A Model of Cell Wall Expansion Based on Thermodynamics of Polymer Networks

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ABSTRACT A theory of cell wall extension is proposed. It is shown that macroscopic properties of cell walls can be explained through the microscopic properties of interpenetrating networks of cellulose and hemicellulose. The qualitative conclusions of the theory agree with the existing experimental data. The dependence of the cell wall yield threshold on the secretion of the wall components is discussed.

INTRODUCTION

The plant cell wall is a complex polymeric sheath, consisting of a network of cellulose microfibrils glued together by a polysaccharide matrix. Because it is mechanically strong, the cell wall is the major determinant of cell mechanical properties and plant cell shape. It allows plant cells to attain high turgor pressure (internal hydrostatic pressure), which puts the wall under high tensile stress. The primary wall is secreted by the growing cell and is maintained in a "plastic," extensible state during the period of cell growth, before the cell matures and the wall loses its ability to expand. Plant cells typically expand 10–100-fold in volume after leaving the meristem but before reaching mature size. Such cell expansion is largely constrained by and regulated by the ability of the cell wall to increase in surface area. In this paper we consider the physical basis for such irreversible wall expansion.

Several important processes accompany plant cell growth. New wall material is synthesized by the cell and secreted into its extracellular space. The existing wall is extended and rearranged, largely by slippage (shearing) of the wall polymers. Newly secreted material is integrated into the extending wall, largely by noncovalent mechanisms of polymer adhesion, although some covalent cross-linking and integration may occur.

The literature on plant cell growth contains two traditional views or themes regarding the mechanism of cell wall expansion. On the one hand, a large literature considers growth to result from a biochemical/biophysical "loosening" of the wall to permit turgor-driven extension of the wall network (Taiz, 1984; Carpita and Gibeaut, 1993; Passioura, 1994). This view can be traced back to the seminal work of Heyn (1940), who showed that auxin, a growth hormone, caused the wall to become more "plastic" or extensible. This wall plasticity was thought to be maintained by wall loosening enzymes (e.g., wall hydrolases), but more recent work indicates that the nonenzymatic protein expansin may be key to wall loosening (McQueen-Mason et al., 1992; Taiz, 1994; McQueen-Mason and Cosgrove, 1995). Lockhart (1965) developed a biophysical model of cell enlargement to obtain an equation (the "Lockhart equation") that accounted for the rate of cell enlargement in terms of the processes of wall expansion and water uptake. This model has been extended by subsequent authors (Cosgrove, 1981; Silk and Wagner, 1980; Cosgrove, 1985; Ortega, 1985) and has served as the conceptual framework for much of the experimental work on plant growth biophysics (Green et al., 1977; Cosgrove, 1986, 1993a; Frensch and Hsiao, 1995; Nonami and Boyer, 1990). Lockhart and subsequent authors used an empirical equation to model the rate of wall expansion as

$$r = \phi(P - Y) \tag{1}$$

where ϕ is a yield rate coefficient (usually termed "extensibility"), P is the turgor, and Y is the yield threshold. Although this constitutive equation has proved useful for characterizing growth responses (i.e., how does ϕ, P, or Y change during a growth alteration?), it fails to ascribe a meaning to the wall parameters ϕ and Y in terms of the structure, behavior, and interactions of the polymers that make up the cell wall. Ultimately, we would like to relate these parameters to the molecular structure of the wall or to replace the equation with another model more closely related to wall structure.

The second theme found in the literature considers wall expansion to be the direct or indirect result of wall polymer biosynthesis and secretion (Brummell and Hall, 1984; Carpita and Gibeaut, 1993; Roberts, 1994). This view accounts for the coupling of wall synthesis and wall extension, and for the observation that walls generally do not become thinner as they extend. However, this view of growth fails to explain how the secretion of polysaccharides can lead to an extension of the wall network that resists the large tensile forces generated by cell turgor. Moreover, this view does not identify the wall polymers that might promote extension of the wall network and those that might resist it. Most cells in the plant body grow in a pattern called "diffuse growth,"

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in which the whole cell wall surface expands by slippage of the polymer network (Carpita and Gibeaut, 1993; Cosgrove, 1993b). Walls isolated from such tissues can extend by 50–100% in vitro when clamped under favorable conditions in an extensometer (greater extension leads to breakage). Such extension indicates that the addition of new wall material is not an essential process for wall extension, at least over the short time scale (Cosgrove, 1996, 1997).

These two conceptions of wall growth, biophysical and biosynthetic, have little in common with each other, but they are not mutually exclusive. Here we take the first steps in integrating these two concepts: we explain wall growth biophysics in terms of wall structure, and we show how wall polymer secretion might influence wall growth biophysics.

An important notion in this integration is the *hierarchy of time scales*. There are two distinct time scales related to wall stretching:

1. *Long time scale*, associated with biosynthesis of the wall components. This time scale presumably spans a period from hours to days. To model the events at this time scale one must have detailed information about the internal workings of the biosynthetic machinery of the cell.

2. *Short time scale*, associated with the cell expansion at a given composition. This time scale presumably spans a period from seconds to hours. One can assume the cell wall composition to be *constant* at this time scale. The great advantage of modeling this stage of cell expansion is that one does not need the details of the cell's biosynthesis machinery; the only information necessary is the cell wall composition at the given moment of the cell's life. Furthermore, this short time scale coincides with the extension behavior of isolated cell walls in extensometer experiments, where wall biosynthesis is lacking (e.g., McQueen-Mason et al., 1992; Okamoto and Okamoto, 1995; Rayle and Cleland, 1972).

In this paper we model cell wall extension on the short time scale. Accordingly, we will consider the cell wall composition to be constant and account for the working of the cell machine through this cell wall composition.

Even in this restrictive formulation the problem seems to be prohibitively complex. Therefore we will not try to make *exact* predictions of the cell wall behavior. Rather, we will discuss *trends* in the cell wall properties as determined by the cell wall structure. The state of the art in the theory of hydrogen-bonded polymers (see Coleman et al., 1991; Coleman and Painter, 1995) is such that this qualitative and semiquantitative prediction is the best thing we can expect from a microscopic theory.

THERMODYNAMICS OF CELL WALL STRETCHING

The aim of this paper is to make the connection between the microscopic parameters of the cell wall and its macroscopic behavior. This section considers the macroscopic description of cell wall expansion. We rederive here the Lockhart equation for cell extension rate (see, e.g., Cosgrove, 1993b) in a somewhat unconventional way. We do this to recast the equation in a more convenient form for the further calculations and to obtain better insight into the thermodynamic meaning of its terms.

The results of the calculations depend on the shape of the cell. In this paper we will restrict ourselves to *a long cylindrical cell*. However, it should be easy to see how to adjust the model for other cell shapes (spherical, etc.).

Let us consider a cylindrical cell of length L and diameter D (see Fig. 1). Suppose it is surrounded by a wall of thickness h. We will consider the wall to be relatively thin:

$$h \ll D \tag{2}$$

and the cell to be relatively long:

$$D \ll L \tag{3}$$

Let G be the free energy of the wall. In general, for cylindrical cells it can be represented as the sum of three contributions: from the side surface of the cylinder, and from its top and bottom surfaces. However, if the condition of Eq. 3 is satisfied, the last two terms are much smaller than the first one. Therefore we can neglect them, and account only for the side surface contribution.

