grass leaf and root cells (Carden et al. 2003; Cuin et al. 2003). Therefore, membrane potential is sufficient to accumulate K from an apoplastic few mM to close to 100 mM in the cytosol through facilitated diffusion through K-channels.

There exists a range of channels (Maser et al. 2001; Pilot et al. 2003; Very and Sentenac 2002), which could potentially function in facilitating K-uptake into growing leaf tissues. Probably the best characterized candidate channel involved in growth associated K-accumulation is ZmK1 from maize (*Zea mays*). ZmK1 operates at the plasma membrane, the cellular control point for entry and exit of solutes, and has been proposed to play a key role in growth-associated K uptake in the coleoptile epidermis of maize and in the auxin-mediated growth response to gravity (Bauer et al. 2000; Philippar et al. 1999). The coleoptile is not a true leaf but it is related to a leaf through its ontogeny. Furthermore, ZmK1 is inward-rectifying and allows uptake of K into cells. It has also high homology to AtAKT1, a K channel that has been charac-

terized in the model plant *Arabidopsis thaliana* and that plays a key role in the K nutrition of plants at low external concentrations of K (Hirsch et al. 1998) and accounts for 50% of K uptake currents in leaf mesophyll cells (Dennison et al. 2001). The outward-rectifying channel KCO may function in the retrieval of K from the vacuole of cells (van den Wijngaard et al. 2005). Together with K transporters, KCO would enable the vacuolar compartment, particularly in the epidermis, to buffer demands of the mesophyll and growing tissues.

In elongating cotton fiber cells, elongation is accompanied by an increased expression of sucrose and K transporters (Ruan et al. 2001). In maize leaves, different developmental zones show different surface fluxes of K, Ca and protons (Zivanovic et al. 2005), and light-stimulated growth of poplar depends on ion transport mechanisms, which are possibly specific to growing tissue (Stiles and Van Volkenburgh 2002). Stiles and Van Volkenburgh (Stiles et al. 2003) concluded that light-dependent uptake of K into growing tobacco leaf tissues is not so much required for increase in cell solute load and generation of osmotic force, but for extrusion of protons, which in turn aids cell expansion through modifying wall properties.

Different mechanisms must operate at the tonoplast. The electric potential difference between cytosol and vacuole is close to zero (Carden et al. 2003; Cuin et al. 2003). If K moved only by diffusion (through channels) across the tonoplast, it would not be possible to establish a concentration difference across the tonoplast, yet it has been shown that vacuolar K exceeds cytosolic K by more than 100 mM or is lower by 20–40 mM (Carden et al. 2003; Cuin et al. 2003). Transporters must be responsible for this differential accumulation of K. The most likely candidates are transporters belonging to the KUP/HAK/HKT family of K transporters (Maser et al. 2001; Vallejo et al. 2005; Santa-Maria et al. 1997; Schachtman 2000). Differences in HAK1 expression have been reported for root developmental zones in barley (Vallejo et al. 2005).

Transport of Na into the vacuole can be achieved through Na^+/H^+ antiporters of the NHX family (Xue et al. 2004; Zhu 2003). Loading of the vacuole with Na through NHX is a mechanism to cope with salinity and maintain cytosolic levels of Na low, but it is also potentially a way to provide the osmotic force for water uptake in environments with lower, non-toxic levels of Na.

3
Aquaporins

3.1
Aquaporins and Water Movement Through Cellular Membranes

As detailed above, cell expansion requires continuous uptake of water to maintain turgor pressure. This water movement is driven by a gradient of wa-

ter potential between cellular membranes established through accumulation of solutes. Water molecules move from cell to cell through the phospholipid bilayer by diffusion. However, the high water permeability found in most biological membranes, including the tonoplast, cannot be explained by purely diffusional processes but by the presence of water channels (aquaporins). Aquaporins represent an important selective pathway for water (and/or small neutral solutes) movement across cellular membranes and a large number of aquaporins has been found in plants (Chaumont et al. 2001; Johanson et al. 2001; Sakurai et al. 2005). This probably reflects the importance of aquaporins in maintaining sufficient water movement through membranes in physiological processes such as long-distance water transport from roots to leaves or cell osmoregulation (reviewed in Chaumont et al. 2005; Hachez et al. 2006a; Luu and Maurel 2005; Maurel et al. 2002; Tyerman et al. 2002).

