

REVIEW / SYNTHÈSE

Plant and fungal cytomechanics: quantifying and modeling cellular architecture¹

Anja Geitmann

Abstract: Biomechanical studies aim at understanding the relationship between the mechanical properties of biological structures and their function. In cytomechanical investigations, this approach is brought down to the scale of cells and sub-cellular structures. In plant cells and the hyphae of fungi and water molds, interactions between turgor pressure, the cell wall, and the cytoskeleton are considered of primary importance. This review is an overview of how the mechanical properties of these individual features and their interactions have been measured and how the experimental data are used to produce theoretical mechanical models of cellular architecture and dynamics. Several models are discussed, and focusing on the example of tip-growing cells, various approaches to understanding the mechanical aspects of cellular morphogenesis are analyzed.

Key words: cytomechanics, biomechanics, cell wall, turgor, cytoskeleton, tip growth, modeling.

Résumé : Les études biomécaniques ont pour but de comprendre la relation entre les propriétés mécaniques des structures biologiques et leur fonction. Les recherches en cytomécanique se font à l'échelle des cellules et des structures subcellulaires. Chez les cellules des plantes et les hyphes des champignons et des oomycètes, les interactions entre la pression de turgescence, la paroi cellulaire, et le cytosquelette ont une importance primaire. L'auteur revoit comment les propriétés mécaniques de ces caractéristiques et leurs interactions ont été mesurées et comment les données expérimentales sont utilisées pour produire des modèles mécaniques théoriques de l'architecture cellulaire et de sa dynamique. On discute plusieurs modèles et, en mettant l'accent sur les cellules de croissance apicale, on analyse diverses façons de comprendre les aspects mécaniques de la morphogenèse cellulaire.

Mots clés : cytomécanique, biomécanique, paroi cellulaire, turgescence, cytosquelette, croissance apicale, modélisation.

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Introduction

Living organisms are architectural beings. While biochemical interactions and signalling processes are important features that help to understand physiology, actual function of the organism is closely linked to mechanical and physical properties of its organs, tissues, and even every individual cell. During their lifetime, plants have to achieve mechanically demanding tasks such as resisting wind and gravity or anchoring themselves in the soil. The physical properties of numerous plant organs are also of significant commercial interest. The mechanical behaviour of wood, the physical resistance of tomatoes to compression stress, and the capacity of wheat stems to resist forces exerted by wind are three out of countless examples that illustrate how important the

understanding of the biomechanical properties of plant tissues is for agriculture as well as for construction and food industries. Numerous devices have been developed to assess the physical properties of plants on the organ level such as those to monitor bending and compression in which pieces of tissue are clamped and subsequently deformed. A principal aspect of biomechanics is the analysis of the relationship between the mechanical properties assessed using these physical experiments and the architecture and molecular composition of a biological structure.

Architecture and mechanics determine the functioning of an organism on multiple scales. While biomechanics and mechanical modeling at the organ level are rather well-established disciplines, the attempt to quantify physical properties on the level of individual cells, cytomechanics, is relatively new. However, since cellular mechanics not only determine the physical behaviour of mature organs but also the formation of organs in the first place (Green 1980), a closer look at the scale of individual cells is bound to contribute important information to the understanding of plant functioning.

Moving the size scale down to objects in the micro- and nanometer range implies a technical challenge, since experimental devices in the appropriate dimension that can be

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used to carry out physical measurements have to be developed. The present review aims at giving a glimpse into the techniques and methodological approaches that have been undertaken to do so. It will concentrate on data obtained on plant and hyphal cells while occasionally referring to progress achieved with mammalian cells. For simplicity, I will talk about hyphae when describing fungi and oomycetes, since their mechanics are very similar albeit they have evolutionary distant origins. Focus will first be on the cellular components that are the structural pillars of plant and hyphal cellular architecture: turgor pressure, the cell wall, and the cytoskeleton. In a second part, the interaction between these components during cellular morphogenesis and growth will be investigated and approaches to theoretically model cell architecture and growth processes will be discussed.

Turgor pressure establishes a hydroskeleton

A significant difference between animal and plant cells concerning their architecture and mechanics is the presence of turgor pressure in the latter. In plant cells, establishment of this internal pressure is possible due to the presence of the surrounding cell wall that withstands the forces exerted by the pressure. By putting cell walls under tension stress, the turgor functions like a hydroskeleton, thus contributing significantly to the mechanical properties of plant organs, especially those that are herbaceous. Wilting is basically the collapse of plant tissue because of the lack of internal pressure in the individual cells. It is surprising, however, that turgor pressures are often higher than is necessary for supporting aerial organs (Mouliat et al. 1994). Unfortunately, few experiments have been done relating the absolute values of turgor to the physical demands of tissues. More quantitative investigations are therefore warranted.

