CHAPTER 11

HOW CELLULOSE SYNTHASE DENSITY IN THE PLASMA MEMBRANE MAY DICTATE CELL WALL TEXTURE

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Abstract

Cellulose microfibrils are deposited by cellulose synthases into the cell wall in often strikingly regular patterns. Here we discuss several mechanisms that have been put forward to explain the alignment of cellulose microfibrils that gives rise to ordered cell wall textures: the hypothesis that *cortical microtubules align cellulose microfibrils* during their deposition, the *liquid crystal hypothesis* in which cellulose microfibrils self-assemble into textures after their deposition, the *templated incorporation hypothesis*, and *the geometrical theory* in which the density of active cellulose synthase complexes inside the plasma membrane may dictate the architecture of the cell wall.

Keywords

cell wall architecture, cellulose microfibrils, cellulose synthase, cortical microtubules, geometrical model.

Abbreviations

cellulose microfibril (CMF), cellulose synthase activation domain (CSAD).

1 TEXTURES OF CELLULOSE MICROFIBRILS

The cell wall texture is a composite of cellulose microfibrils (CMFs) arranged in one CMF thick lamellae. The orientation of the CMFs within a lamella is constant, but may vary from lamella to lamella. The most striking texture is the helicoidal wall, which consists of subsequent lamellae in which the orientation of the CMFs

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R.M. Brown, Jr. and I.M. Saxena (eds.), Cellulose: Molecular and Structural Biology, 183–197.

changes by a constant angle. Other wall textures are the axial, helical, crossed-polylamellate, transverse and the random wall textures, and combinations of these. Since wall texture is cell type and developmental stage specific, it must be highly regulated by the cell it embraces. Irrespective of the type of wall texture (review Emons 1991), cellulose microfibrils are produced by plasma membrane embedded cellulose synthase complexes (Kimura et al. 1999) which in freeze fracture images are observed as particle rosettes (first observation: Mueller and Brown, Jr. 1980).

Being crystalline and outside the plasma membrane, CMFs of plant cell walls were among the first structures that were reliably visualized with electron microscopy (Frey-Wyssling et al. 1948; Preston et al. 1948; reviewed by Preston, 1974). Roelofsen and Houwink (1953) showed that CMFs are deposited transverse to the cell elongation direction of elongating plant cells, but that CMFs in outer lamellae have an oblique to longitudinal alignment. They suggested that in previously deposited, older wall layers the originally transverse CMFs rotate to a longitudinal orientation during cell elongation. This is known as the "multi net growth" hypothesis (Roelofsen 1959). For epidermal cells from the style of *Petunia,* Wolters-Arts and Sassen (1991) have shown that this realignment indeed takes place. In a recent publication Refrégier et al. (2004) also suggest realignment of CMFs in older wall layers of elongating hypocotyl cells of dark-grown *Arabidopsis* seedlings after transverse deposition of CMFs in the innermost wall layer.

An alternative to the "multi net growth" hypothesis was the "ordered subunit" hypothesis of Roland and coworkers (review 1977), in which CMFs are laid down during deposition in subsequently different directions. Deposition in subsequently different orientations should surely take place in nonexpanding cells or cell parts having walls with various CMF orientations. The important question of the regulation of the deposition orientation of CMFs is still subject of lively scientific debate.

We have formulated a geometrical, mathematical theory for CMF ordering during their deposition, which allows production of axial, helical, crossed, helicoidal, and random wall textures (Emons 1994; Emons and Kieft 1994; Emons and Mulder 1997; 1998; 2000; 2001; 2001; Emons et al. 2002; Mulder et al. 2004). Before reviewing our theory, we first discuss the most important alternate CMF ordering hypotheses that have been proposed: (1) microtubule-directed CMF orientation, (2) self-assembly like liquid crystals, (3) templated incorporation hypothesis. In addition, we will respond to criticism that has been put forward against the geometrical theory and discuss those predictions from the theory that can be tested experimentally and, therefore, potentially, verify or falsify the theory.