Aquaporins that are present in the plasma membrane (PIPs) and tonoplast (TIPs) are likely to play essential roles in cell expansion. Vacuole biogenesis and enlargement require the transport of osmotically active substances across the tonoplast, followed by a rapid influx of water. Water transport measurements on tonoplast vesicles isolated from tobacco suspension cultures and wheat root cells (Maurel et al. 1997; Niemietz and Tyerman 1997) and on isolated vacuoles (Morillon and Lassalles 1999) showed generally a very high osmotic water permeability coefficient ($P_f > 200\ \mu m\ s^{-1}$) that was inhibited by mercury, an aquaporin inhibitor. These data support an aquaporin-mediated water movement across the tonoplast that probably plays an important role in cell expansion but also in water homeostasis (see below). In contrast, water permeability of the plasma membrane determined for isolated plasma membrane vesicles from tobacco suspension cultures and wheat root cells (Maurel et al. 1997; Niemietz and Tyerman 1997) or from swelling assay of protoplasts from different plant species (Chaumont et al. 2005) was on average much lower ($P_f < 30\ \mu m\ s^{-1}$) than permeability of the tonoplast, although high P_f values were obtained for some cell types and developmental stages (Chaumont et al. 2005; Maurel et al. 2002). It is possible that the comparatively low permeability of the plasma membrane results from experimental approaches. Plasma membrane permeability determined for intact cells within tissues may differ from that determined for isolated vesicles or protoplasts. For example, cell pressure probe measurements suggest that cell permeability values are higher than values of isolated protoplasts (Zhang and Tyerman 1999; Volkov et al. 2006). Despite this uncertainty, it appears justified to conclude that plasma membrane permeability represents the limiting factor in cell-to-cell water movement.

The difference in permeability between plasma membrane and tonoplast is essential for cell water homeostasis. During cell expansion, continuous uptake of solutes and water could perturb cytosol metabolism. Cytosol volume is small relative to vacuole and total cell volume. The volume and osmotic potential of the cytosol have to be rapidly equilibrated (buffered) in response to

changes in external osmotic potential, and this can be achieved best through a much higher water permeability of the tonoplast, making vacuole water readily available as cytosol water is lost or taking up water into the vacuole as cytosol water increases (Maurel et al. 2002; Tyerman et al. 2002).

3.2 Tonoplast Aquaporins and Cell Expansion

The first indication of aquaporin involvement in cell elongation came from the analysis of gene expression patterns in plant organs, tissues and cells (reviewed in Maurel et al. 2002). Using in situ hybridization and transcriptional fusion between the promoter of the *Arabidopsis thaliana TIP1;1* gene, encoding a tonoplast aquaporin, and β-glucuronidase gene, Ludevid et al. (1992) detected a high *AtTIP1;1* expression in root and stem elongating tissues. No expression was detected in the meristems (cell division zones) or older parts of organs. Interestingly, *AtTIP1;1* expression was shown to be up-regulated after application of gibberellic acid, a hormone promoting cell expansion, in *Arabidopsis ga1* dwarf mutant impaired in gibberellin synthesis (Phillips and Huttly 1994). The physiological role of *AtTIP1;1* was further investigated *in planta* by an RNA interference approach (Ma et al. 2004). Plants with down-regulated *AtTIP1;1* displayed pleiotropic phenotypes including a reduced growth of varying severity according to the silencing efficiency.

Maize tonoplast aquaporin ZmTIP1;1, a close homologue of AtTIP1;1, was highly expressed in expanding cells in roots, leaves and reproductive organs. Transcript levels were also abundant in dividing cells (Barrieu et al. 1998; Chaumont et al. 1998). Elevated expression of tonoplast aquaporins has been reported also for elongating tissues in hypocotyls of soybean, castor bean and radish seedlings (Eisenbarth and Weig 2005; Higuchi et al. 1998; Maeshima 1990; Suga et al. 2001), during cold-induced stalk elongation in tulip (Balk and de Boer 1999) and pea fruit growth (Ozga et al. 2002). *HvTIP1;1* transcripts were increased in the *slender* mutant of barley, which is characterized by a faster elongation rate of leaves compared to the wild-type (Schunmann and Ougham 1996). Together these studies demonstrate a positive correlation between tonoplast aquaporin expression and cell elongation and indicate that this process requires a high hydraulic permeability of the tonoplast to support water entry into the vacuole and guarantee cellular water homeostasis.