We will define internal stress σ as the derivative:

$$\sigma = \frac{1}{\pi D h}\frac{\partial G}{\partial L} \tag{4}$$

What happens if the cell length increases by δL? There are several processes:

The turgor pressure P produces the work $\delta A_P = (\pi D^2/4)P\delta L$.

The wall produces the negative work $\delta A_\sigma = -\pi D h \sigma \delta L$.

The cell absorbs the volume $\delta V_\omega = \pi(D^2/4)\delta L$ of water and produces the work $\delta A_\omega = -\Delta\psi(\pi D^2/4)\delta L$, where $\Delta\psi$ is the difference in water potentials inside and outside the cell.

Some heat δQ is produced. We assume that the only irreversible process occurring during cell stretching is the friction of cell components. Therefore δQ is proportional to the stretching rate.

If the cell is in equilibrium (and thus $\Delta\psi = 0$), the total work produced at infinitely slow stretching (when $\delta Q = 0$)

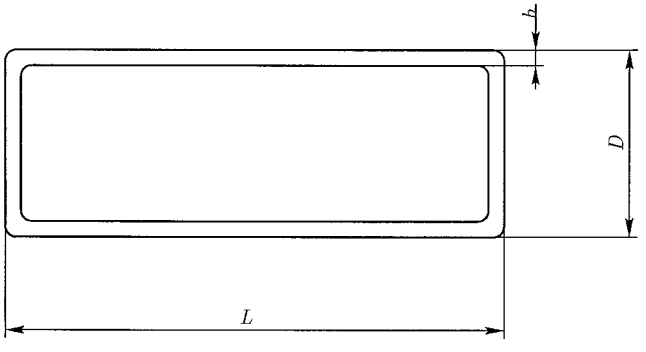

FIGURE 1 A cylindrical cell.

is zero and therefore

$$P = \frac{4h}{D}\sigma \tag{5}$$

At a given turgor P, Eq. 5 gives the equilibrium wall stress σ. Suppose now the cell enlarges slightly, stretching the wall. If the wall stress σ increases, the resulting force will shrink the wall, returning the cell to equilibrium. On the other hand, if σ decreases, the total force will stretch the wall even further, thus leading the cell from the equilibrium. Therefore the cell is stable if and only if

$$\frac{\partial\sigma}{\partial L} > 0 \tag{6}$$

Equation 6 corresponds to the well-known stability condition for the volume dependence of the pressure p: $\partial p/\partial V < 0$ (see, e.g., Landau and Lifshitz, 1980–1981). The critical wall stress (i.e., the threshold stress for wall creep) σ^* is determined by the equation

$$\frac{\partial\sigma^*}{\partial L} = 0 \tag{7}$$

and the corresponding critical turgor P^* is given by Eq. 5. At $P \le P^*$ the cell wall does not creep. At $P > P^*$ it stretches (creeping regime). The schematic diagrams of the function $\sigma(L)$ and the stretching rate dL/dt are shown in Fig. 2.

To determine the stretching rate let us note that if the cell wall is not in equilibrium, its stretching results in excess work, equal to the produced heat δQ:

$$\delta Q = \delta A_P + \delta A_\sigma + \delta A_\omega \tag{8}$$

There are different channels for this process, but it is common (see Cosgrove, 1993b) to assume that the main contribution to δQ comes from the following two:

Friction as water traverses cell walls and cell membrane:

$$\delta Q_\omega = \frac{1}{K_1}\delta V_\omega \frac{\delta L}{L\delta t} \tag{9}$$

where K_1 is the hydraulic conductance and δt is the stretching time.

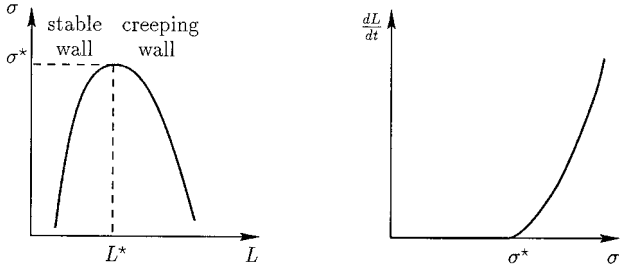

FIGURE 2 Schematic diagram of cell wall stress and stretch rate.

Friction due to polymer movement within the cell wall. We will write it as

$$\delta Q_{\text{wall}} = \frac{1}{K_2}\frac{\pi D^2}{4}\frac{(\delta L)^2}{L\delta t} \tag{10}$$

where K_2 is the wall yielding coefficient. (The factor $\pi D^2/4$ is chosen to simplify the following calculations and to comply with the common notations (Cosgrove, 1993a).)

Then we can write the total heat produced as

$$\delta Q = \frac{\pi D^2}{4}\frac{K_1 + K_2}{K_1 K_2}\frac{(\delta L)^2}{L\delta t} \tag{11}$$

Substituting this equation and the expressions for δA_P, δA_σ, δA_ω in Eq. 8, we obtain

$$\left(\frac{\pi D^2}{4}P - \pi D h\sigma\right)\delta L = \frac{\pi D^2}{4}\frac{K_1 + K_2}{K_1 K_2}\frac{(\delta L)^2}{L\delta t} \tag{12}$$

Rearranging the terms and recalling that $dL/(Ldt) = d\ln L/dt$, we see that the stretching rate obeys a modified Lockhart equation (cf. Cosgrove, 1993b):

$$\frac{d\ln L}{dt} = \frac{K_1 K_2}{K_1 + K_2}\left(P - \frac{4h}{D}\sigma\right) \tag{13}$$

What is the wall stress σ in this equation? The answer depends on the history of the process. If the stretching begins abruptly, then at the first moment σ equals the equilibrium value $\sigma(L)$ for the cell length L at the moment of the beginning of the stretching. On the other hand, if the stretching begins slowly, then at the first moment of creeping $\sigma = \sigma^*$. In this case Eq. 13 would resemble the semiempiric Eq. 1. Indeed, we can write

$$Y = P^* = \frac{4h}{D}\sigma^*, \quad \phi' = \frac{K_1 K_2}{K_1 + K_2} \tag{14}$$

and Eq. 13 has the form of Eq. 1 with the difference that instead of ϕ we now have ϕ'. Note that in the original derivation (Lockhart, 1965) the coefficient ϕ corresponded to what we call K_2. This can be obtained from Eq. 14 by assuming

$$K_1 \gg K_2 \tag{15}$$

However, the mathematical form of Eq. 1 turns out to be more general than the assumptions used to derive it: even if the condition of Eq. 15 is not valid, we only need to redefine the coefficient ϕ to restore Eq. 1.

In both cases the wall stress σ changes in the process of creeping. If the creeping itself is slow enough, wall stress will relax to the equilibrium value $\sigma = \sigma(L(t))$, where $L(t)$ is the cell length in the given moment t. In particular, if $L(t)$ is close to the critical (threshold) value L^*, then the following series expansion holds:

$$\sigma = \sigma^* + \frac{d\sigma}{dL}(L - L^*) + \frac{1}{2}\frac{d^2\sigma}{dL^2}(L - L^*)^2 + \cdots \tag{16}$$

It follows from Eqs. 6 and 7 that at the threshold point $L = L^*$ the first derivative of σ is zero and the second is negative: $d\sigma/dL = 0$ and $d^2\sigma/dL^2 < 0$. This means that Eq. 16 can be written as

$$\sigma = \sigma^* - \alpha(L - L^*)^2, \quad \alpha > 0 \tag{17}$$

and therefore the creeping rate must grow with time. Of course, other effects (like the time dependence of K_1 and K_2 or secretion of new wall material) might screen out or reverse this trend.