2 HYPOTHESES ABOUT CELLULOSE MICROFIBRIL ORDERING MECHANISMS

2.1 Microtubule-directed microfibril orientation

In 1962, Green stated that "…the control of the cylindrical cell form in plants appears to reside in the orientation of the reinforcing CMFs in the side walls…" and that "…control of new synthesis of oriented wall texture is shown to be in

turn related to the orientation of cytoplasmic elements in the cell periphery…" (Green 1962). The first statement may be logical, and is often taken for granted, but has not been proven in a direct way. In the second statement Green foretells the existence of intracellular polymers (i.e., cortical microtubules), but also predicts that "…long elements in the cytoplasm adjacent to the wall can become aligned into the direction of maximum strain…" (i.e., the direction of cell elongation), which is perpendicular to the CMFs being deposited. This orientation of the long cytoplasmic structures in the direction of maximum strain is a logical prediction from a physical point of view. However, when cortical microtubules were indeed observed one year later (Ledbetter and Porter 1963), they appeared, in contrast to Green's prediction, to run in the same orientation as the CMFs. This led to the hypothesis that not their presence but their orientation determines nascent CMF direction. This evoked the still unanswered question of what orders the microtubules; apparently, this is not the direction of maximum strain of a growing cell.

The textbook dogma about the ordering mechanism of nascent CMFs, since 1963 (Ledbetter and Porter), is the "alignment hypothesis" (term given in review of Baskin 2001). This hypothesis was derived from the observation that CMFs run perpendicular to the axis of cell elongation, like the microtubules, and the experimental results that showed altered CMF ordering after microtubule depolymerization in such cells. Later, the theory has been worked out, hypothesizing an ordering mechanism in which the microtubules direct the cellulose synthases (Heath 1974), or channel them through the plane of the plasma membrane (Herth 1980; Giddings and Staehelin 1988). This hypothesis, that cortical microtubules exert control over nascent CMFs, is not supported by the work on nonelongating parts of Equisetum *hyemale* root hairs and other work on nonelongating cells (reviewed in Emons et al. 1992).

Strong evidence against the alignment hypothesis further comes from the recent work of Wasteneys and coworkers. By using drugs and temperature sensitive mutants they showed that CMFs align properly in the absence of normal cortical microtubules (Sugimoto et al. 2003; Himmelsbach et al. 2003). Interestingly, an orientation of both of them perpendicular to the growth axis appears to be a precondition for cell elongation in the right direction. (review: Wasteneys and Galway 2003). Another example of cells with nonparallel cortical microtubules and CMFs is found in the maturation zone of water-stressed *Zea mays* roots, where cortical microtubule arrays turn right handedly, but CMFs left-handedly (Baskin et al. 1999). Baskin and coworkers (2004) have recently demonstrated quantitatively that local CMF alignment does not require cortical microtubules. The growth pattern in cells mildly treated with microtubules drugs shifted from anisotropic in the direction of the root to more isotropic. At the same time, the net alignment of cortical microtubules acquired a less strictly transverse orientation. Polarized light microscopy of CMFs, which gives overall CMF direction of whole cell walls, showed unaltered net CMF orientation, but with deviations from the transverse orientation in the oryzalin-treated cells larger than in the controls. Field emission scanning electron microscopy of innermost wall layers showed local deviations from the transverse orientation in the drug treated cells.

These authors conclude that cortical microtubules are dispensable for CMF alignment locally, but not globally.