Aquaporin activity in the tonoplast appears to affect not only cell elongation but also final cell size. Cauliflower tonoplast aquaporin BobTIP1;1 was fused to the green fluorescent protein (GFP) and expressed in tobacco suspension cells (Reisen et al. 2003). BobTIP1;1-GFP fusion protein was still an active water channel and localized in the vacuolar membrane. The fusion protein did not affect the growth rate of cell suspensions but increased

the size and surface of cells two-fold, parallel to a swelling of the vacuole. It is possible that over-expression of BobTIP1;1 induced a concomitant solute transport which increased the osmotic gradient and water entry into the vacuole (Reisen et al. 2003).

3.3 Plasma Membrane Aquaporins and Cell Expansion

The involvement of plasma membrane aquaporins in the growth of cells in leaves and roots has been deduced from pressure probe and osmotic swelling experiments. Osmotic water permeability of elongating epidermal and mesophyll cells from barley leaf was 31 to 55% higher than that of non-expanding cells (Volkov et al. 2006). Interestingly, the increased P_f in epidermal elongating cells correlated with the expression of barley *HvPIP1;6* gene encoding an active plasma membrane water channel (Fricke et al. 2006). Treatment of maize roots with mercury chloride, an aquaporin inhibitor, reduced maize root elongation by around 75% as well as the hydraulic conductivity of growing cells in the distal region of the elongation zone (Hukin et al. 2002). Recently an extensive study of plasma membrane PIP gene and protein expression has been performed in maize roots grown aeroponically (Hachez et al. 2006b). Twelve of the 13 maize *PIP* genes identified (Chaumont et al. 2001) were expressed in primary roots. Expression was found to be dependent on the developmental stage of the root with an increase in expression towards either the elongation or mature zone (Hachez et al. 2006b). Aquaporins present in the plasma membrane of expanding root cells might have a dual function; they facilitate water entry into cells and maintain turgor pressure as the mechanical force driving wall expansion, and they participate in radial movement of water from soil to xylem vessels.

Although most PIPs are widely expressed in plant tissues, preferential expression in elongating tissues has been reported (Maurel et al. 2002). *Arabidopsis* PIP1;2 is expressed in expanding and differentiating cells comprising the root elongation zone, vascular bundle sheaths, filaments of stamen and young siliques (Kaldenhoff et al. 1995). Plasma membrane aquaporin expression has also been reported for the elongation zone of tobacco roots (Otto and Kaldenhoff 2000), castor bean and radish hypocotyls (Eisenbarth and Weig 2005; Suga et al. 2002), expanding cells of reproductive tissues (Bots et al. 2005; O'Brien et al. 2002), and for the elongation zone of barley leaves (Hollenbach and Dietz 1995; Fricke et al. 2006; Wei et al., 2006, personal communication). De-regulation of plasma membrane aquaporins by gene silencing, gene knock-out or over-expression leads to several phenotypes related to water relations but no exhaustive study on cell expansion has been conducted yet (reviewed in Hachez et al. 2006a).

There is accumulating evidence that aquaporin activity is regulated through many different post-transcriptional and post-translational mechan-

isms, and this provides another means through which cell expansion can be modified (Chaumont et al. 2005; Luu and Maurel 2005). Differential elongation of cells at the upper and lower side of gravitropically bending roots in pea was caused by a difference in the water-uptake rate and cell or tissue hydraulic conductivity rather than a difference in the driving force (Miyamoto et al. 2002, 2005). No significant difference in the levels of putative aquaporins between the upper and lower side of roots was observed using general aquaporin antibodies. The increased water conductivity measured in the (faster) elongating part of the root might have resulted from activation of pre-existing aquaporins, for example through aquaporin heteromerization (Fetter et al. 2004), phosphorylation (Johansson et al. 1998; Maurel et al. 1995; Weaver and Roberts 1991), deprotonation (Tournaire-Roux et al. 2003) and/or subcellular trafficking (Vera-Estrella et al. 2004). All these regulatory mechanisms have been extensively described in recent reviews (Chaumont et al. 2005; Luu and Maurel 2005).