This discussion of the wall stretching thermodynamics is purely phenomenological. Our derivation of the Lockhart Eq. 13 shows that it is model-independent. No assumptions about the structure of the wall were made, and therefore Eq. 13 is valid for *any* realistic wall structure.

Our next task is to relate the phenomenological coefficients in Eq. 13 with the structure of the cell wall. We cannot say much about P; it is determined by the physiology of the cell. The coefficients K_1 and K_2 are determined by the cell wall and the cell membrane, but a consistent microscopic theory of a multicomponent viscous flow is rather difficult. Moreover, it seems that the coefficients K_1 and K_2 remain roughly constant during stretching.

The wall stress σ seems to be a more promising object. So our aim is to calculate $\sigma(L)$ and its dependence on the concentration of glucans and cellulose in the cell wall. By doing so we can determine the threshold for stretching (P^*) and the stretching rate near the threshold.

MICROSCOPIC THEORY

A simple picture of the wall

For the purposes of this paper, we employ a bare-bones model of the plant cell wall (Fig. 3). We represent the wall as a network of cellulose microfibrils embedded in a matrix of hemicellulose and pectin polymers. We assume that the hemicelluloses are all of one kind, that they are long relative to the distances between microfibrils, and that they can reversibly adhere to the surface of the microfibril, e.g., through hydrogen bonding (Carpita and Gibeaut, 1993). It should be noted that the specific nature of the bonds between hemicelluloses and glucans is irrelevant for the purposes of this study. Actually any selective reversible bonding will produce the discussed effects. To simplify the language we will use the term "hydrogen bonding" throughout this paper. One should keep in mind, however, that this bonding might also include all other selective interactions between the components. The exact nature of these interactions is an interesting problem itself, but it is outside the framework of this paper.

We further assume that the pectins do not adhere to either the cellulose microfibrils or the hemicelluloses; they act as inert fillers.

The theoretical results presented in this paper show that this wall should exhibit a yield threshold (P^*) that depends on the ratio of cellulose to hemicellulose. Remarkably, the yield threshold does not depend on the bond strength between cellulose and hemicellulose.

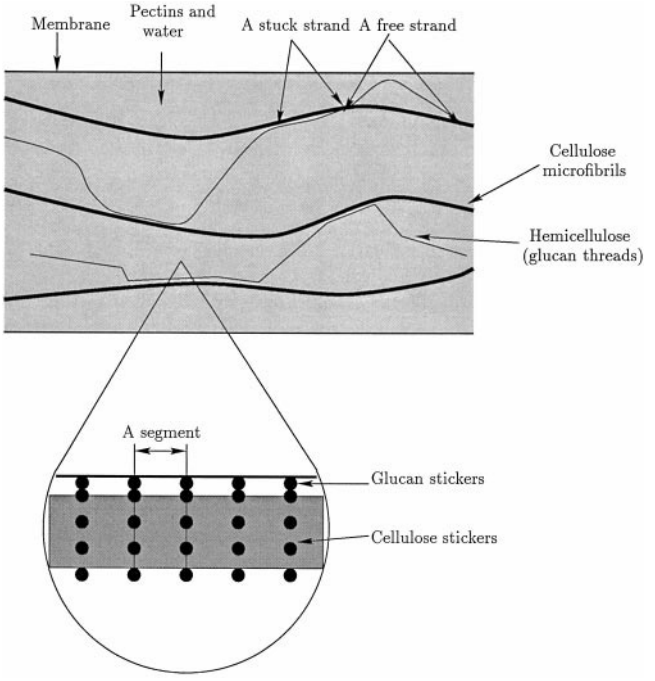

FIGURE 3 The simplified model of the cell wall.

Free energy of the wall

Equation 4 shows that to calculate the wall stress σ we need the free energy of the wall G. The latter has the following main components:

1. Translational entropy of cellulose, hemicellulose, pectins, and water
2. Energy of van der Waals interactions between the molecules
3. Energy and entropy of hydrogen bonds between molecules of different kinds.

A comprehensive calculation of the free energy that takes into account all of these contributions is a difficult task. Moreover, most of the force constants that govern these contributions are not known. Therefore we will employ a number of assumptions. These assumptions are rather drastic, but they are in accordance with our general intent to predict right *trends* in the composition dependence of the cell wall yielding.

First, let us discuss the translational entropy of the cell wall components. The lattice approximation by Flory (1941, 1942) gives the following estimate for the contribution to the translational entropy from the component i:

$$S_i = -kT\frac{V}{M_i v_i}\Phi_i \ln \Phi_i \tag{18}$$

where k is the Boltzmann constant, T is the temperature, V is the volume of the system, v_i is the volume of one subunit of the component i, M_i is the number of such units per molecule of this component, and Φ is the volume fraction of the component. Most of the components of the cell wall (cellulose, hemicellulose, pectins) are highly polymerized; therefore the number M_i for them is large. Therefore we can neglect their translational entropy. The only exception is water. It has low molecular weight, and therefore its translational entropy is not negligible. However, because membranes are permeable to the water molecules, the chemical potential of water *inside* the wall equals that *outside* it to a close approximation (Cosgrove, 1986). Therefore water molecules do not contribute to the wall stress, and we can disregard their free energy.

Second, we will neglect the van der Waals energy of interaction between the molecules. To justify this assumption we can note that this energy is roughly the same for a variety of organic compounds, and therefore it does not change significantly when the cell wall composition changes because of wall extension.

Third, there are several kinds of hydrogen bonds in the system. We will explicitly account for only two kinds of hydrogen bonding:

- Between cellulose fibrils and glucan threads, *and*
- Between water molecules.

We do not include hydrogen bonds between water and cellulose or glucan directly, but we do this *indirectly* by assuming that the effective constant of hydrogen bond formation between glucans and cellulose is measured in a water environment. In other words, the formation of a hydrogen bond between glucan and cellulose is actually a four-stage process consisting of

1. Opening of a hydrogen bond between water and glucan
2. Opening of a hydrogen bond between water and cellulose
3. Formation of a hydrogen bond between glucan and cellulose
4. Formation of hydrogen bonds between water molecules

and therefore the free energy of hydrogen bond formation we will discuss is actually the total outcome of this complex process.

In the framework of these approximations the free energy of the wall can be written down as

$$G = G_{el} + G_h \tag{19}$$

where G_{el} is the contribution of the elasticity of macromolecules and G_h is the contribution of the hydrogen bonds.