The mechanical principle of the hydroskeleton is an important mechanism that is also employed for plant movement. The technical principle behind many movements is based on the ability of certain strategically located cells to rapidly increase or reduce cellular volume by uptake or excretion of water. The opening and closing of stomatal guard cells and leaf and branch movements caused by bulliform cells and pulvini are only a few examples where this mechanical principle has been realized to allow movements on individual cellular or on the organ level.

To understand turgor-based mechanical behaviour, it is pivotal to quantify the pressure in individual cells. Measurements of turgor pressure have been attempted with various indirect approaches based on the quantification of the osmotic pressure such as vapour point psychrometry, isopiestic psychrometry, and the pressure bomb. Other indirect methods used to assess turgor include incipient plasmolysis, incipient cytorrhysis or cell collapse, freezing point osmometry, melting point osmometry, and vapour pressure deficit osmometry (summarized by Kaminskyj et al. 1992; Money 1997).

Direct measurement of turgor pressure on the single cell level in real time, however, was only made possible with the development of the pressure probe. The first pressure probe was a water- and air-filled glass microcapillary devised to make direct measurements of turgor in the giant-celled alga *Nitella* (Green 1968). The compression of an air

bubble allowed cellular pressure to be estimated using Boyle's Law. The technique was then improved by the group of Zimmermann and Steudle (1978) who replaced the air bubble with an electronic pressure sensor attached to an oil-filled capillary. Their model also included a piston that allowed turgor in the cell to be varied artificially, thus allowing for the assessment of parameters such as the half-time for water exchange, hydraulic conductivity, and cell wall volumetric elastic modulus. These parameters were first determined for a number of giant-celled algae such as *Nitella*, *Chara*, and *Acetabularia* (Zimmermann and Steudle 1978). In these cells, turgor pressures were measured to typically range from 0.1 to 0.6 MPa (Tomos 1988). Further improvements of the device finally allowed the measurement of much smaller cells, and thus, higher plant cells such as stomatal guard cells could be investigated (Franks 2003; Hüskens et al. 1978). Values in well-watered higher plants were found to range from below 0.1 MPa to over 1 MPa. To appreciate these numbers, it might help to realize that an inflated car tire typically has a pressure of 0.2 MPa and household water pressure amounts to around 0.3 MPa. Plant cell walls therefore have to provide considerable mechanical resistance against these pressures.

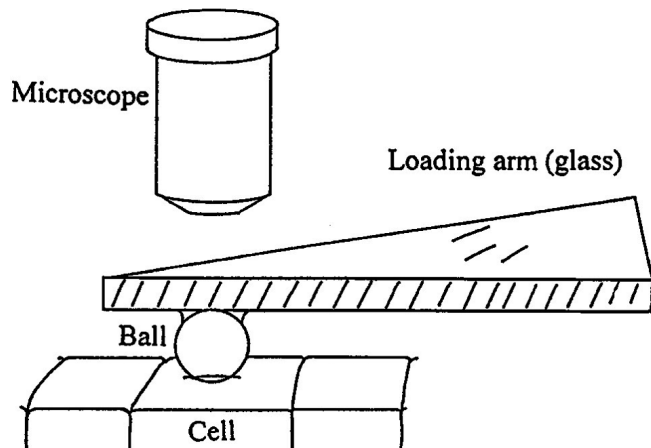
This accomplishment is topped, however, by the pressure observed in the penetration organs of fungal hyphae such as *Magnaporthe grisea*, which was measured to be between 6 and 8 MPa, thus 30–40 times the pressure of a typical car tire (Howard and Valent 1996). To determine the pressure in these cells, a cell collapse assay was used that calculated the concentration of polyethylene glycol required to collapse an appressorium. Special thick and rigidified cell walls are necessary to resist the kind of pressure observed in appressoria (Emmett and Parbery 1975; Howard and Valent 1996). Furthermore, these structures must be able to accumulate large amounts of solute to actually drive the water influx into these cells. In *M. grisea*, the solute providing the osmotic potential for turgor generation is glycerol, which accumulates to concentrations in excess of 3 mol·L⁻¹ (de Jong et al. 1997).