4 Conclusions

Solute transport has received the least attention of the three main biophysical variables—walls, water and solutes—potentially limiting growth. This surprises given the importance of solute transport for osmotically driven water uptake by cells. In contrast to water, which accumulates in each cell and tissue in the same chemical form, solute composition and concentration differs between cells and tissues. Study of the role of solute transport in growth must take this heterogeneity into consideration through analyses at cell- and tissue level. The two basic ways through which solutes accumulate differentially between tissues or accumulate preferentially in growing compared to non-growing tissues (Fricke 2004a) are (i) differential supply of solutes to cells or (ii) differential transport properties of cells. In the first instance, we will have to focus on loading and un-loading of xylem and phloem; in the second instance, we need to focus on solute transporters and channels of growing cells.

To the best of our knowledge, there exists not a single study in which solute transport properties specific to growing leaf or root tissue has been studied at the molecular level. The best characterized system is the maize coleoptile, for which the shaker-type, inward-rectifying K-channel, ZmK1 has been shown to be involved in growth-associated uptake of K (Bauer et al. 2000; Philippar et al. 1999). Until we have further evidence for other tissues and organs, we can only speculate about candidate channels and transporters. Mechanisms are expected to differ between tonoplast and plasma membrane. At the plasma membrane, a significant trans-membrane electrical potential difference exists, and K could move into cells through either, active transport by transporters or facilitated diffusion through channels.

Channels can be voltage gated and show characteristic voltage-current relationships. Therefore, it is possible to predict based on electrophysiological analyses (patch-clamping) which channels are involved. Small (20–40 mV) changes in membrane potential can cause several-fold changes in K-uptake, and we need to obtain information on membrane potential in growing tissues and how it differs from that in non-growing tissue. At the tonoplast, where transmembrane potential between cytosolic and vacuolar compartments is close to zero, little driving force exists for (facilitated) diffusion through channels; active transport is required to accumulate vacuolar K above cytosolic K concentration. This transport is aided by the proton gradient across the tonoplast.

Expansion growth of cells must be matched by adequate rates of solute supply or solute uptake (Van Volkenburgh 1999), and either rate can become growth-limiting. This applies in particular to situations where environmental stress imposes extra demand on solute provision. For example, in barley exposed to high external NaCl (Fricke and Peters 2002), high salt overloads the capacity of epidermal cells to maintain osmolality during growth-dilution and to adjust osmotically to the large decrease in external water potential. The reduction in leaf cell expansion may not be so much a detrimental effect of salinity on the plant, but a mechanism through which the plant assures that solutes accumulate sufficiently and guarantee osmotic adjustment in an expanding cell. Similarly, Frensch (1997) concluded that growth in osmotically stressed maize roots is limited by solute supply. It appears that limitation of growth by solute supply occurs particularly in plants exposed to large decreases in external water potential, or in tissues that depend on a high rate of radial transport of phloem-borne solutes (discussed in Cosgrove 1993).

There is accumulating evidence that tonoplast and plasma membrane aquaporins mediate water uptake into growing cells and tissues. Tonoplast osmotic water permeability is by a factor 10 to 100 larger than plasma membrane osmotic water permeability. This assures that vacuole and cytosol are in osmotic equilibrium and suggests that the plasma membrane constitutes the main hydraulic barrier and is therefore the prime target for increasing growth and yield of plants through (genetic) modification of aquaporin activity. Several studies support a role of specific TIP or PIP isoforms in growth, based on expression profiles. “Hard” evidence that water channel activity of these isoforms is actually limiting water uptake into cells is scarce, particularly since the generally short half time of water exchange of plant cells questions the possibility that water transport limits cell and organ growth in the first place! Several technical approaches need to be combined to obtain unequivocal evidence. This will involve analyzing osmotic water permeability of cells and protoplasts/vesicles, monitoring expression levels and tissue localization of PIPs and TIPs, testing their post-translational regulation and, ultimately, testing the significance of altered expression levels of a candidate