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Review

Wall Relaxation and the Driving Forces for Cell Expansive Growth

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ABSTRACT

When water uptake by growing cells is prevented, the turgor pressure and the tensile stress in the cell wall are reduced by continued wall loosening. This process, termed *in vivo* stress relaxation, provides a new way to study the dynamics of wall loosening and to measure the wall yield threshold and the physiological wall extensibility. Stress relaxation experiments indicate that wall stress supplies the mechanical driving force for wall yielding. Cell expansion also requires water absorption. The driving force for water uptake during growth is created by wall relaxation, which lowers the water potential of the expanding cells. New techniques for measuring this driving force show that it is smaller than believed previously; in elongating stems it is only 0.3 to 0.5 bar. This means that the hydraulic resistance of the water transport pathway is small and that rate of cell expansion is controlled primarily by wall loosening and yielding.

The expansive growth of plant cells can be viewed as a 'push-pull' system. The protoplast pushes against the cell wall and the wall yields, thereby reducing the pressure within the cell and enabling the cell to pull water in from its surroundings. Prolonged water uptake by expanding cells is unusual because nongrowing cells ordinarily equilibrate with the surrounding water potential in just a few seconds or minutes and thereafter cease net water uptake. Nevertheless, during growth plant cells absorb water for many hours. This comes about because the cell continuously weakens its wall, relaxing the mechanical restraint of the protoplast, thereby reducing cell turgor pressure and maintaining the low internal water potential necessary for water uptake.

This brief review is about the connection between water absorption and wall yielding, and the forces driving these two distinct but interdependent processes essential for growth. Discussion is restricted to the results of recent studies on this topic; many of the basic concepts originated in older works which are referenced extensively in recent reviews (3, 13).

YIELDING, CREEP, AND STRESS RELAXATION OF THE CELL WALL

As a mechanical structure (Fig. 1), the growing wall consists of elastic elements in series with inelastic or plastic elements. The elastic elements are polymers or polymer networks which return to their original shape when tension is relieved whereas plastic elements shear or distend irreversibly when placed in tension. Both elements bear the mechanical wall stress produced by cell turgor pressure.

Growth starts when wall yielding reduces the wall stress. This process may be visualized as a lengthening of the plastic elements and a simultaneous contraction of the elastic elements in the wall (point 2a in Fig. 1). The contraction of the elastic elements means that wall stress and turgor pressure have decreased. Note that the wall dimensions have remained constant up to this point, even though the plastic elements have expanded.[1] The wall can expand only when the cell absorbs water, and this starts only after the elastic elements contract, and thereby reduce wall stress and cell turgor pressure. As a consequence, cell water potential is reduced, a water influx ensues, and the cell enlarges. At steady state, wall stress and turgor pressure are maintained constant, despite continued wall yielding, by water absorption. Thus, further stress relaxation is not observed; instead the wall creeps.[2]

In recent studies wall relaxation has been measured in living tissues by blocking cell expansion without directly interfering with wall loosening. This was done either (a) by isolating the growing tissue from an external water supply and thereby preventing water uptake (1, 2, 5) or (b) by using a pressure chamber to apply just sufficient pressure to prevent the growing tissue from taking up water (4). When cell size is held constant, wall yielding induces a gradual reduction in wall stress and turgor pressure. Up to now, *in vivo* relaxation has been measured by monitoring turgor pressure, or some property which correlates with turgor pressure, as an indication of wall relaxation. This is valid so long as evaporation and other processes which may affect turgor are insignificant.

In vivo stress relaxation, as described above, differs in two important ways from the older technique of stress relaxation carried out with isolated (dead) wall specimens. First, wall stress is generated by cell turgor, not by an external force, and so the stress pattern is the native, multiaxial pattern. Second, because living tissues are metabolically active, *in vivo* stress relaxation can measure the physiological and time-varying aspects of wall loosening. Hence, this technique measures more than the simple rheological properties of the wall.

Usually, wall growth properties are described in terms of a 'physiological wall extensibility' (ϕ) and a yield threshold (Y),

[1] Actually there will be a tiny expansion due to the very slight compressibility of water.