The pressure probe method necessitates the insertion of a micropipette into the cell, thus being invasive and prone to artefacts. A less invasive method of assessing turgor on the single-cell level was developed by Lintilhac and Wei, although its applicability is limited to surface cells with rather thin cell walls. They calibrated the cell's ability to bear a spherical load and related the load-induced deformation of its surface with the pressure inside the cell (Fig. 1), the same principle as a device used to measure eye pressure. This tonometer approach has been successfully applied to plant epidermal cells in which, if the cells were turgid, this noninvasive method produced results similar to those obtained with the pressure probe (Lintilhac et al. 2000; Wei et al. 2001).

The cell wall provides support as an exoskeleton

The cell wall represents the most prominent structural feature that distinguishes plant and hyphal cells from animal cells. By surrounding themselves with a more or less rigid shell, plant cells generate tissues that are structurally very

Fig. 1. Setup of the ball tonometer probe in relationship to the microscope axis and target tissue. A turgid cell is compressed by a ball-shaped indenter, resulting in a spherical, cup-shaped contact. The size of the contact patch can be related to the internal pressure of the compressed cell. (From Lintilhac et al. (2000), reproduced with permission of J. Plant Growth Reg., Vol. 19, p. 92, © 2000 Springer Science and Business Media.)



different from their animal counterparts. From a mechanical point of view, in plant tissues, the cell wall is the dominant load-bearing feature that determines structure. Two mechanical strategies can be identified. (i) The ability of the cell wall to resist tensile stress allows for the establishment of the turgor-based hydroskeleton and thus erectile forces that are able to withstand applied stresses. This principle is predominantly realized in cells with primary cell walls. (ii) Secondary cell walls typical for sclerenchymatic tissue are equipped to not only resist tensile but also compressive stress. In this case, the living protoplast and thus the turgor are dispensable for the cell's mechanical functioning, and fully differentiated cells are often devoid of any cell component other than the cell wall.

Plant cell walls are composed of a variety of polymers that differ in their biochemical and biomechanical properties. The mechanically dominant feature in many cell walls is cellulose, which forms microfibrils with an estimated stiffness of 130 GPa (Vincent 1999). Considering its specific gravity, this value is comparable with that measured in high-performance materials produced in industry. Cellulose forms microfibrils that can be up to 20 nm in diameter. Their arrangement determines the cell wall's capacity to resist forces in different directions as shown by measurements of the anisotropic extensibility of algal cell walls (Probine and Preston 1962; Wei et al. 2006) or single-layer tissues (Kerstens et al. 2001). Microfibrils, if arranged in parallel, would only be able to confer strength in one particular direction. To resist shear stress and stress in other directions, it is assumed that they are linked into a network, with the crosslinks formed by hemicelluloses. These are tethered to cellulose microfibrils by hydrogen bonds and have been proposed to make them into a "sticky" network (Cosgrove 2000). Alternative models for the arrangement of microfibrils and matrix polymers in the cell wall include the "multicoat model" and the "stratified hybrid model" (Cosgrove 2000; Thompson 2005). Three other networks have been identified in the

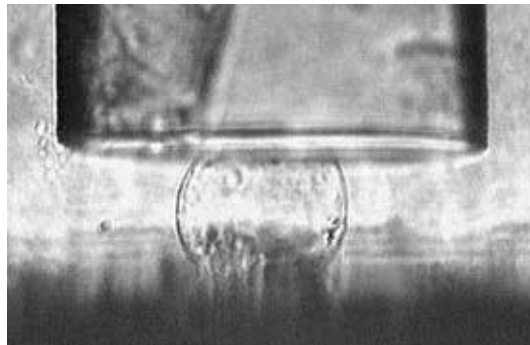
plant cell wall that can be considered more or less independently (Brett and Waldron 1996). (i) Pectins are composed of homopolymeric partially methylated poly- α -(1 \rightarrow 4)-D-galacturonic acid residues and rhamnogalacturonan I and (or) II sidechains. In their deesterified configuration, they can be gelled by calcium ions forming more or less rigid gels. (ii) Glycoproteins can have crosslinking activities, but little is known of their function. (iii) Lignin makes walls more hydrophobic and therefore stiffer, partly because water is a plasticizer or lubricant and partly because the charged groups that would interact with water, were it present, interact with each other, thus increasing the degree of crosslinking.