It is important to realize that, in most instances, the elongation direction changes after microtubule depolymerization as well (Baskin 2001) and this was not checked in studies relating cortical microtubule orientation to the orientation of nascent CMFs. Thus, microtubule depolymerization has apparently two effects that may or may not be related: change of CMF orientation and change of cell elongation direction, i.e., cell form acquisition. One cannot infer from the results whether cortical microtubule depolymerization has an effect on both parameters independently, or on CMF orientation and, therefore, cell elongation direction, or rather cell elongation direction and, therefore, CMF orientation. This problem is not merely the problem of correlation that we often come upon in cell biology, like the suggestion that if cortical microtubules align with nascent CMFs their orientations should have a causal relationship, or even that the one orients the other. In the drug experiments in which one actor, the cortical microtubule presence, changes two items, orientation of nascent CMFs and cell elongation direction, this goes a step further. In logical reasoning, if A influences B and C, one cannot conclude that B influences C, or C influences B, or that the two are independent. Therefore, elongating cells are not the ideal cells to study the "microtubule – microfibril syndrome" with microtubule drug application. Full-grown cells do not have this problem and are the cells of choice to solve this question. For such an investigation, not the local banded secondary wall deposition in xylem cells, in which the deposition is so dense that individual cortical microtubules and CMFs cannot be discerned, should be used, but the smooth and constant secondary cell wall deposition in most full-grown plant cells.

2.2 The liquid crystalline self-assembly hypothesis

Under suitable thermodynamical conditions, many substances composed of or containing highly elongated chiral molecules form a state of matter known as the cholesteric liquid crystalline phase. In this otherwise liquid phase the molecules spontaneously align, with the direction of alignment rotating in a manner akin to a helical staircase with a pitch (= repeat distance) typically in the order of 500 nm. The apparent structural similarity between the ubiquitous helicoidal texture of fibrous extracellular matrices in nature and the cholesteric liquid crystalline phase first led Bouligand (1976) to propose the hypothesis that cell wall texture could arise from a liquid crystalline self-organization principle. Although in essence an idea based solely on analogy, it nevertheless captured the imagination of many researchers. Especially intriguing was the observation by Abeysekera and Willison (1987) of apparently spontaneous helicoidal order in the pre-release mucilage of quince. Later, several groups established that suspensions or melts, containing cellulose or cellulose derivatives, can form cholesteric liquid crystalline phases (Vian et al. 1994).

In our view, however, liquid crystalline self-organization is a highly unlikely mechanism for cell wall texture formation. In order to obtain a thermodynamically self-organized state, of which a liquid crystal is just one example, a number of requirements need to be met. First of all, a sufficient number of molecules must simultaneously interact. Secondly, the thermodynamical equilibrium state must be reached, requiring the molecules to exhibit both sufficient mobility and changes of conformation to equilibrate all pertinent degrees of freedom. It is not clear that any of these conditions hold at any given stage of cell wall deposition. The CMFs are deposited sequentially from membrane-bound cellulose synthases. They are co-deposited with matrix material into the limited space between the plasma membrane and the already extant cell wall. Under these circumstances their mobility is extremely reduced, if not nonexistent. The same holds a fortiori for the conformational changes. A CMF whose length can safely be assumed to be many microns is essentially a macroscopic object. Even when such an object is in good thermal contact with its environment (e.g., in a low molecular weight solvent) the relaxation times, corresponding to slow long-wavelength modes, become exceedingly large. Moreover, liquid crystalline arrangements are highly sensitive to boundary conditions and equilibrium configurations are readily suppressed by unfavourable pinning of particle orientations at interfaces. The conditions of extreme confinement under which CMF deposition takes places are extremely unlikely to be conducive to the formation of bulk equilibrium phases. Finally, the hypothesis appears limited to addressing the formation of helicoidal textures, and thus begs the question of how other common textures, such as helical and crossed-polylamellate, that can even occur side-by-side with the helicoidal texture within the same cell wall, could be explained by the same mechanism.

