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How Do Plants Enlarge? A Balancing Act

Cells of plants are surrounded by strong walls that prevent rupture from internal pressures that can be two or three times that of an automobile tire. In this way, the walls protect the cytoplasm. However, at the same time, the cells can enlarge as they grow. How this balancing act works and how it enlarges the plant were the subject of a recent conference at the University of Delaware in Lewes. The aim was to identify areas for future research that could explain the enlargement of whole plants. It followed a meeting in Tamarron, Colorado devoted mostly to the molecular aspects of enlargement, which was recently reviewed (Carpita et al., 1996). There is a large practical need to predict and modify plant enlargement but the additional processes that overlie the molecular ones need to be integrated with the molecular information before a picture will emerge. How best to accomplish this involved input from cross-disciplinary areas in biomechanics, physics and engineering as well as molecular biology, biochemistry and ultrastructure.

Enlargement is one of the most fundamental processes in plants. It increases the size of the plant by producing new cells and enlarging existing ones in localized regions, the meristems. The larger volume that results is caused mostly by an increased water content of the cells rather than an increased dry mass, and the increased water content can be dramatic. New cells about to divide again can double in size in a few hours, and others that do not re-divide can rapidly enlarge until they become 10 to 100 times their original size. The latter ones contribute most to plant growth and thus to the development of form in plant organs such as roots or flowers.

Without enlargement, plants cannot acquire nutrients or reproduce, and their presence in the next generation is diminished or prevented. The ability to enlarge is thus central to their success. However, this ability is often one of the first to fail when conditions become unfavorable. The earliest evidence of a deficiency in a mineral nutrient is decreased growth, and water deficits decrease shoot growth before any visible symptoms occur such as wilting. The failure to grow is thus a critical but subtle event.

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Cellular Processes

In this setting, the cell wall must balance between preventing cell rupture and enlarging the compartment of the cell. The wall is made up of cellulose microfibrils embedded in a matrix of polysaccharides and proteins, and the constraining effects are thought to be controlled mostly by the microfibrils acting as reinforcement while the enlarging activity seems to be mostly caused by events in the matrix. Pressure in the cells puts these polymers under tension, and enlargement would involve slippage or cutting of some of the matrix molecules. As one of the participants, John Passioura, put it, the wall appears to be held together by molecular tethers continuously being cut, but disaster may be averted by adding new tethers.

Many of our concepts of how these balances are achieved began with plants having large cells, particularly the charophyte algae. These plants contain cells large enough to study individually and are the closest marine progenitors of land plants. Experiments were done by Probine and Preston (1962) with extension in isolated cell walls and by Green et al. (1971) with pressure in living cells. Richmond and coworkers studied load-bearing in the walls (see review by Taiz, Metraux and Richmond, 1981). The experiments showed that the walls are deformable by turgor pressure and determine the rate of enlargement. At the cell level, the wall is thus the focus of the growth process. Lockhart (1965a,b) noted the similarity between polymer deformation and wall deformation, and suggested a simple extensibility equation $R = m(P-Y)$ to describe growth. R is the rate of growth measured as an increase in size, P is the turgor pressure, Y is the minimum turgor for growth, and m is the wall extensibility, that is, the coefficient describing the ability of the wall to be physically and irreversibly deformed by turgor. In this formulation, growth is controlled by the physical properties of the wall (m and Y). Elastic effects are not included and there is no provision for synthesis of new cell wall, as Lockhart (1965a, b) pointed out. The equation is limited to steady conditions in single cells and cannot be directly applied to rapid changes in cell dimensions or to multicellular tissues. Lockhart (1965a, b) also included an analysis of water uptake, but most subsequent studies use only the extensibility equation because water transport is often considered nonlimiting (Taiz, 1984; Cosgrove, 1993).

Passioura and Fry (1992) and Passioura (1994) extended this model to include dynamic aspects of "wall loosening". They considered the tension on individual macromolecules, which placed the model within the context of the molecular architecture of the wall. During the workshop, Passioura pointed out that the model is based on a balance between the cutting rate of macromolecules such as xyloglucans acting as tethers between cellulose microfibrils, and the recruitment of new macromolecules. A prediction of the model is that when the balance is disturbed by increasing the turgor, there should be a transiently increased growth rate that would return to the original rate within a few minutes as a new balance occurs. Examples of this were given in leaves of grasses. The model is attractive for its molecular description of m and Y and its ability to account for transients in growth.

McCann and Roberts (1994) related polymer orientation to wall enlargement and McCann reported at the workshop that despite a thickness of only 3-4 microfibrils in some primary walls, the wall is not a uniform structure. Individual cells have a layer of pectins on their outer surface and epidermal cell walls contain cutins where the wall is on the outside of the organ. There often is specialized molecular deposition where the walls of adjacent cells meet intercellular spaces, and the orientation of macromolecules varies with position in the wall and with development. Orientation can be detected with polarized infrared analysis of the wall macromolecules but the exact location of proteins is not yet clear nor is the migration mechanism known for components such as pectins in special wall locations. Analysis of enlargement mutants showed that many involved the pectin component of the walls, and treating with auxin caused increased expression of pectate lyase suggesting that pectins contribute to the regulation of growth rate.