Individual cell wall components have been analyzed in vitro (Jarvis 1984) and cell wall extensibility has been investigated by measuring the deformation of cell walls, cells, or tissues in response to force applied externally (Cosgrove 1989, 1993; Kutschera 1996). Such investigations have revealed several factors that affect cell wall extensibility. For example, stress relaxation in cell walls was accelerated by pretreating the tissues with plant growth substances (Masuda 1990) and expansins increased the rate of long-term cell wall extension under acidic conditions (Cosgrove 1996).

While approaches to study cell wall components in vitro or entire cell walls in the form of tissue blocks have provided important information, quantitative physical data obtained on the level of individual living cells are scarce. There are several reasons for this lack of information. Most plant cells are present in the form of aggregates forming a tissue. Isolation of a single cell from such a tissue almost inevitably involves a disturbance of the cell wall structure, even though some authors have succeeded in minimizing this effect by peeling off individual cells instead of using chemical maceration techniques (Keckes et al. 2003). Second, while it is relatively easy to clamp a piece of tissue for a creep or bending experiment, the same manipulation on the cellular level is much more difficult, simply because of the size of the specimen. The development of micro- and nanomanipulation methods has therefore been an important step to obtain information on this scale. Compression of individual cells using a micromanipulation probe has allowed the estimation of the Young's modulus of their cell walls (Wang et al. 2004). The authors placed tomato cells grown in suspension culture into a device that compressed the entire cell between two plates, and using a theoretical model for the force deformation data, they were able to conclude on the physical properties of the cell wall of these living cells (Fig. 2). A similar principle has been realized by using a displacement transducer to obtain the volumetric elastic modulus of the cell walls of the huge *Chara* internode cells (Stuedle et al. 1982).

Compression stress is also the concept behind nanoindentation and modulation force microscopy (an application of the atomic force microscope), which have been applied to measure the elastic properties of the cell wall of fungal hyphae (Zhao et al. 2005) and wood fibres. Given that wood fibres do not contain a living protoplast at maturity, the authors were able to cut open the cells horizontally and apply pressure in the direction parallel to the cell surface. This method allowed them to assess the elastic properties of the individual layers present in the fibre cell wall (Clair et al.

Fig. 2. Photograph of a single tomato cell from a suspension culture being compressed between a micromanipulation probe and a glass surface. The comparison between the force deformation curve and a theoretical model allows the calculation of the Young's modulus of the cell wall. (From Wang et al. (2004), reproduced with permission of *Ann. Bot.*, Vol. 93, p. 445, © 2004 Oxford University Press.)



2003; Wimmer and Lucas 1997; Wimmer et al. 1997). The mechanical properties of wood fibre cell walls were also assessed using tensile forces in combination with X-ray analysis of the microfibril angle. Clamping isolated fibres and applying strain using a piezoelectric mechanical stage led to the conclusion that upon exceeding a certain shear stress, the molecular bonds linking cellulose microfibrils break and, interestingly, locking at the new position thus causing a plastic deformation similar to a stick-slip mechanism (Keckes et al. 2003).

Another method that has been used to measure the elastic modulus of the cell wall in living cells is the turgor pressure probe. Under conditions of changing turgor (ΔP), the cell volume (V) will change by an amount (ΔV) determined by the volumetric elastic modulus

$$\epsilon = (\Delta P / \Delta V) \times V$$

The elastic modulus is therefore measured by determining the relative change in volume (measured by microscopy) in response to a change in turgor applied by the pressure probe. Measurements have demonstrated that the phenomenon of stress hardening of ϵ (a stiffening of the material in response to being stretched) occurs in certain but not all plant cells (Tomos and Leigh 1999). Another major finding is that the steady-state expansion rate of cells is modulated without a change in turgor that can be detected by the pressure probe. This indicates that at least in the cells that were investigated, the cell wall properties and not turgor pressure are regulating expansion (Cosgrove and Cleland 1983; Ray et al. 1972; Rich and Tomos 1988; Tomos and Pritchard 1994). This concept cannot be generalized, however, since in other systems, the amount of turgor has been related to the velocity of growth (Proseus et al. 2000).

The cytoskeleton as internal scaffold

The cytoskeleton plays various mechanical roles in cell functioning. In addition to forming the tracks for intracellular transport, the cytoskeletal elements provide the force for cells to contract or to move their edge forward and are involved in the mechanotransduction of mechanical stresses;

at least this is the case in animal cells. The mechanics of wall-less mammalian cells is of course less complex, since essentially only two factors contribute to the physical properties of most animal cell types: the plasmamembrane and the cytoskeleton. Almost all existing data on cytoskeletal mechanics have therefore been obtained from analyzing animal cells.