There are two different components in G_{el} that reflect the fact that macromolecules do not want to be either too stretched or too compressed. We will assume that the macromolecules in the cell wall are in the compressed state—i.e., that if not for constraints and hydrogen bonds they would naturally expand. The elastic component of free energy of a polymer with radius of gyration R_g confined in a gap of width L_ω is proportional to (see, e.g., Doi and Edwards, 1986, Chapter 2.3.2)

$$G_{el} \propto kT\frac{R_g^2}{L_\omega^2} \tag{20}$$

From this formula we see that the largest contribution to G_{el} is provided by macromolecules with the largest radius of gyration. We will assume that these are cellulose fibrils, and their radius of gyration exceeds the cell dimensions. Noting that we have a three-dimensional problem and putting the all proper coefficients in Eq. 20, we obtain (cf. Doi and Edwards, 1986)

$$G_{el} = \frac{kT\pi^2 b^2 \mathcal{M}}{6}\left(\frac{1}{L^2} + \frac{1}{h^2} + \frac{2}{\pi D^2}\right) \tag{21}$$

where b is the Kuhn segment length of the cellulose fibrils, and $\mathcal{M}$ is the total number of cellulose Kuhn segments inside the wall. The Kuhn segment length b characterizes flexibility of the fibrils: a part of a fibril shorter than b resembles a stiff stick, whereas on the length scales larger than b a fibril is bent.

Let us calculate G_h. We will use a mean field theory analogous to the one discussed by Veytsman (1990).

We will divide glucan threads into segments with one "sticker" per segment, i.e., each segment is a glucose residue capable of hydrogen bonding to cellulose. Cellulose microfibrils, however, have several stickers per segment because many glucans make up a microfibril. Let the segment volumes be v_c for cellulose and v_g for glucans. Let us assume that we have $\mathcal{C}$ cellulose stickers and $\mathcal{G}$ glucan stickers on M threads, so the average number of stickers per thread is $\mathcal{G}/M$. We will divide glucan chains into *free strands* and *stuck strands* (see Fig. 3). The former are not hydrogen-bonded to the cellulose fibrils, the latter are bonded to them. Any glucan chain can be represented as a sequence of free and stuck strands. If we neglect the ends of the threads, the number of free strands equals the number of stuck strands. Let us assume that we have N pairs of free and stuck strands. Let the free strands have $l_1, l_2, \ldots, l_N$ stickers, and the stuck strands have $s_1, s_2, \ldots, s_N$ stickers. Let

$$\mathcal{L} = \sum_{i=1}^{N} l_i, \quad \mathcal{S} = \sum_{i=1}^{N} s_i \tag{22}$$

Obviously,

$$\mathcal{L} + \mathcal{S} = \mathcal{G} \tag{23}$$

The free energy of the network of hydrogen bonds contains two basic terms: the sum of negative contributions from each hydrogen bond formed and the combinatorial entropy related to the number of ways of arranging these bonds in the system. The calculation of the latter is a tedious (albeit straightforward) combinatorial problem; therefore we put it in an appendix (see the Appendix). The only thing

we need here is the result of these calculations, which is given by the following equation:

$$\frac{G_h}{kT} = N \ln\left[\frac{V(z_g-1)N^2}{(N-\mathcal{L})(\mathcal{S}-N)(\mathcal{S}-N-\mathcal{C})v_g z_n^2}\right] + \mathcal{L}\ln\left[\frac{\mathcal{L}-N}{\mathcal{L}(z_g-1)}\right] + \mathcal{S}\ln\left[\frac{(\mathcal{S}-N)(\mathcal{S}-N-\mathcal{C})ev_g}{K\mathcal{S}(\mathcal{S}-\mathcal{C})}\right] + \mathcal{C}\ln\left[\frac{\mathcal{S}-\mathcal{C}}{\mathcal{S}-N-\mathcal{C}}\right] - M\ln\left[\frac{Mv_g}{V}\right] \quad (24)$$

where K is the equilibrium constant of the hydrogen bond formation, V is the volume of the cell wall, v_g is the volume of a glucan segment, and z_g and z_n are the parameters related to the flexibility of the glucan threads (see the Appendix), and e is the base of the natural logarithms.

Expression 24 depends on the number of pairs of strands N and the total length of stuck strands $\mathcal{S}$. Minimizing it with respect to N and $\mathcal{S}$, we obtain the following *stoichiometry equations*:

$$N^2V(z_g-1) = e(N-\mathcal{L})(\mathcal{S}-N)(\mathcal{S}-N-\mathcal{C})v_g z_n^2$$
$$K(\mathcal{L}-N)\mathcal{S}(\mathcal{S}-\mathcal{C}) = ev_g\mathcal{L}(\mathcal{S}-N)(\mathcal{S}-N-\mathcal{C})(z_g-1) \quad (25)$$

Wall stress

The wall stress is given by Eq. 4. It is interesting that the last term in Eq. 19 depends only on the *volume* of the cell wall V, but not on its shape, as determined by the parameters D, L, and h. This corresponds to the general property of hydrogen bonds: at room temperature a network of hydrogen bonds usually can support liquid-like order (like the liquid order in water) but is less likely to produce solid-like order. Therefore the network of hydrogen bonds in a cell wall can preserve the wall volume, but not the wall shape. (We are grateful to Prof. Paul Painter for this remark.) The shape of cell walls is determined by the pattern of cellulose secretion and the history of wall expansion.

When differentiating Eq. 19 we should note that, generally speaking, the wall thickness h varies with the change in L. Therefore we must write

$$\frac{\partial G}{\partial L} = \left(\frac{\partial G}{\partial L}\right)_{h=\text{const.}} + \frac{\partial G}{\partial h}\frac{\partial h}{\partial L} \quad (26)$$

The term $\partial h/\partial L$ describes the thinning of the wall during stretching. To estimate this term let us discuss a small change in the wall thickness δh. The turgor will produce the work $\pi DLP\delta h$, which should be compensated for by the change in the free energy G. By analogy with the stress σ (Eq. 4) we can introduce lateral stress σ_h as

$$\sigma_h = \frac{1}{\pi DL}\frac{\partial G}{\partial h} \quad (27)$$

and the equilibrium condition will be

$$\sigma_h = -P \quad (28)$$

(Note the difference in signs between Eqs. 5 and 28. It means that the cell wall is stretched along the cell axis, but is compressed in the normal direction.) If the wall stretching is performed at quasiequilibrium conditions, Eq. 28 is still satisfied, and therefore the proper derivative in Eq. 26 is $(\partial G/\partial L)_{\sigma_h}$. Using the method of Jacobians (see, e.g., Landau and Lifshitz, 1980–1981), we obtain

$$\left(\frac{\partial G}{\partial L}\right)_{\sigma_h} = \frac{\partial(G,\sigma_h)}{\partial(L,\sigma_h)} = \frac{\partial(G,\sigma_h)}{\partial(L,h)} \div \frac{\partial(L,\sigma_h)}{\partial(L,h)} = \left(\frac{\partial G}{\partial L}\right)_h - \left(\frac{\partial \sigma_h}{\partial L}\right)_h \left(\frac{\partial G}{\partial h}\right)_L \div \left(\frac{\partial \sigma_h}{\partial h}\right)_L \quad (29)$$

Let us estimate the last term in Eq. 29. By the order of magnitude

$$\left(\frac{\partial \sigma_h}{\partial L}\right)_h \approx \frac{\sigma_h}{L}, \quad \left(\frac{\partial \sigma_h}{\partial h}\right)_L \approx \frac{\sigma_h}{h} \quad (30)$$

and using Eqs. 27 and 28, we see that the last term in Eq. 29 is by the order of magnitude equal to

$$\frac{h}{L}\left(\frac{\partial G}{\partial h}\right)_L = \pi DhP \quad (31)$$

On the other hand, from Eqs. 4 and 5 we can conclude that the total value of $\partial G/\partial L$ is

$$\pi Dh\sigma = \frac{\pi D^2}{4}P \quad (32)$$

We see that the ratio of the last term in Eq. 26 to the total sum is h/D. Thus if Eq. 2 is satisfied, this term is small, and the wall thinning effect on the wall stress is negligible. Therefore when calculating σ we will take the derivative $\partial G/\partial L$ at constant h.