2.3 Templated incorporation hypothesis

In his review Baskin (2001), outlines his ideas for a unifying model of CMF alignment. In this, he proposes a "templated incorporation" mechanism, in which templating molecules guide the orientation of nascent microfibrils. These templating molecules attach either to previously deposited CMFs or to plasma membrane proteins that bind cortical microtubules. In this way, both the case in which microtubules apparently do not play a role in the CMF orientation and the case where it is believed they do, can be dealt with in a single conceptual framework. Although at present there is no evidence for the existence of the templating molecules, the hypothesis is an intriguing one. However, an explanation for the sustained orientational order over distances of micrometers, as observed in cell wall lamellae, would require in our view an unrealistic degree of correlated alignment between the templating molecules. To transmit orientational information from one CMF to another CMF or from a microtubule to a CMF in a reliable fashion would require that the templating molecules always bind in fixed orientation to the fibers involved. It is not clear that the relatively disordered surface of a CMF or the inevitable molecular flexibility of the hypothesized

membrane-microtubule associated proteins allow this requirement to be met. Furthermore, the "guiding" fibers (microtubules or microfibrils) are themselves not perfectly aligned to begin with, which causes nearby templating molecules to have a distribution of orientations. We believe that these effects will accumulate so that the inherent molecular disorder will be amplified to destroy any original imposed ordering after the deposition of just a few lamellae. Himmelspach et al. (2003) reported that CMFs recovered in transverse patterns, without a well-ordered preexisting microfibril template in *Arabidopsis mor1–1* with disrupted cortical microtubules. These authors conclude that cellulose microfibril orientation is largely generated by mechanisms that do not rely on any templates.

3 THE GEOMETRICAL MODEL FOR CELLULOSE MICROFIBRIL ORIENTATION

In his review Baskin (2001) has also assessed the generality of the hypothesis that microtubules align CMFs. In that paper he states that "alignment of CMFs can occur independently of microtubules", showing that an alternative to the alignment hypothesis must exist. We have proposed that the default mechanism, which determines the orientation of CMFs as they are deposited in the absence of other influences, is geometrical in origin. Based on the observation that CMFs always appear approximately evenly spaced in close-packed lamellae and that their average distance apart does not depend on their orientation with respect to the cell axis, the geometrical close packing rule was formulated (Emons 1994):

$$\sin \alpha = \frac{Nd}{2\pi R}$$

This formula relates the CMF winding angle α to the number of CMFs being deposited (N), the distance d between them and the radius R of the cell. This explicit mathematical rule is the corner stone of a dynamic developmental model, which rests on the assumption that new active cellulose synthases insert into the plasma membrane through exocytosis of Golgi vesicles, or else, are activated within moving localized regions along the cell, the cellulose synthase activation domains (CSAD) (Figure 11-1). The rate at which new synthases become active is under cellular control and regulated, and the microtubules may well play a yet unknown role in this process, as discussed before (Emons and Mulder 1998, 2001). Once activated in the plasma membrane, the cellulose synthases move forward propelled by the forces generated in the CMF deposition and/or crystallization process. In the course of time, their angle of motion with respect to the cell axis is continuously adapted to the changing number of other cellulose synthases in their neighborhood in order to satisfy the geometrical close packing constraint. The CMFs deposited follow the tracks of the cellulose synthases and as such constitute a "recording" of their motion. The final ingredient of the model is that cellulose synthases have a finite active lifetime.