[2] A note about terminology: wall loosening denotes the breakage of load-bearing bonds in the wall; wall yielding is the irreversible extension or shearing of plastic elements in the wall; wall creep is the physical change in wall dimensions at constant wall stress; wall relaxation is the reduction in wall stress at constant cell wall dimensions. Thus wall loosening gives rise to wall yielding, which in turn gives rise either to wall relaxation or to wall creep, the latter occurring when the cell is able to absorb water and expand.

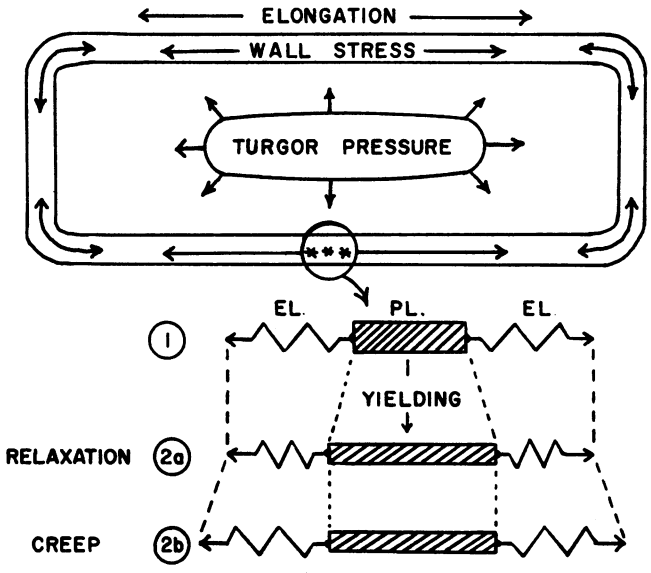

FIG. 1. Mechanical model of the growing cell wall. Turgor pressure exerts a force against the wall and sets up a tensile stress in the plane of the wall. Point 1 shows that both the elastic (EL) and plastic (PL) elements bear the wall stress. The elastic elements are shown as springs in which extension is proportional to stress. The transition from point 1 to point 2a involves yielding of the plastic elements without net change in wall length. The contraction of the springs means wall stress has relaxed. In point 2b the wall stress is maintained constant, so the wall expands to the same extent that the plastic elements lengthen.

which are defined by a functional relationship between growth rate (r) and turgor pressure (P):

$$r = \phi(P - Y) \quad (1)$$

(units: P and Y in bar; ϕ in $bar^{-1}\ h^{-1}$; r in h^{-1}). Note that the theory behind this equation assumes that the wall yields at a rate which depends linearly on the wall stress in excess of a minimum yield stress (see Refs. 3 and 13 for review). However, wall stress is a complex function of cell geometry and wall thickness and is not easily measured, so instead wall properties are expressed in equivalent terms of turgor pressure (P) and the minimum turgor needed for growth (Y), which are more readily measured. It should be kept in mind, nevertheless, that P in equation 1 is the cause of and a substitute for the unmeasured wall stress.

The traditional evidence cited in favor of equation 1 has been experiments in which turgor pressure and wall stress are reduced by osmotica or by dehydration and growth is found to decrease in corresponding fashion (6, 9). Typically, growth ceases when turgor is reduced to a value still well above zero. This minimum or critical turgor needed for growth is termed the yield threshold, and is generally in the range of 2 to 4 bars. Sometimes growth-*versus*-turgor curves are curvilinear near the yield threshold, and so an exact value for Y is difficult to establish (9). This deviation from ideal behavior may arise (a) because the growth process does not conform exactly to equation 1, (b) because of changes with time in the parameters of equation 1, or (c) because of variations among cells in turgor pressure and wall properties. In these cases, the concept of a yield threshold is only an approximation, though still useful.

The results of *in vivo* stress relaxations confirm the existence of a yield threshold. In elongating stems of several species and in expanding bean leaves, wall relaxation ceased when turgor pressure was reduced to 1 to 3 bars (2, 4, 14). Thus, the concept of a yield threshold for growth seems well established by independent methods.