Differentiating Eq. 19, we obtain the wall stress:

$$\sigma = -kT\frac{\pi^2 b^2 \mathcal{M}}{3VL^2} + kT\frac{N}{V} - kT\frac{M}{V} \quad (33)$$

Equation 33 has a simple interpretation. The first term corresponds to the elastic contribution of cellulose microfibrils (as discussed above, we neglected elastic contributions of other polymers). Because it is negative, it prevents the cell wall collapse. The last term is the osmotic pressure of the glucan macromolecules. The term kTN/V corresponds to the contribution of hydrogen bonds. It is positive, so hydrogen bonds tend to contract the cell wall. The surprising fact is that this contribution is very simply related to the number of strands of the glucan threads N, which is determined by Eq. 25.

We see that the larger is the number N, the stronger is the wall. This means that a strong wall should contain many relatively short glucan strands (of course, their lengths

should be still larger than the distance between microfibrils). It is interesting that the sum of the last two terms in Eq. 33 is positive as long as $N > M$. This inequality means that each thread has several strands, and therefore the glucans actually sew together cellulose microfibrils. In the opposite case of $N < M$, each glucan thread is connected on average to only one cellulose microfibril, and the cell wall cannot hold together.

Equation 33 shows that the wall stress depends only on several well-defined physical quantities: the number of glucan strands N, the number of glucan threads M, and the number of cellulose Kuhn segments $\mathcal{M}$. Let us discuss various limiting cases for this equation.

First let us discuss the case of a relaxed cell wall. In this case Eq. 33 is dominated by the negative first term. Because the absolute value of the first term in Eq. 33 decreases as the wall cell volume increases, the wall stress grows with L.

In the opposite case of a very stretched cell wall (close to mechanical failure), the first term in Eq. 33 is negligible. Obviously the number of glucan threads N does not increase as L increases. Because the cell wall volume V grows with L (i.e., the cell wall absorbs water), the wall stress decreases as L increases.

We see that at small L, σ increases, and at large L, σ decreases with L. This means that somewhere at the intermediate values of L the function $\sigma(L)$ has a maximum. Therefore the qualitative picture in Fig. 2 is correct, and the description of the thermodynamics of stretching in the second section of this paper is confirmed. There indeed exists the threshold value σ^* where the stable regime changes to the creeping regime.

Dimensionless equations

The usual way to describe cell wall expansion is to choose some cell wall length as a reference point and measure the extension of the wall with respect to this reference point. To compare our theory with the experimental findings let us recast our equation in this way.

Accordingly, let L_0 be the length of the cell wall at some point in the stable regime (the reference point). The corresponding cell wall volume is

$$V_0 = \pi D L_0 h \tag{34}$$

The wall extension can be measured by the dimensionless ratio

$$u = \frac{L}{L_0} = \frac{V}{V_0} \tag{35}$$

We will divide all extensive values by V_0, so, e.g., M/V_0 gives the number of glucan threads per unit volume when the wall cell occupies the volume V_0. Because it is still a dimensional quantity, we will normalize it by the characteristic volume v_g. In this way we will introduce the following set of dimensionless quantities:

$$m = \frac{v_g M}{V_0}, \quad n = \frac{v_g N}{V_0}, \quad g = \frac{v_g \mathcal{G}}{V_0}, \quad c = \frac{v_g \mathcal{C}}{V_0}, \quad l = \frac{v_g \mathcal{L}}{V_0}, \quad s = \frac{v_g \mathcal{S}}{V_0} \tag{36}$$

The physical meaning of the dimensionless variables c, g, l, and s is simple. Suppose we enclose each segment in a sphere of volume v_g. Then these variables give the volume fraction of cellulose stickers, glucan stickers, free glucan strands, and stuck glucan strands correspondingly with respect to the cell wall volume V_0. Analogously, m and n give the volume fractions of *ends* of glucan threads and strands correspondingly. If each glucan thread has λ stickers, then

$$m = \frac{g}{\lambda} \tag{37}$$

In the dimensionless variables Eqs. 23 and 25 can be written as

$$l + s = g \tag{38}$$

$$n^2 u = \zeta (n - l)(s - n)(s - n - c) \tag{39}$$

$$(l - n)s(s - c) = \varepsilon l (s - n)(s - n - c) \tag{40}$$

Here ε and ζ are dimensionless constants equal to

$$\varepsilon = \frac{e(z_g - 1)v_g}{K}, \quad \zeta = \frac{e z_n^2}{(z_g - 1)} \tag{41}$$

The wall stress becomes

$$\sigma = \frac{kT}{v_g}\tilde{\sigma} \tag{42}$$

Here $\tilde{\sigma}$ is the dimensionless wall stress:

$$\tilde{\sigma} = -r\frac{c}{u^3} + \frac{n - m}{u} \tag{43}$$

where

$$r = \frac{\pi^2 b^2 \mathcal{M}}{3 L_0^2 \mathcal{C}} \tag{44}$$

Strong bonding limit

The solution of Eqs. 38–40 depends on the values of the dimensionless constants ζ and ε. The latter describes the hydrogen bonding in the system. In nonaqueous systems it is rather small—$\sim 10^{-2}$. In the aqueous systems the hydrogen bonding constant K is in fact an *effective constant* describing the difference between cellulose glucan hydrogen bonds and other types of hydrogen bonding. One can therefore expect K to be smaller and ε to be larger than in nonaqueous systems. Still one can argue that ε is small enough even in aqueous systems. One of the indirect indications of this is the fact that cell walls do exist, and

therefore the bonding between cellulose and glucans must be strong enough to support cell walls.

Therefore in this section we will discuss the limit

$$\varepsilon \to 0 \tag{45}$$

From Eq. 40 we see that in this case either $s \to 0$ or $(l - n) \to 0$ or $(c - s) \to 0$. What do these possibilities mean?

If $s \to 0$, the glucans are not connected to cellulose fibrils. This case corresponds to the broken wall. We are not interested in this case.

If $(l - n) \to 0$, then from Eq. 39 we see that both $l \to 0$ and $n \to 0$. Then Eq. 43 gives $\tilde{\sigma} < 0$. This case corresponds to the situation in which the number of cellulose stickers is greater than the number of glucan stickers. In this case glucan threads just lie along the cellulose microfibrils. They do not actually connect microfibrils in a network and cannot support the wall.

We are left with the case $(c - s) \to 0$. This case corresponds to the situation in which there are not enough cellulose stickers to put all glucan threads on. This is probably the case that is realized in the real cell walls.

For this case we have in the lowest order by ε:

$$\begin{aligned} s &= c \\ l &= g - c \end{aligned} \tag{46}$$

and n is determined by the following quadratic equation:

$$nu = \zeta(c - n)(g - c - n) \tag{47}$$

The wall stress is given by Eqs. 42 and 43. The remarkable feature of these equations is that they do not depend on ε. This means that the wall stress does not depend on the strength of the hydrogen bonding K, once K is large enough to ensure the limit $\varepsilon \to 0$. It means also that the factors changing the equilibrium constant of hydrogen bond formation (like *pH* or temperature) do not affect the wall stress significantly, as long as the constant remains high enough. On the other hand, the wall stress depends on the number of cellulose and glucan functional groups c and g. This means that a factor that changes them might significantly influence σ. Among such factors is, e.g., some enzyme that blocks a number of functional groups, thus lowering c or g.