Numerous micromechanical models explaining the physical functioning of the cytoskeleton have been proposed over the past decade. Whereas simple models have considered the cytoplasm as viscoelastic homogeneous material, more sophisticated models incorporate microstructure and assume that the cytoskeleton is organized of a porous network composed of discrete structural elements. Among the latter, two classes of structural mechanical models have been advanced: the open-cell foam networks and the stress-supported structure (Stamenović and Ingber 2002). In the open-cell foam, stress within the cytoskeleton arises primarily from deformation of individual cytoskeletal filaments (e.g., stretching, bending, and torsion) under the action of externally applied load to the cell (Satcher and Dewey 1996). In the stress-supported structures, a preexisting mechanical stress within the cytoskeleton plays the central role in resisting applied loads. This configuration is also known as tensegrity architecture (Ingber 2003a; 2003b). It takes into account contributions from collective interactions among different cytoskeletal filament systems or from the extracellular matrix. This latter aspect especially makes it an interesting model, the validity of which should be tested for plant cells, since it allows to integrate cytoskeletal mechanics with the mechanics of the cell wall.

A tensegrity architecture is a tensed network of structural members that resists shape distortion and self-stabilizes by incorporating other support elements. In the model, tensional forces are borne by actin filaments and intermediate filaments, and these forces are balanced by interconnected structural elements that resist compression, most notably internal microtubule struts, bundles of crosslinked actin filaments, and adhesions to the extracellular matrix. To illustrate this system of prestressed tension and compression elements, one just has to imagine a camp tent whose membrane (tension element) is made stiff by pulling it between fixed tent pegs and tent poles (compression elements) and possibly tethers to an overlying tree (which would represent attachments to the extracellular matrix). This illustrates that cytoskeletal mechanics cannot be separated from the mechanics of the cell wall and the connection between the two. In this context, it will therefore be essential to advance the understanding of the links between these two components, which in plant cells is still rather limited, albeit for recent progress (Baluska 2003; Wasteneys and Galway 2003).

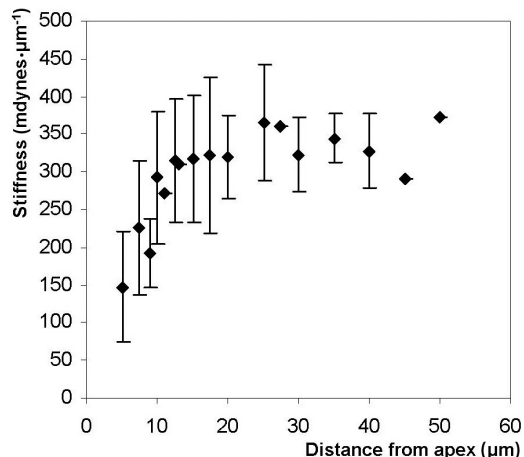
An example that illustrates that experimental data are consistent with the tensegrity model is the mitotic spindle, the mechanism of which is likely to be similar in animal and plant cells. Severing kinetochore fibres using a UV microbeam resulted in movement of the pole towards the equator, thus being consistent with the interpretation of microtubules being compression struts in a system that is under tension (Pickett-Heaps et al. 1997). Unequivocal evidence that distinguishes this model from one based on force balance remains to be shown, however.

Various techniques have been applied to animal cells to determine the physical properties of the cytoplasm structured by the cytoskeleton and surrounded by the plasma membrane. Micropipet aspiration, manipulation with micro-needles or optical tweezers, magnetic bead rheometry, magnetic rotational microrheology, and atomic force microscopy have provided valuable information on the physical properties of different types of animal cells. Not all of these techniques would be applicable for plant cells and almost none of them has been tested on them so far. In fact, the cytoskeleton is certainly the least studied of the structural elements determining plant cell architecture. Of course, numerous studies have investigated the link between the cytoskeleton and cell wall deposition (Baskin 2001; Himmelspach et al. 2003; Wasteneys 2004), but investigations attempting to actually quantify cytoskeletal forces in plant cells are virtually absent. A first approach has been done using microindentation, a technique that has been successfully used to characterize the cytoskeleton-based stiffness of animal cells such as leukocytes (Zahalak et al. 1990). Over the past years, it has been applied several times on thin-walled plant cells such as pollen tubes. While the measured cellular stiffness (Fig. 3) in these cells coincided well with the nonuniform distribution of actin filaments (Fig. 4A), this parameter has yet to be discriminated from the effect of the nonuniform distribution of the mechanical properties of the surrounding cell wall (Geitmann and Parre 2004).