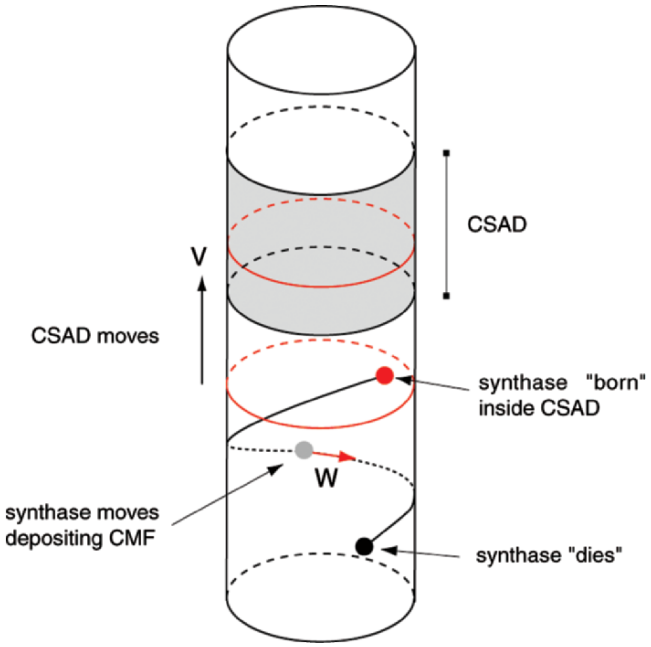

Figure 11-1. The cellulose synthase life cycle. After being inserted into the plasma membrane within a cellulose synthase activation domain (CSAD: located between the red circles at the time of deposition) the synthase moves with an average speed w within the plasma membrane, leaving a cellulose microfibril in its wake. The direction of motion and hence the angle the deposited CMF makes with the cell axis is determined by the local density of other synthases. The CMF synthase becomes inactive after a characteristic lifetime $t^{\dagger}$, which determines the length of the microfibrils. The CSAD itself, here shown in grey, moves with a speed v in the direction opposite to that of the CMF synthases (*See Color Plate of this figure beginning on page 355*)

The elements outlined above are cast into the form of a partial differential equation describing the evolution, both in space and in time, of the density of active cellulose synthases present in the plasma membrane. This equation takes the following form on a cylindrical cell of radius R

$$\frac{\partial N(z,t)}{\partial t} - \frac{wd}{2\pi R}\, N(z,t)\, \frac{\partial N(z,t)}{\partial z} = \varphi(N,z,t) - \varphi^{\dagger}(N,z,t)$$

where w is the speed with which the synthase moves and d the effective width of a CMF plus adherent matrix material, i.e., the distance between neighboring CMFs. φ is the local rate of synthase production for which we choose the following form

$$\varphi(N,z,t) = \frac{N_*}{t_*(1-\gamma)}\left(1-\frac{N(z,t)}{N_{\max}}\right)^{\gamma} = \text{if } N(z,t) < N_* \text{ and z is located inside a CSAD}$$

In all other cases $\varphi = 0$. The parameter γ controls the shape of the synthase production curve and ranges between zero and one. Synthase production stops when the maximum density

$$N_{\max} = \frac{2\pi R}{d}$$

is reached, which for stationary CSAD would happen after time t_*. The insertion domains are assumed to have a length l and travel at a speed v. Finally, the local rate of rosette de-activation $\varphi^\dagger$ needs to be determined. This rate depends on the full evolution of the density in a time interval of length $t^\dagger$ (= the synthase lifetime). Fortunately, the resultant equations are of a type that can be readily solved with entirely classical techniques. The solutions of these equations can be reinterpreted in terms of the tracks of the cellulose synthases, and hence the orientations of the deposited CMFs, thus leading directly to the cell wall texture. Because of its geometrical origin, the model has only a small number (4) of relevant parameters (Table 11-1). We have shown that by varying these parameters several known cell wall textures can be reproduced by this fully predictive mathematical model: the axial, helical, helicoidal, and crossed wall texture (Figure 11-2). Recently arguments were put forward to relate it also to the random texture. In this view the random cell wall in fact is a helicoidal wall, however with such large spacings between the microfibrils that the texture looks to be random (Mulder et al. 2004).

The geometrical model provides a conceptual framework for the alignment mechanism of CMFs, which unites examples where cortical microtubules are and are not parallel to nascent cellulose microfibrils, and in which they do not directly move or channel the synthases but may be involved in their activation inside the plasma membrane. The basic line is as follows: by *default* CMFs go straight unless obstructed and their alignment depends mainly on the number of cellulose synthases simultaneously active at any position in the plasma membrane. The geometrical model does not rule out that cortical microtubules bind to the plasma membrane so tightly that synthase movement is obstructed, which could be the case in elongating cells in which both polymers are always in line with each other and transverse to the cell elongation direction, (Emons and Mulder 1998; Emons et al. 2002).