Is the rate of wall expansion linearly proportional to turgor above the yield threshold? Here the growth behavior of plants is more variable. In some cases equation 1 represents plant behavior exceptionally well, whereas in other cases the expansion rate appears to reach a plateau at high turgor pressures (reviewed in Cosgrove [2]). In instances in which the growth rate remains constant despite alterations in turgor pressure, it is likely that the wall yielding properties adjust to compensate for the altered turgor. This phenomenon of apparent growth regulation has been suggested in numerous studies, but remains poorly characterized.

In vivo stress relaxation provides new support for the concept that wall stress drives wall yielding. The rate of *in vivo* relaxation at any moment is given by:

$$dP/dt = -\epsilon\phi(P - Y) \quad (2)$$

where ϵ is the volumetric elastic modulus of the growing tissue of the cell (2, 11). This equation, like equation 1, assumes that wall stress in excess of the yield threshold is the driving force for wall yielding. Equation 2 indicates that the rate of turgor relaxation depends on the value of turgor pressure, so that as relaxation proceeds and turgor declines, the rate of further relaxation should proceed more slowly. Assuming that ϵ, ϕ, and Y remain constant, turgor pressure should decay exponentially to the yield threshold, with a time course given by:

$$P(t) = Y + (P_0 - Y)e^{-\phi\epsilon t} \quad (3)$$

where $P(t)$ is turgor at time t and P_0 is turgor pressure at the start of relaxation.

In stems and in leaves undergoing relaxation, turgor pressure declined towards an asymptotic value with a time course approximating an exponential decay (2, 5, 14), as predicted by equation 3. Thus, the kinetics of relaxation generally support the view that wall yielding is a function of wall stress.

In most experiments which characterize wall expansion in terms of P, both turgor pressure and water potential are altered, so one might argue that reduced water potential, not reduced turgor pressure or wall stress, causes slower wall yielding (8, 15). The results of two experiments indicate that wall stress and turgor pressure govern wall yielding, not water potential per se. When water was withheld from freshly excised growing regions of the pea epicotyl, turgor was found to decrease to 3 bars, corresponding to a water potential of −5 bars (5). When the segments were preincubated on water, their cell sap became more dilute, partly by solute leakage and partly by water uptake. Nevertheless, when the tissue underwent relaxation, turgor pressure again fell to 3 bars, which corresponded to a water potential of only −3 bars. Thus, wall yielding appeared to depend on turgor pressure, rather than water potential.

In a different type of experiment, relaxation was induced by holding cell size constant by means of a hydrostatic pressure applied to the growing tissue, that is, by the new pressure-block technique (4). With this technique, wall stress and turgor pressure fall during the course of relaxation, yet water potential remains high because of the applied pressure. Under these conditions the amount of relaxation is the same as with the technique in which both turgor and water potential fall. Again, this result supports the view that turgor pressure creates the wall stress which drives wall yielding, and that water potential, per se, is not directly involved.

Finally, it is worth noting that wall properties may sometimes be more dynamic than assumed for equation 3. For example, in stems undergoing relaxation by the pressure-block technique, the rate of relaxation was observed to increase 5 to 10 min after the start of the procedure (4). This enhanced relaxation rate is not predicted by equation 3 and appears to be a response to the blockage of cell expansion. This result implies that relaxation procedures may sometimes induce an alteration in the rate of

wall loosening. The mechanism for this alteration and the relation between the dynamic wall properties and the steady state wall properties remain to be explored, but such studies hold great promise for our understanding of cell expansion.

DRIVING FORCES FOR WATER UPTAKE

For single cells, water uptake depends simply on the water potential difference across the plasma membrane. This difference can be miniscule—as little as 0.003 bar—and still sustain the water influx needed for growth (3). In multicellular tissues water uptake is more complicated because water must be transported across multiple cell layers and because water may move through the cell wall as well as cell-to-cell. In an elongating stem, water must be transported radially from the xylem to the epidermis. This long and complex path offers a greater hydraulic resistance than that of a single plasma membrane, and requires a greater driving force to supply water to the expanding cells (Fig. 2).

The motive force for water uptake into expanding tissue arises because wall yielding tends to reduce cell turgor pressure and water potential. As a result, water is taken up from the apoplast, and this removal of water creates a tension or a negative pressure in the apoplast. The archaic term 'suction force' imparts an intuitive feeling for this phenomenon. Apoplast pressure and protoplast water potential become more negative as a function of distance from the xylem (Fig. 2). The full gradient, from xylem to epidermis, constitutes the effective force drawing water into the tissue. I will refer to this driving force as $\Delta\Psi_e$, the water potential difference supporting cell expansion.