The main reason for the lack of experiments and information is the presumption that in most plant cell types, the cell wall and the turgor are so dominant in determining the forces implicated in cell architecture that neither the plasma membrane nor the cytoskeleton could possibly have a direct mechanical role. While this might be true for plant cells exhibiting secondary and (or) lignified cell wall layers, the situation might be different in rapidly growing and dynamic cells, where the relationship between the turgor, or rather volume increase by water uptake, and the expanding primary cell wall can be assumed to be delicate and the cytoskeleton might have a mechanical function. Since calculations show that forces exerted by turgor are in a higher order of magnitude than those created by cytoskeletal dynamics, this function might not be directly quantitative in the form of propulsive forces. Instead, the cytoskeleton might direct growth and expansion activities and thus contribute to the anisotropy of growth processes.

Knowledge is also still extremely sketchy on how plant cells perceive mechanical cues. For a cell to perceive mechanical signals, some cellular structure needs to be physically stressed and that stress must be transmitted to receptors that in turn trigger a signal response cascade. In mammalian cells, the cytoskeleton is known to be involved in this function, and in plant cells, this is also rather likely to be the case. For example, the gravitropic response of roots is supposedly based on an interaction between statoliths and the actin cytoskeleton. Numerous models for the statolith–statocyte interaction have been proposed over the years. A popular model, referred to as the “tethered” model, postulates that the statoliths are physically connected to the actin filaments, which in turn are anchored to the plasma membrane and (or) endoplasmic reticulum. However, new evidence has indicated that statoliths might not be bound

Fig. 3. Graph plotting cellular stiffness versus distance from the apex of a typical growing *Papaver rhoeas* pollen tube. The values were obtained by microindentation. In the apical 20 μm , a steep increase of stiffness can be noted, whereas no significant variations occur in the distal area. (From Geitmann and Parre (2004), reproduced with permission of Sex. Plant Reprod., Vol. 17, p. 11, © 2004 Springer Science and Business Media.)



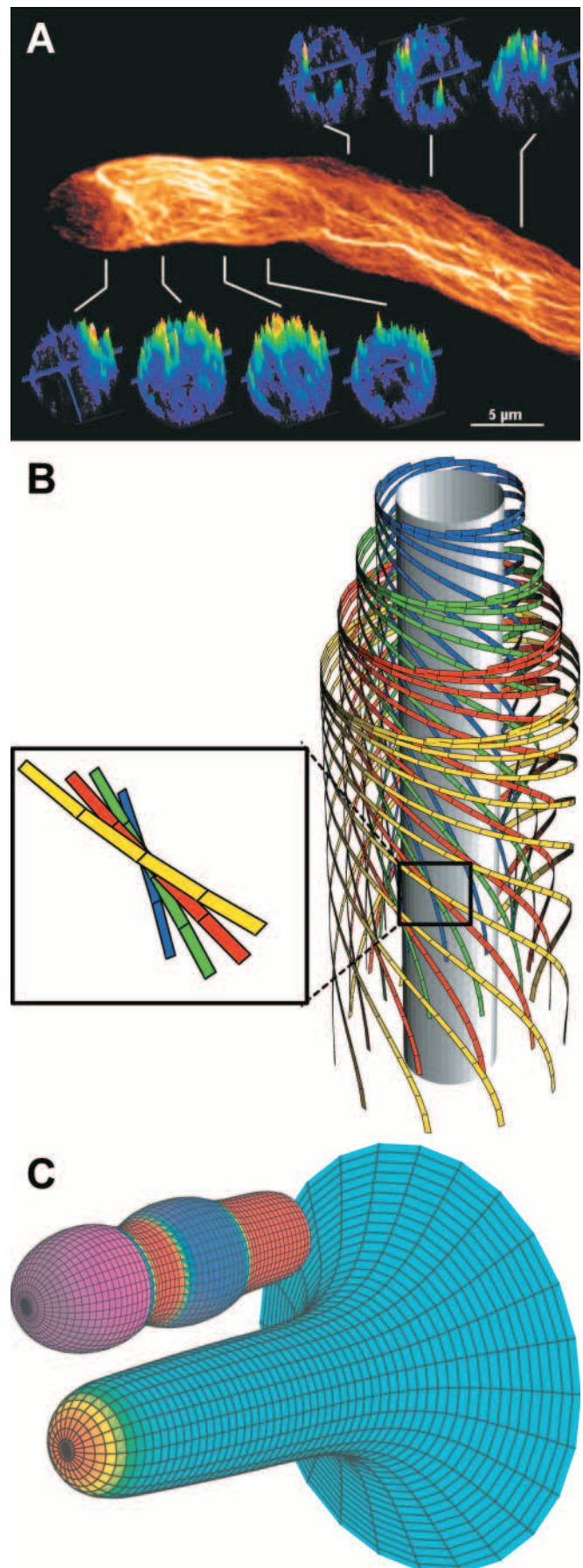
but function by locally disrupting an actin network that is postulated to be linked to stretch-sensitive receptors in the plasma membrane (Yoder et al. 2001). Signal perception would therefore be based on an alteration in the distribution of tension within the cytoskeletal network as a whole, which if confirmed would be perfectly consistent with a tensegrity architecture “in action”.