Table 11-1. Relevant parameters of the geometrical model

Length of the CSAD	$\lambda = \frac{l}{wt_*}$
Speed of the CSAD	$\beta = \frac{v}{w}$
Synthase lifetime	$\tau^\dagger = \frac{t^\dagger}{t_*}$
Synthase production curve shape	γ

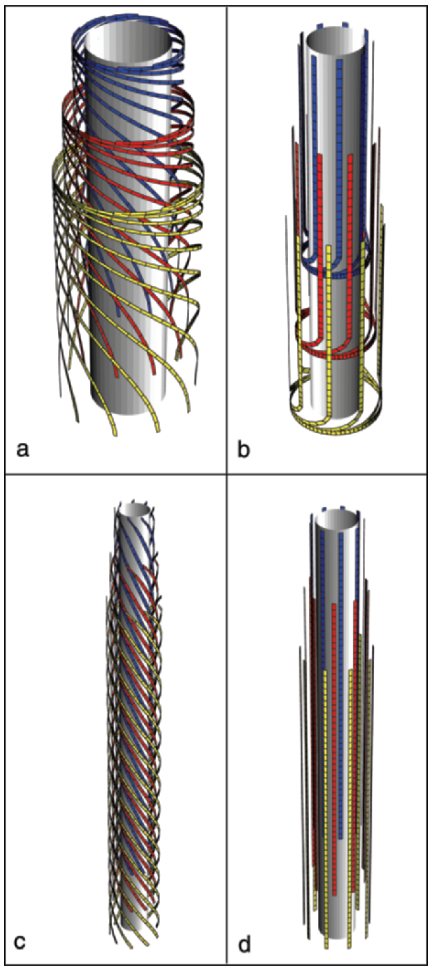

Figure 11-2. Different cell wall textures as predicted by the geometrical model. The ribbons shown represent the tracks of CMFs, obtained from the explicit solutions to the CMF evolution equation. (**a**) The helicoidal texture in which the angle of orientation between subsequent lamellae changes by a constant amount. (**b**) A crossed polylamellate texture with alternate lamellae with transverse and axial oriented CMFs. (**c**) A purely axial texture. (**d**) A helical texture in which the CMFs have an almost constant winding angle (*See Color Plate of this figure beginning on page 355*)

4 A ROLE FOR CORTICAL MICROTUBULES IN LOCALIZING CELL WALL DEPOSITION

Green came to the idea of transverse CMFs determining cell elongation direction because he viewed plant cell growth primarily as "…the yielding of cell wall to the turgor pressure of the cell vacuole …" (Green 1962). However, apart from wall yielding to turgor pressure, a second, equally important process is involved in cell elongation, which is the wall deposition itself. For anisotropically longitudinally elongating cells, we not only have to look for a mechanism that allows wall yielding in the right direction, but also for one that channels new wall material to the right cell faces. In an anisotropically longitudinally elongating cell, these should be the sidewalls. Transversely aligned microtubules are in a very

good arrangement to be part of this positioning mechanism. A putative glycosylphosphatidylinositol (GPI)-anchored protein COBRA, which is mainly localized at the longitudinal sides of elongating root cells (Schindelman et al. 2001) and a kinesin-like protein (Zhong et al. 2002) may be involved in this process. Since wall material enters the cell wall as the content of Golgi vesicles, the microtubules, looping around the cell's sidewalls, and not the transverse walls, could be part of a system that locates the Golgi bodies or the Golgi vesicles to those cell faces. Evidence for microtubules, possibly acting in such a way, is the localization under the bands of cellulose in xylem cells, reviewed by Baskin (2001), and in *Arabidopsis* mutants shown by Gardiner et al. (2003). One should realize, however, that determining exocytosis or activation sites, by a yet unknown mechanism, is a completely different function for cortical microtubules than orienting cellulose synthases during CMF deposition, either by directing the synthases or channeling them through the plasma membrane. Recent work by a consortium of plant researchers (Roudier et al. 2005) shows COBRA to be required for the oriented deposition of cellulose microfibrils and to be aligned in narrow bands perpendicular to the long axis of diffuse anisotropically elongating cells in a pattern different from, but depending on, cortical microtubule organization.