Carpita related findings that there are stable and changing wall polysaccharides in the walls of expanding cells, and he highlighted the finite lifetime of many polymers in the wall, expanding on earlier work (Carpita and Gibeaut, 1993). In dicotyledonous species, xyloglucans are major constituents of the wall matrix. In grasses, arabinoxylans are present and only small amounts of the xyloglucans. During enlargement, mixed linkage glucans ((1→3), (1→4) β-D- glucan) appear in grasses in the matrix polysaccharides and disappear after enlargement is completed. The xyloglucans and arabinoglucans likely have a hydrogen bonding role with cellulose, and the mixed linkage glucans may play a specific

role in enlargement. It seems that the breaking and re-bonding of hydrogen bonds together with the cleavage and reattachment of the covalent links in the matrix glycans can be controlled with enzymes such as xyloglucan endotransglycosylase (Fry et al., 1992; Nishitani and Tominaga, 1992, Nishitani, 1995) or possibly by proteins like expansin (McQueen-Mason and Cosgrove, 1995). This control may be some of the molecular manifestation of the Passioura and Fry (1992) model. Also, pectin carboxyl groups appear blocked by esterification during enlargement but become de-esterified during maturation, which allows cross-bridging of the carboxyl groups by calcium ions. Evidence was presented that inadequate water availability can inhibit the synthesis of specific wall constituents (Sweet et al., 1990; Iraki, et al. 1989).

Braam reported that mechanical stimulation of plants inhibited growth and she tested for mRNA expression that could explain the inhibition. Touch-sensitive mRNAs appeared within 0.5h and were degraded within a few hours. Many of these were also expressed after exposure to dark or temperature shocks (Braam and Davis, 1990; Polisensky and Braam, 1996). However, these stimuli may act through distinct cis-regulatory elements. One of the genes encodes a xyloglucan endotransglycosylase that is upregulated by auxin. Others encode calcium-sensitive calmodulin-like proteins found in expanding cells, the epidermis, or in guard cells. There is a need to identify plants with defective TCH genes so that the physiological roles of the TCH gene products can be assessed. It would be predicted that the TCH genes may function during plant development and in plant responses to environmental stimuli.

Richmond had earlier used large-celled algae to show that the increased area of the growing wall is balanced by the biosynthesis of new wall that maintains wall thickness (Taiz, Metraux and Richmond, 1981). At the workshop, he showed that much of the synthesis occurs at the inner layer of the wall, and there is evidence that the primary load-bearing region is near the inner surface although there was debate about how much load also was borne by the outer layers. In the inner surface, microfibrils of cellulose are deposited in a more or less transverse direction relative to the long axis of the cells. As elongation progresses, there is realignment of the microfibrils that can be followed *in vivo* using polarization techniques, and the microfibrils tend to become increasingly parallel to the long axis. If

cellulose deposition is prevented with inhibitors, the cells burst emphasizing the need for cellulose to reinforce the wall matrix.

Baskin described his work concerned with the relation between expansion in length and radius, sometimes called growth anisotropy (Baskin et al., 1994; Baskin and Bivens, 1995). He presented unpublished data for maize roots showing that alignments of cortical microtubules and cellulose microfibrils are perpendicular to the axis of major expansion but do not appear to specify the observed different rates of radial expansion. As a result, he argued that the same types of biochemical processes usually considered only for elongation in fact regulate wall extensibility differentially in orthogonal directions.

Processes Between Cells

As cells combine to form multicellular organs, water for growth must be transported among many cells that cause increased resistance to flow. Here, the value of cross-disciplinary approaches was particularly apparent. The usual concept that growth is a physical extension of the wall by turgor implies that growth will increase as turgor becomes higher. However, because turgor is generated by the strength of the wall, it is also clear that as the wall enlarges, the turgor necessarily decreases. The lower turgor lowers the cell water potential and creates the force for water to enter. A balance develops between the need for turgor to be above a threshold but low enough to bring water into the cell. Viewing growth simply as a wall extension by turgor would overlook the turgor lowering that gives the most rapid growth. There was discussion about whether this lowering could be large enough to indicate that water transport limits rates of growth. Matthews provided an example where water uptake appeared nonlimiting in leaves (Serpe and Matthews, 1994) but Boyer showed that uptake could limit leaf or stem growth (Tang and Boyer, 1996; Boyer et al., 1985), and Ortega emphasized the need to augment the wall equation with an equation for water uptake (Ortega, 1990).

Because most of the increase in cell size is caused by increased water content, attention was given to how water enters growing regions. Boyer reported that water potential gradients exist in growing regions and correlate with leaf growth in maize. Pressurizing roots to reestablish the gradients can fully

return growth to high rates in dehydrated soils. Silk showed that many of the vessels are inactive in water transport in the growing regions of sorghum leaves. There were surprisingly large distances between the active vessels which would cause water to be more impeded that previously thought as it moved into the growing cells between the vessels.