Estimates for the model parameters

We will assume that the wall is extended at the room temperature $T = 300$ K and the molar volume of glucan monomers *is* $v_g \approx 100$ cm^3/mol. The dimensionless parameters ε and ζ, introduced in Eq. 41, are $\varepsilon \approx 10^{-2}$, $\zeta \approx 10$.

The parameter r in Eq. 43 could be estimated if we assume the Kuhn length of cellulose segments to be $b \approx 0.5L_0$ and the number of stickers per cellulose segment to be $\mathscr{C}/\mathscr{M} \approx 10$. Then from Eq. 44 we obtain $r \approx 0.1$.

The parameter λ measures the number of glucan stickers per thread; let us take it to be about $\lambda \approx 100$.

To estimate the parameters g and c we enclose each sticker in a sphere of volume v_g; then c is the total volume of such spheres per unit volume of the wall. If the spheres were not overlapping, the parameters c and g would represent the volume fractions of cellulose and glucans. However, because the spheres overlap, they should be several times greater than the actual volume fractions; we assume them to be on the order of $c \approx 0.2$, $g \approx 0.8$.

Dependence of wall stress on the concentration of glucans and cellulose in the cell wall

The wall stress depends on the composition of the cell wall. Equations 42 and 43 allow us to estimate this dependence. For simplicity we will discuss only the *strained cell wall in the strong bonding limit.* In this case we can write down

$$\tilde{\sigma} \approx \frac{n - m}{u} \tag{48}$$

where n is determined by Eq. 47. Differentiating Eq. 47 with respect to g and c, we obtain

$$u\frac{\partial\tilde{\sigma}}{\partial g} = \frac{n(c - n)}{2c(g - c) - gn} - \frac{1}{\lambda} \tag{49}$$

$$u\frac{\partial\tilde{\sigma}}{\partial c} = \frac{n(g - 2c)}{2c(g - c) - gn} \tag{50}$$

Signs of the derivatives $\partial\tilde{\sigma}/\partial g$ and $\partial\tilde{\sigma}/\partial c$ determine the direction of the cell wall stress change when additional glucans or cellulose is secreted.

The number of free glucan threads n is always smaller than the number of free glucan "stickers" $g - c$, because each free thread should contain at least one free "sticker." Moreover, because each free strand terminates at a cellulose "sticker," n is smaller than c. We see that

$$n \le \min(c, g - c) \tag{51}$$

It follows from this inequality that the denominator in Eqs. 49 and 50 is always positive. (Indeed, if $c < g - c$, then $n \le c$ and $2c(g - c) - gn \ge 2c(g - c) - gc = c(g - 2c) > 0$. On the other hand, if $c > g - c$, then $n < g - c$, and again, $2c(g - c) - gn \ge 2c(g - c) - g(g - c) = (2c - g)(g - c) > 0$.)

Let us first assume that the glucan threads are infinite in length ($\lambda \to \infty$). Then it is easy to see that the right-hand side of Eq. 49 is always positive. This means that secretion of glucans strengthens the wall.

If we now introduce a finite length of glucan threads, then Eq. 49 changes sign at large enough g. This means that when the concentration of glucans is really huge (on the order of magnitude $\lambda c/\zeta \approx 10c$), the additional secretion of glucans will only weaken the wall.

Equation 50 predicts an interesting dependence of cell wall stress on the concentration of cellulose. Its right-hand side is positive if $2c < g$ and negative otherwise. This means that there is an optimal concentration of cellulose. In the dimensionless variables (Eq. 36) it is determined by the

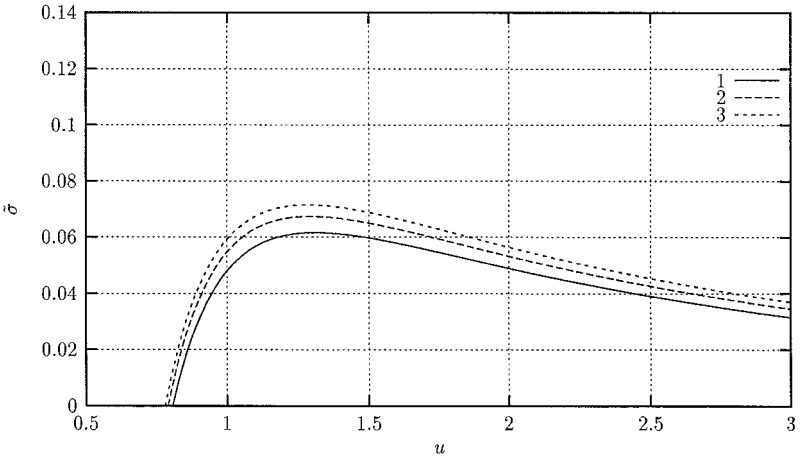

FIGURE 4 Effects of glucan concentration on wall stress. Dimensionless wall stress $\tilde{\sigma}$ as the function of stress expansion u. $r = 0.1$, $\zeta = 10$, $\lambda = 50$, $c = 0.2$. 1: $g = 0.7$; 2: $g = 0.8$; 3: $g = 0.9$.

equation $g = 2c$. At this concentration the cell wall stress is highest. If the concentration of cellulose is either lower or higher than this optimal number, cell wall stress decreases, and cell wall is weaker.

Calculations

First let us look at how the concentration of glucans influences wall strength. In Fig. 4 we plotted the function $\tilde{\sigma}(u)$ at various g. We see that this function has the shape of Fig. 2, with the maximum corresponding to the threshold wall stress $\tilde{\sigma}^*$. Additional secretion of glucans increases the yielding threshold, as discussed above. Note that this prediction challenges the commonly stated (but unproven) idea that newly secreted matrix components enhance wall loosening and wall extension. Our thermodynamic model indicates that secretion of glucan threads may confer a higher yield threshold on the wall and therefore cause it to extend *more* slowly. However, such a wall is also stronger in the sense that it is capable of more prolonged extension before breakage.

Now let us explore the influence of the amount of cellulose microfibrils. The corresponding graphs are shown in Fig. 5. We see that there is an optimal value of c that corresponds to the highest yield threshold. Deviations from this value decrease the cell wall stress and yield threshold.

The threshold value of wall strain is close to 20%. The absolute value of the wall stress is determined by Eq. 42. From the figures we can conclude that the maximum value of $\tilde{\sigma} \approx 10^{-1}$. Taking this value we obtain

$$\sigma^* \approx 25 \text{ bar} \tag{52}$$

which seems to be quite reasonable. For example, measurements of the turgor threshold (Y in Eq. 1) for plant cell growth typically fall in the range of 1–3 bar (reviewed in Cosgrove, 1986). The corresponding wall stress σ^* can be calculated by Eq. 5 to be in the range 10–30 bar (given $h = 0.5$ μm and $D = 20$ μm).