The geometrical model also does not rule out, even favors the idea, that cortical microtubules are (part of) the mechanism that regulates the sites and or amounts of cellulose synthase insertion, i. e., exocytosis or activation areas in the plasma membrane. Inferring from our knowledge of tip growing cells this would require modulation of the actin cytoskeleton (Miller et al. 1999; de Ruijter et al. 1999; Ketelaar et al. 2002, 2003), as well as of calcium ion gradients at those sites (de Ruijter et al. 1998). However, we cannot rule out that exocytosis goes on everywhere and that synthases are activated, or even assembled, locally inside the plasma membrane. The crucial factor in the geometrical model is that density of active cellulose synthases in the plasma membrane is the default determining factor for CMF direction control. Intuitively and scientifically, this factor is directly linked to CMF ordering since the CMF synthase complexes are the nanomachines that spin out the fibrils themselves. This self-ordering mechanism is tightly controlled by the cell, which controls cellulose synthase activation in the plasma membrane.

5 CRITICISM ON THE GEOMETRICAL MODEL

In his review, Baskin (2001) presents a criticical discussion of the geometrical model. On page 157, he states: "…several of the model's assumptions appear to contradict observations". The points he specifically mentions are:

(1) The geometry of the root hair changes with colchicine treatment but the helicoid does not (Emons et al. 1990). (2) and the density of neither the CMFs (Emons 1989) (3) nor the rosettes (Emons 1985) changes with the distance from the apex according to the model's assumptions." Here we take the opportunity to comment on the issues he raises.

Ad (1). As shown in Emons et al. 1990, the geometry of the new part of the hair is wider after colchicine treatment than before colchicine treatment. In this article, we state that the type of texture has not changed; it has remained helicoidal. Of course, not every change in morphology is enough to change the *type* of texture. Furthermore, a detailed analysis of this wall after colchicine treatment, nor measurements of angles between CMFs in subsequent lamellae and with the long axis of the hair, nor a mathematical working out of these measurements have been carried out. Of much more interest is the fact that the cell wall texture in old *Equisetum hyemale* root hairs, in which the cell dimension has changed drastically because the lumen of the cell has almost completely been filled with cell wall, has become axial (Emons and Wolters-Arts 1983). This is like the geometrical model would predict (Emons 1994; Emons and Mulder 1998) and this change is gradual with a helical transition phase in between the helicoidal and axial textures, (Emons and Wolters-Arts 1983), apparently depending on the cell width.

Ad (2). In fact, the areal density of CMFs (= total length of CMF per unit area, measured on a scale sufficiently large with respect to the mean distance between the CMFs) within a lamella does not increase at all, not in reality and neither in the model. One of the striking observations made on the helicoidal cell wall of *Equisetum hyemale* root hairs was that the distance between the cellulose microfibrils within lamellae does not depend on the CMF orientation in those lamellae. Moreover, the length of an area with a certain orientation as measured along the plasma membrane also does not depend on the CMF orientation (Emons 1989). Therefore, although the resulting cell wall locally seems to consist of lamellae with microfibrils having regularly rotating CMF angle, the deposition mechanism could never be that of helices with a constant pitch being wound around the plasma membrane at consecutively different angles. This would namely give rise to short areas having transverse microfibrils and long areas with longitudinal microfibrils. The cornerstone of the geometrical model (Emons 1994; Mulder and Emons 2001) is the change in the *number of active synthases* at a given location. These changes arise from the interplay between the motion of synthases, the creation of new synthases inside the CSADs and the inevitable deactivation of synthases. In our view a CSAD encompasses the whole circumference of a cell. When a CSAD passes any location in the cell, the number of cellulose synthases locally increases. An increased number of synthases implies an increase in the winding angle. In this way, lamellae with different angles are being formed in the model.

Ad (3). The density of rosettes in freeze fracture images cannot be measured in areas of the plasma membrane that are sufficiently large. We hope to have a GFP-cellulose synthase fusion construct soon. The only observations that could be made in the freeze fracture study are densities of rosettes in areas with good platinum shadowing, which in a bent surface can never be optimal for the whole surface. Areas with and areas without rosettes were observed; and when there were rosettes present their density was up to 15 per μm^2 (Emons 1985).