The maintenance of wall thickness during cell growth implies that solute for wall biosynthesis is an important part of the growth process. Without a supply of solute, there would be depletion and dilution of existing solute pools and decreases in the osmotic force for water uptake and turgor development. Silk emphasized that growth zones are the strongest sinks for most solutes (Sharp et al., 1990). As a consequence, the root growth zone probably affects the chemistry of the rhizosphere more than do the mature parts of the root system that are less metabolically active. Nutrient delivery to the enlarging cells takes place without a well-developed vascular network, and the mechanism is not well understood (Bret-Harte and Silk, 1994). Work has shown that delivery generally balances use (McNeil, 1976; Schmalstig and Cosgrove, 1990; Meyer and Boyer, 1981), but the balance may be disturbed particularly by changes in water availability (Munns et al., 1979; Meyer and Boyer, 1981). Growth decreases first but this is followed by an imbalance between solute use and uptake (Munns et al., 1979; Meyer and Boyer, 1981), and solute accumulates.

Cramer investigated the transport of solute in salinized plants and reported that salinity induced decreases in leaf growth (Cramer and Bowman, 1991) that can be entirely reversed by pressurizing the roots in maize. This suggested that salinity acted mostly osmotically perhaps by altering tensions in the shoot. However, polymers were secreted more slowly in salt-treated plants than in controls, and this inhibition could be simulated with inhibitors of Golgi secretion. In consequence, effects on secretion may be important for the balance between oligosaccharide transport and oligosaccharide incorporation into the wall.

Coordination

There are signals that coordinate the development of various plant parts and thus the rates of enlargement of various plant parts and these were explored during the workshop. Sharp addressed the changes in partitioning between roots and shoots that occurs during water deficits. Roots are less inhibited in their growth than shoots, and the use of ABA mutants and inhibitors showed that this differential development is likely to be under the control of ABA (Saab et al., 1990). Importantly, the results indicated that water deficits alter the responsiveness of growing tissues to ABA (Sharp et al., 1994). Subsequent work with ethylene mutants and inhibitors implicates an interaction of ABA with ethylene biosynthesis in the partitioning response. Holbrook reported grafting experiments with mutants that further exemplified how mutants can be powerful tools for investigating growth regulation. She showed that ABA-deficient roots when grafted to wildtype shoots showed no difference in stomatal behavior from the wildtype in tomato as the soil dehydrated. This indicates that ABA is unlikely to be the main form of root-shoot communication in plants exposed to dehydrating soil, but for the grafting experiments there is a need to determine the ABA content to ensure that the grafts did not alter mutant expression of ABA.

Conclusions

The participants spent the last hours of the meeting trying to systematize their knowledge and identify over-arching concepts that need more investigation. Passioura, Matthews, and Silk pointed out that our work looks at different time scales and spatial-temporal relationships, and processes that dominate behavior at one scale might be much less important at another. An example is the rapid variation in growth on a minute by minute timescale that averages to give the final size of the plant. in addition to these differences in scale, there are perturbation experiments that look at the sequence of events following the imposition of a treatment, descriptions of gradients in the wall and between tissues, and dynamic experiments involving temperature, pressure and growth regulators. Several of these experimental systems would benefit from mutants to perturb the models and dynamic tests to determine rate-limiting steps. A conceptual framework is needed to account for these diverse issues, and Ortega

argued that the framework should be built on first principles in order to identify mechanisms underlying the growth process.

It was generally agreed that the wall cannot be viewed simply as a passive plastic material. Synthesis and deposition take place and cause changes in the molecular architecture of the wall. Enzymes are present that could participate not only in synthesis but also in slippage and reattachment of wall polymers. However, a threshold turgor generally exists below which there is no enlargement much as in some plastic materials. Has wall synthesis stopped when turgor is below the threshold? Have wall enzymes lost their activity? Are the cross-links and bonding forces in the wall no longer the same? Some enlargement features must be affected by turgor that are not now known and that apparently are rate-limiting or necessary steps in the growth process.

Additional questions arise from the fact that the wall is not a constant or uniform structure but undergoes highly organized changes during enlargement while bearing a considerable load. How do surface polymers like pectins make their way to the surface through the intervening matrix? Does insertion of polymers in existing wall (intussusception) contribute to enlargement? What is the exact role of microtubule/microfibril orientation? Is there a relation between wall pore diameters and the insertion of polymers, especially cellulose microfibrils whose diameter is on the same order as the pore diameters?

Recent advances in signaling, biochemical analysis and ultrastructure visualization are beginning to relate to the molecular load-bearing and enzymatic activities in the wall. Clearly a balance occurs between loosening and load-bearing in the walls, and scaling up to the whole plant raises new issues, especially how multicellularity affects processes needed for each cell. How can results with single cells be applied to multicellular plants where many cells compete for water and solute? Because the load for an organ may be borne by surface tissues more than by individual cells within the tissue, where are the most appropriate sites controlling enlargement?

The participants agreed that there probably is enough information to begin developing a comprehensive model that would balance wall effects with the limitation of growth by transport, especially for water, and this could help clarify events occurring at different time scales and places.

Beyond that, there seems to be a need to resolve problems of solute transport and wall behavior that are poorly understood in growing regions, leaving many promising areas for future experiments. Understanding each balancing act seems to be just the beginning.

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