Mathematical modeling of cellular architecture

Numerous attempts to model the cell or its components in terms of their physical structure and mechanical behaviour have been made. A theoretical biomechanical model serves at least two purposes: (i) the prediction of the behaviour of a structure under stress and (ii) the inspiration for novel experimental approaches. Mechanical models can therefore make an important contribution to the structure–function debate. Any model is an idealization of the real system, so its predictive abilities are constrained by the realism with which the structure and materials are abstracted. Depending on the modeling approach used, it may or may not cover several levels of structural hierarchy, from the dimension of the entire cell down to the interactions between structural molecules. Most available cytomechanical models focus on one of the individual cellular elements such as the cytoskeleton or the cell wall.

Progress on modeling the plasma membrane and the cytoskeleton has been made using mammalian cells. While the membrane has been modeled as a rubber-like membrane surrounding a fluid (Evans and Yeung 1989; Hiramoto 1963), various approaches to mechanically represent the cytoskeleton have resulted in competing models that include that of a tensegrity structure (Ingber 2003a; 2003b), a sol–gel transition phase (Fabry 2001), and open-cell foams (Satcher and Dewey 1996; Stamenović and Coughlin 1999) as mentioned in the previous section. For a review of recent cytomechani-

Fig. 4. (A) z -Projection of confocal laser scanning micrographs of a *Papaver rhoeas* pollen tube labeled with Alexa-488-phalloidin for actin (false colour representation). Surface plots for fluorescence intensity of xz -sections demonstrate that in the subapical region, the actin forms a dense meshwork that could contribute to the increase in mechanical resistance to external deformation observed in microindentation experiments. (B) Visualization of the solution of the geometrical model for the deposition of cellulose microfibrils in a cylindrical cell. Four “generations” of cellulose microfibrils are spun by synthases that were activated at the tip of an insertion domain at four equally spaced moments in time. The displacement of the starting points along the cell is a consequence of the motion of the insertion domain. Each of these generations contributes locally to a single lamella. The inset shows how the angle of deposition between adjacent lamellae is nearly constant, the hallmark of helical texture. (From Emons and Mulder (2000), reproduced with permission of Trends Plant Sci., Vol. 5, p. 38, © 2000 Elsevier.) (C) Three-dimensional representation of the model of growing hyphae developed within the framework of large deformation membrane theory in which the cell wall is represented as a growing elastic membrane with geometry-dependent elastic properties. The inset shows the beading of the model hypha obtained by allowing variations of the elastic modulus of the wall along the hypha. The beading simulates the effect of lytic action of lysozyme on the cell wall. (From Goriely and Tabor (2003b), reproduced with permission of J. Theor. Biol., Vol. 222, pp. 216–217, © 2003 Elsevier.)



cal models relevant for mammalian cells, see Stamenović and Ingber (2002) and for a fundamental approach to their mechanics in terms of flexible polymers, networks, and membranes, refer to Boal (2002).

Modeling of plant cells has almost exclusively covered the mechanics of the cell wall. Depending on the questions to be answered, the cell wall as a material has been treated either as a continuum material with linear elastic stress-strain characteristics or as a material with properties that depend on its polymeric nature. In the latter case, mechanical models generally interpret the different cell wall molecules as constituents of a fibre matrix composite or treat them with theories derived for entangled polymers. Since cellulose microfibrils are understood to play a major role for cell wall mechanics representing the fibre phase, it is not surprising that mathematical modeling of the cell wall mostly focused on their properties and behaviour. An excellent overview of different approaches to model the plant cell wall as a material is given in Bruce (2003).