The literature on plant growth biophysics contains several reports that the turgor pressure Y can change after various physiological and biochemical responses of growing plant tissues (e.g., Cramer and Bowman, 1991; Frensch and Hsiao, 1995; Nakahori et al., 1991; Okamoto and Okamoto, 1995). The molecular basis for such changes in Y has not been elucidated, but our theory offers the possible explanation that specific alterations in the structure of the hemicellulose-cellulose network may underlie these changes in Y.

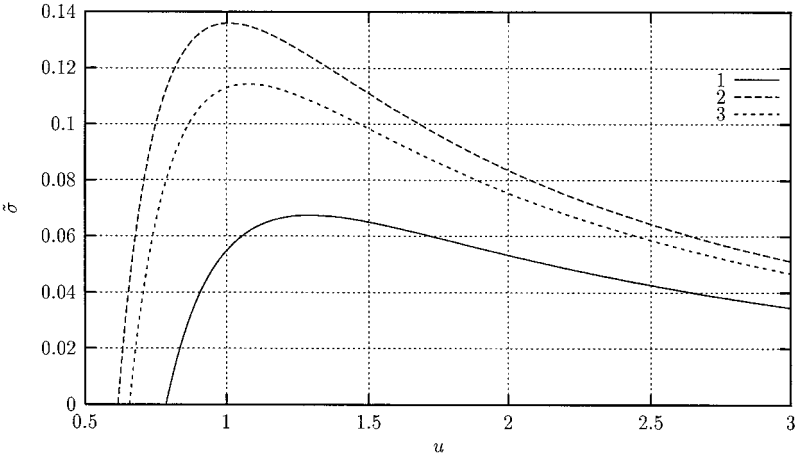

FIGURE 5 Effect of cellulose concentration on wall stress. Dimensionless wall stress $\tilde{\sigma}$ as the function of stress expansion u. $r = 0.1$, $\zeta = 10$, $\lambda = 50$, $g = 0.8$. 1: $c = 0.2$; 2: $c = 0.4$; 3: $c = 0.5$.

For instance, secretion of additional matrix hemicelluloses ("glucans" in the simplified terminology of our model) would be predicted to increase the yield threshold, whereas cutting the hemicelluloses into smaller pieces ought to lower the threshold and increase the growth rate. Xyloglucan—the most abundant cellulose in growing walls of pea seedlings—was found to undergo large, reversible changes in molecular size upon treatment of pea seedlings with the growth hormone auxin (Talbott and Ray, 1992). Our theory could also be tested by measuring the yield threshold in walls treated so as to alter the ratio of hemicellulose to cellulose, e.g., by treatment with special inhibitors of wall biosynthesis before the wall is collected for extension assays.

CONCLUSION

In this paper we explored a simple thermodynamic model for the expansion of cell walls. The goal of this model is the qualitative understanding of the processes taking place in the growing cell wall. Our hypothesis is that the main contribution to the cell wall stress is caused by the interpenetrated hydrogen-bonded networks of cellulose fibrils and glucan threads. The consequences of this hypothesis, presented in this paper, agree with the existing experimental data—at least qualitatively.

A surprising finding is that the cell wall yield threshold and strength are determined mostly by the concentration of glucans and cellulose, and not by the strength of the hydrogen bonding. At a given level of glucans, either increase or decrease of the concentration of microfibrils decreases the yield threshold. This model proposes that the yield threshold is a net outcome of the thermodynamics of wall polymer interactions. Previous biophysical models of wall extension have generally taken this threshold as a "given" without inquiring into its underlying nature (Lockhart, 1965; Green et al., 1977). The recent model of Passioura (1994) treats the yield threshold in a more detailed way, but still considers it to "represent the unzipping properties of hydrogen bonds" between connected polymers. We offer a new interpretation of the yield threshold.

Of course, there are many effects and phenomena not covered by this simple model. We hope to discuss them in subsequent works. Here we just mention some of the most interesting ones:

This model shows that *flexibility* (i.e., the Kuhn segment length of the cellulose fibrils) has a significant effect on cell wall stress. Because hemicelluloses are thought to become trapped within cellulose microfibrils during their formation, with consequences for microfibril structure and flexibility (Hayashi, 1989), this effect deserves special study.

We considered the networks to be completely unordered. Of course, this is an oversimplification. It might be interesting to study the orientation ordering of cellulose and its influence on cell wall extension.

We assumed the pectins to be just neutral fillers. Actually their role is probably more active.

The influence of the network of hydrogen bonds in water deserves a better treatment.

The evolution of the cell wall thickness h is one of the most important questions not answered by this theory. It warrants experimental and theoretical study.

The chemical nature of selective reversible interactions between the cell wall structural elements is an important problem from both theoretical and experimental points of view. In this paper we swept all of these interactions under the broad term "hydrogen bonding." However, a better understanding of their origins might give better insight into the mechanism of cell wall enlargement.

APPENDIX: FREE ENERGY OF HYDROGEN BONDS BETWEEN GLUCANS AND CELLULOSE

Let us calculate the contribution of glucan fibers. The method we use is close to the one proposed by Veytsman (1990) for hydrogen-bonded mixtures (see also Sanchez and Panayiotou, 1994).

We will neglect the end-of-chain effects and assume that we have one long chain of $\mathcal{G}$ segments.

First, we will divide $\mathcal{S}$ stuck segments into N stuck strands. It can be done in

$$\mathcal{N}_1 = \frac{\mathcal{S}!}{(\mathcal{S}-N)!N!} \tag{53}$$

ways. Next, we will put them on the cellulose fibrils. Suppose we have $\mathcal{C}$ stickers on the cellulose fibrils. Then the number of ways to put N strands of the combined length $\mathcal{S}$ on $\mathcal{C}$ linearly arranged stickers is equal to the number of ways of putting N strands of length 1 each on $\mathcal{C} + N - \mathcal{S}$ stickers, i.e.,

$$\mathcal{N}_2 = \frac{(\mathcal{C}+N-\mathcal{S})!}{(\mathcal{C}-\mathcal{S})!} \tag{54}$$

Each segment of glucan residing on a cellulose sticker has an excess partition function $K/(ev_g)$, where K is the hydrogen bonding constant, and v_g is the volume of a glucan segment (see Veytsman, 1990). Therefore the partition function of *stuck* strands is only

$$\Xi_s = \mathcal{N}_1\mathcal{N}_2\left(\frac{K}{ev_g}\right)^{\mathcal{S}} \tag{55}$$