An important point to note is that the driving force for water movement within the apoplast is not a water potential difference, but $(\Delta P - \sigma\Delta\pi)$. Because the reflection coefficient (σ) of the wall is close to zero, an osmotic pressure difference $(\Delta\pi)$ within the apoplast is not effective for water transport (3, 12); water moves primarily in response to a pressure difference (ΔP). I point this out because expanding tissues contain apoplastic solutes which lower the tissue water potential. My definition of $\Delta\Psi_e$ specifically excludes the effects of such apoplastic solutes because (a) they do not arise from wall yielding and (b) they are ineffective for water transport (3).

Another point worth emphasizing is that $\Delta\Psi_e$ originates from and is maintained by wall yielding. If wall yielding were to cease, $\Delta\Psi_e$ would quickly dissipate and water uptake would cease. Nevertheless, the tissue water potential could remain below zero

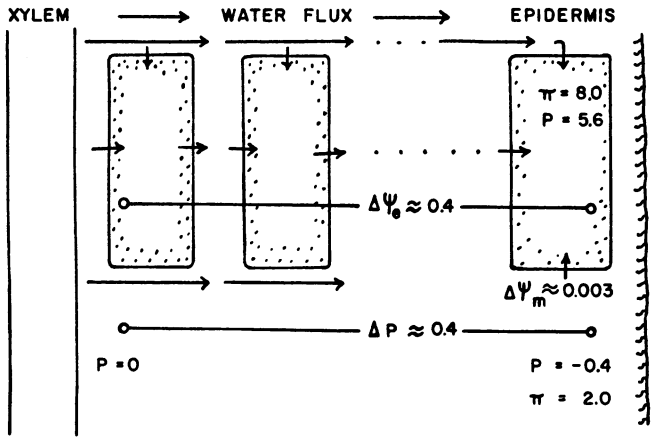

FIG. 2. Radial water transport in an elongating stem, with representative values (in bars) for the driving forces in the pea epicotyl. Water moves both through the wall (long arrows) and cell to cell (short arrows). The water potential difference across a single plasma membrane, $\Delta\Psi_m$, is small compared with the total driving force between the xylem and the epidermis, $\Delta\Psi_e$. Assuming the reflection coefficient of the wall to be zero, $\Delta\Psi_e$ is equal to the negative pressure gradient in the wall, ΔP.

if solutes were maintained in the apoplast (3). Boyer and coworkers (1, 15) have argued that it is possible to collapse $\Delta\Psi_e$ and thereby to inhibit growth, by reducing the xylem water potential. However, this treatment by itself would only momentarily stop growth. If the rate of wall yielding were unaffected, then $\Delta\Psi_e$ would soon be reestablished, albeit at a lower water potential, and water uptake would resume at the previous rate. Hence, the interesting point should be whether and how low water potentials reduce wall yielding. As discussed above, a major mechanism appears to be through reduction of wall stress (turgor pressure). Other mechanisms may involve long-term modification of the rate of wall loosening (6, 8, 9), but this topic is beyond the scope of this limited review.

The magnitude of $\Delta\Psi_e$ has attracted some interest, in part because it provides a way of assessing whether the hydraulic resistance of the water pathway is great enough to limit the cell expansion rate. In a tissue with high resistance, a large $\Delta\Psi_e$ is needed to sustain water uptake, but a large $\Delta\Psi_e$ reduces turgor pressure and hence the rate of wall yielding (by equation 1). When $\Delta\Psi_e$ exceeds $(P-Y)$, growth is primarily restricted by water transport. Note that this argument depends on wall yielding being proportional to turgor pressure, which decreases as $\Delta\Psi_e$ increases.