Another problem one could have with the geometrical model is that it would not be able to account for local differences in texture in different faces of the same cell, as are seen in epidermal cells of leaves. However there is no reason to suppose that a cell would not be able to regulate the cellulose to matrix ratio and, therefore, its wall texture in different wall facets.

6 OUTLOOK ON THE VERIFICATION/FALSIFICATION OF THE GEOMETRICAL THEORY

The geometrical theory predicts definite effects on the CMF angle and hence on the resultant wall texture following changes in the amount of active synthases (N), the cellulose to matrix ratio (d) and cell geometry (D). The amount of active synthases, moreover, is determined in a definite fashion by the intrinsic parameters of the model shown in Table 11-1: the length of the CSAD, the speed of movement of CSAD, the cellulose synthase lifetime and synthase production curve shape. To verify, falsify, or improve the model we should measure these parameters and relate them to the types of textures formed.

Based on the theoretical results, a next round of experiments has been defined and is being carried out in our laboratory: (1) wall texture of root hairs of wild type and *rsw1* mutant of *Arabidopsis* is analyzed, (2) insertion or activation sites of cellulose synthases in the plasma membrane of diffuse growing cells are being determined, (3) measurements of physical parameters of CMFs *in vitro* are being performed, and (4) the theory is further being worked out. The geometrical model for cell wall texture formation is gaining importance now that, from work on *Arabidopsis* mutants, the microtubule or microfibril paradigm does not seem to be as straightforward as once thought and cannot explain CMF orientation regulation in general.

A kinesin-like protein (FRA1) influences cell wall strength and the oriented deposition of CMFs, at least in fiber cells, without effecting cortical microtubule alignment (Zhong et al. 2002). Fibers are fragile, stems are stronger than in the wild type and the plants are shorter caused by short cells, although wall composition is unchanged. Still, an ordered, helicoidal-like (Zhong et al. 2002, Figure 4), cell wall is being produced. Our conclusion from the FRA1 phenotype is not necessarily in favor of cortical microtubules functioning in the regulation of CMF orientation. The interesting results of this work rather show that (1) CMF orientation determines mechanical cell and tissue properties, (2) transverse CMF orientation correlates with the degree of cell elongation, (3) cortical microtubule orientation by itself cannot determine CMF orientation, nor degree of cell elongation, but can be involved in determining elongation direction, (4) a kinesin-like protein that binds tubulin is needed for CMF patterning transverse to the elongation direction and may well be involved in determining the location of the CSADs inside the plasma membrane. A change in patterning of cellulose synthases in the plasma membrane in our model would give rise to a different wall texture. How the geometrical model behaves in elongating cells is a task we still have to undertake.

Plant cell walls have tremendous commercial value. Understanding and manipulation of their properties will greatly enhance their application. We are not close to understanding the complete process. However, the future is bright. Now that we have mutants, GFP-constructs, and advanced microscopes, we have the tools to verify or falsify existing hypotheses and build up the basis of a consistent theory.

After writing this chapter new information about the movement of the cellulose synthase complexes came from the laboratory of Somerville in Stanford (Paredez et al. 2006). The work of Paredez et al. proves that the synthase complex indeed moves inside the plasma membrane, steered by the propulsive force of cellulose microfibril generation, its own product, and that in the cells examined, the microtubules are guide tracks. The work also shows that it is highly improbable that a direct attachment exists between the cellulose synthase complex and the cortical microtubule, since the complexes move along microtubules bidirectionally. The microtubules could be fences for the complexes and once the complexes bump into them have to follow them, which brings us to the starting point of our hypothesis: "rosettes go straight unless obstructed."

Indeed, in this recent work, it is shown in addition that when the cortical microtubules are completely depolymerized, cellulose synthase complexes move in highly ordered patterns!

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