Let us now discuss free strands. First, we will divide $\mathcal{L}$ stuck segments into N stuck strands. This can be done in $\mathcal{N}_3$ ways, where

$$\mathcal{N}_3 = \frac{\mathcal{L}!}{(\mathcal{L}-N)!N!} \tag{56}$$

In the next step we will connect stuck strands with free strands. Let us divide the space V into

$$\mathcal{V} = \frac{V}{v_g} \tag{57}$$

lattice cells with the coordination number z_g. Each of the N free strands connects two stuck segments (remember, we have already placed all stuck segments!). If this *connectivity condition* were absent, the partition function of the free strands would be $\mathcal{V}_g^N(z_g-1)^{\mathcal{L}-N}\mathcal{N}_3$. However, the fact that free strands must begin and end in predefined places significantly decreases this number. First, let us satisfy only half of the connectivity condition: let the first segment of each free strand be in the vicinity of the corresponding stuck segment, but let the last one be anywhere. Let z_n be the

number of possible positions for a free segment following the stuck segment. The partition function of the free strands therefore will be

$$\Xi_{f,0} = z_n^N (z_g - 1)^{\mathcal{L}-N} \mathcal{N}_3 \quad (58)$$

Now let us recall the fact that the free strands must *end* in the vicinity of the corresponding stuck strands. The partition function of this arrangement depends on the correlations between the stuck strands. We will discuss the simplest possible situation: the *random network model.* In this model we will assume the cellulose fibrils to be placed *completely randomly* in the volume V. Then the probability of a free strand ending in the right place is $z_n/\mathcal{V}$, and the partition function of free strands is

$$\Xi_f = \Xi_{f,0} \left(\frac{z_n}{\mathcal{V}} \right)^N \quad (59)$$

If we want to account for the fact that there are M chains, we must multiply the partition function by $\mathcal{V}^M/M!$. Therefore the total partition function is

$$\Xi = \Xi_s \Xi_f \frac{\mathcal{V}^M}{M!} \quad (60)$$

The free energy is equal to

$$G_h = -kT \ln \Xi \quad (61)$$

where k is the Boltzmann's constant, and T is the absolute temperature. Using the Stirling formula, we obtain Eq. 24.

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REFERENCES

Brummell, D. A., and J. L. Hall. 1984. The role of cell wall synthesis in sustained auxin-induced growth. *Plant Physiol.* 63:406–412.

Carpita, N. C., and D. M. Gibeaut. 1993. Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. *Plant J.* 3:1–30.

Coleman, M. M., J. F. Graf, and P. C. Painter. 1991. *Specific Interactions and the Miscibility of Polymer Blends.* Technomic, Lancaster, PA.

Coleman, M. M., and P. C. Painter. 1995. Hydrogen bonded polymer blends. *Prog. Polym. Sci.* 20:1–59.

Cosgrove, D. J. 1981. Analysis of the dynamic and steady-state responses of growth rate and turgor pressure to changes in cell parameters. *Plant Physiol.* 68:1439–1446.

Cosgrove, D. J. 1985. Cell wall yield properties of growing tissues. Evaluation by *in vivo* stress relaxation. *Plant Physiol.* 78:347–356.

Cosgrove, D. J. 1986. Biophysical control of plant cell growth. *Annu. Rev. Plant Physiol.* 37:318–320.

Cosgrove, D. J. 1993a. How do plant cell walls extend? *Plant Physiol.* 102:1–6.

Cosgrove, D. J. 1993b. Tansley review no. 46. Wall extensibility: its nature, measurement and relationship to plant cell growth. *New Phytol.* 124:1–23.

Cosgrove, D. J. 1996. Plant cell enlargement and the action of expansin. *BioEssays.* 18:533–540.

Cosgrove, D. J. 1997. Relaxation in a high-stress environment: the molecular bases of extensible cell walls and cell enlargement. *Plant Cell.* 9:1031–1041.

Cramer, G. R., and D. C. Bowman. 1991. Kinetics of maize leaf elongation. I. Increased yield threshold limits short-term, steady state elongation rates after exposure to salinity. *J. Exp. Bot.* 42:1417–1426.

Doi, M., and S. F. Edwards. 1986. *The Theory of Polymer Dynamics.* Clarendon Press, Oxford.

Flory, P. J. 1941. Thermodynamics of high polymer solutions. *J. Chem. Phys.* 9:660–661.

Flory, P. J. 1942. Thermodynamics of high polymer solutions. *J. Chem. Phys.* 10:51–61.

Frensch, J., and T. C. Hsiao. 1995. Rapid response of the yield threshold and turgor regulation during adjustment of root growth to water stress in *Zea Mays. Plant Physiol.* 108:303–312.

Green, P. B., K. Bauer, and W. R. Cummins. 1977. Biophysical model for plant cell growth: auxin effects. *In* Water Relations in Membrane Transport in Plants. A. M. Jungreis, T. K. Hodges, A. Kleinzeller, and S. G. Schultz, editors. Academic Press, New York. 30–45.

Hayashi, T. 1989. Xyloglucans in the primary cell wall. *Annu. Rev. Plant Physiol. Mol. Biol.* 40:139–168.

Heyn, A. N. J. 1940. The physiology of cell elongation. *Bot. Rev.* 6:515–574.

Landau, L. D., and E. M. Lifshitz. 1980–1981. *Statistical Physics.* Pergamon International Library of Science, Technology, Engineering and Social Studies. E. M. Lifshitz and L. P. Pitaevskii, editors. Pergamon Press, Oxford and New York.

Lockhart, J. A. 1965. An analysis of irreversible plant cell elongation. *J. Theor. Biol.* 8:264–275.

McQueen-Mason, S., and D. J. Cosgrove. 1995. Expansin mode of action on cell walls: analysis of wall hydrolysis, stress relaxation, and binding. *Plant Physiol.* 107:87–100.

McQueen-Mason, S., D. M. Durachko, and D. J. Cosgrove. 1992. Endogenous proteins that induce cell wall expansion in plants. *Plant Cell.* 4:1425–1433.

Nakahori, K., K. Katou, and H. Okamoto. 1991. Auxin changes both the extensibility and the yield threshold of the cell wall of *Vigna* hypocotyls. *Plant Cell Physiol.* 32:121–129.

Nonami, H., and J. S. Boyer. 1990. Wall extensibility and cell hydraulic conductivity decrease in enlarging stem tissues at low water potentials. *Plant Physiol.* 93:1610–1619.

Okamoto, A., and H. Okamoto. 1995. Two proteins regulate the cell wall extensibility and the yield threshold in glycerinated hollow cylinders of cowpea hypocotyl. *Plant Cell Environ.* 18:827–830.

Ortega, J. K. E. 1985. Augmented growth equation for cell wall expansion. *Plant Physiol.* 78:318–320.

Passioura, J. B. 1994. The physical chemistry of the primary cell wall: implications for the control of expansion rate. *J. Exp. Bot.* 45: 1675–1682.

Rayle, D. L., and R. E. Cleland. 1972. The in-vitro acid-growth response: relation to in-vivo growth responses and auxin action. *Planta.* 104: 282–296.

Roberts, K. 1994. The plant extracellular matrix, in a new expansive mood. *Curr. Opin. Cell Biol.* 6:688–694.

Sanchez, I. C., and C. G. Panayiotou. 1994. Equation of state thermodynamics of polymer and related solutions. *In* Models for Thermodynamics and Phase Equilibria Calculations. S. I. Sandler, editor. Marcel Dekker, New York, Basel, Hong Kong. 187–285.

Silk, W. K., and K. K. Wagner. 1980. Growth-sustaining water potential distributions in the primary corn root. A noncompartmented continuum model. *Plant Physiol.* 66:859–863.

Taiz, L. 1984. Plant cell expansion: regulation of cell wall mechanical properties. *Annu. Rev. Plant Physiol.* 35:585–657.

Taiz, L. 1994. Expansins: proteins that promote cell wall loosening in plants. *Proc. Natl. Acad. Sci. USA.* 91:7387–7389.

Talbott, L. D., and P. M. Ray. 1992. Changes in molecular size of previously deposited and newly synthesized pea cell wall matrix polysaccharides. *Plant. Physiol.* 98:369–379.

Veytsman, B. A. 1990. Are lattice models valid for liquids with hydrogen bonds? *J. Phys. Chem.* 94:8499–8500.