There is some disagreement in recent studies about the value of $\Delta\Psi_e$ and whether it is large enough to diminish wall yielding. Using a thermocouple psychrometer, Boyer *et al.* (1) measured the water potential of the growing region of intact soybean hypocotyls to be about −2 bars. This measurement includes the effects both of apoplastic solutes and $\Delta\Psi_e$. By assuming that apoplastic solutes were negligible, they equated the low water potential with $\Delta\Psi_e$, and concluded that the resistance to water flow (between the xylem and epidermis) restricted the growth rate by about 50%. Previous work, however, showed that wall solution extracted from growing soybean hypocotyls had an osmotic pressure of at least 1.6 bar (reviewed in Cosgrove [3]) and raises doubt about the interpretation by Boyer *et al.* (1). Other work with growing pea epicotyls concluded that $\Delta\Psi_e$ was small, about 0.4 bar, but that apoplastic solutes reduced tissue water potential to about −2.5 bar (2, 3).

Two pressure-chamber methods have recently been used to measure $\Delta\Psi_e$ without the complications arising from apoplastic solutes and wall relaxation (4). In the pressure-block method, a position transducer was mounted inside a pressure chamber and was used to monitor stem length. The applied pressure which was just sufficient to block growth—before relaxation took place—was taken as a measure of $\Delta\Psi_e$. It was 0.4 bar in peas and 0.35 bar in soybean. In cucumber and zucchini seedlings $\Delta\Psi_e$ was about 0.5 bar. In another technique, the growing region of a soybean hypocotyl was sealed in a pressure chamber, excised, then immediately cooled to about 8°C to inhibit further relaxation. The balance pressure (0.3 bar) was taken as an estimate of $\Delta\Psi_e$. The results from both of these methods indicate that $\Delta\Psi_e$ is less than 0.5 bar in these tissues. This is small compared with the value of $(P-Y)$, which is typically in the range of 2 to 3 bars. Calculations indicate that the resistance to water flow restricts the rate of cell expansion rate only by about 10% in these tissues (3, 4).

Katou and Furumoto (7) have added a new aspect to the discussion of water transport during growth. They proposed that water uptake is enhanced by solute pumps located in cells immediately surrounding the xylem. These plasma-membrane pumps are hypothesized to remove solutes from the apoplast and thereby raise the water potential of the apoplast. The result, according to their model, is enhanced water uptake, higher turgor pressure, and consequently faster wall expansion. Katou and Furumoto propose that this mechanism of respiration-dependent water uptake may be responsible for a large fraction of the growth

stimulation by auxin. The experiments of Okamoto *et al.* (10) were cited as evidence. They found that after *Vigna* hypocotyls were perfused with 200 mM sorbitol, shrinkage could be induced by anaerobic conditions and halted by aeration. They proposed that aeration maintains respiration-dependent water uptake by the xylem pumps. Although this evidence is consistent with the xylem-pump model, it is also consistent with the trivial view that oxygen starvation simply allows passive solute leakage by all the cells in the hypocotyl. Thus, a convincing test of the model remains to be made. In the longer term, where cell volume more than doubles, solute uptake or production is certainly required to maintain turgor in the face of dilution, but how this is coordinated with cell expansion is not well understood.

FINAL COMMENTS

In the work discussed here, turgor pressure is generally conceived as the driving force for wall yielding and extension. However, with the possible exception of water stress, there do not appear to be well established cases in which a growth response is mediated directly by a change in turgor pressure (although this has been proposed). Thus, turgor pressure is appropriately termed a passive driving force for growth in the limited sense that it provides the mechanical force needed for wall yielding, but the regulatory aspects of cell growth appear to be controlled (in a looser sense of the word, 'driven') through changes in the cell wall properties.

The coupling of water uptake to wall yielding is an essential aspect of growth because the protoplast is incompressible and consists mostly of water. Wall relaxation creates the initial driving force for water uptake by reducing cell turgor pressure. Unfortunately, many techniques used to measure tissue water potential do not block wall relaxation after tissue excision, and so are prone to measure artefactually low water potentials. When relaxation and apoplastic solutes are taken into consideration, $\Delta\Psi_e$ is shown to be so small that water transport restricts cell expansion by only 10% under nontranspiring conditions. When transpiration proceeds rapidly, this figure will be larger. In tissues which rapidly alter wall properties to compensate for changes in turgor pressure, the resistance of the water transport path would seem to be unimportant as a limiting factor for growth. More research is needed to understand how cell wall properties are dynamically regulated and how dynamic wall properties relate to steady state properties.

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