Because of their commercial importance, plant cells and tissues with secondary cell walls have been studied in depth. While numerous models of wood tissue exist, models on the level of single cells have been developed only recently. Wood fibres, which contain an extremely thick cell wall rich in cellulose, have been modeled as thick-walled concentric cylinders in which each layer is made from an orthotropic material with a given filament winding angle. These layers represent the architecture of the secondary cell wall, which exhibits different microfibril orientations. The analysis was performed using linear elasticity theory and allowed the prediction of stress-strain responses that pointed out the resemblance of the plant cell wall structure to industrial materials such as glass fibre reinforced composites (Davies and

Bruce 1997). A different analogy was used when applying the concept of slip planes occurring in single crystals to model the shear deformation caused by tensile loads on single wood fibre cells (Fratzl et al. 2004). These approaches demonstrate the successful application of modeling techniques originally developed in materials sciences for the analysis of cell wall mechanics.

Since the orientation of the fibres in a composite material determines the anisotropy of its physical properties, the deposition of the microfibrils is an essential process during cell morphogenesis. An approach to model the changing orientation of microfibrils in the different layers of the cell wall was presented by Emons and Mulder (1998, 2000). The authors proposed a geometrical model for cellulose microfibril deposition that quantitatively relates their deposition angle to the density of active cellulose synthases in the plasma membrane, the distance between individual microfibrils within a wall lamella, and the geometry of the cell (Fig. 4B). Since this model does not acknowledge the widely recognized concept of microtubules determining the orientation of microfibrils, it will be an exciting challenge to actually validate it experimentally.

Mechanical principles of cell growth

While understanding and modeling static cellular architecture represents a challenge, a new dimension is added when considering the dynamic processes that change cell shape. Contrary to many animal cells that are able to migrate, most plant cells have a shape that will not change once functional maturity is achieved. However, the way to maturity is generally accompanied by numerous processes that involve a remarkable increase in cell size and significant changes in cell shape, both of which are based on the irreversible expansion of cell walls. This expansion is generally considered to be driven by a turgor-controlled volume increase and involves stress relaxation and polymer creep in the wall (Cosgrove 1997, 2000). Cell walls of growing cells therefore have unique rheological properties that may be measured in various ways (Cosgrove 1993). Numerous approaches have revealed that it is important in this context to distinguish between elastic (reversible) and plastic (irreversible) deformation of the cell wall (Proseus et al. 1999).

Depending on the cell type, cellular morphogenesis has to produce a particular spatial pattern. While the amount of turgor can affect growth velocity (Proseus et al. 2000), pressure is a nonvectorial force. Therefore, it cannot by itself produce shape deformations other than isotropic ones. To produce anisotropic shape changes and thus particular spatial growth patterns, the cell wall has to enter the equation by exhibiting regions that are more easily deformable than others or by possessing preferential directions of expansion. This can be produced by a combination of the following: a nonuniform distribution of cell wall components with different physical properties, a nonuniform localization of the insertion points of new cell wall material, or an anisotropic expansibility determined by the orientation of fibrillar cell wall components. Numerous studies have focused on the latter, the role of the orientation of microfibrils for cell growth. Early models include the concept of “multinet growth” (Roelofsen and Houwink 1953; Green 1960), which for cylindrical cells

postulated the addition of new microfibrils by apposition in a transverse direction to the inner cell wall and their passive reorientation from a transverse to an axial arrangement during growth. This model explained the different orientation of microfibrils found in the cell wall layers of many cells. The question then arose as to how the oriented deposition of microfibrils is controlled. Since the discovery of cortical microtubules, various models for their implication in this process have been proposed, the most widely accepted being the cellulose synthase constraint hypothesis (Giddings and Staehelin 1991). However, while research during the last couple of decades has shown that cortical microtubules clearly participate in the regulation of anisotropic cell wall expansion, their precise role in microfibril alignment has been intensively discussed, since experimental evidence is not unequivocal (Baskin 2001; Himmelspach et al. 2003; Sugimoto et al. 2003). A recent model might consolidate the apparently conflicting experimental evidence. It proposes that not only the orientation of microfibrils but also their length is essential for the direction of cellular expansion and that microtubules have a function in determining the effective functional length of the microfibril (Wasteneys 2004). An exhaustive overview over the principles of the anisotropic expansion of the plant cell wall that also discusses the role of the microtubules has recently been published by Baskin (2005).

Modeling of cellular dynamics

When attempting to represent dynamic processes such as growth and movements, biomechanical modeling has to accommodate an additional group of parameters. Typically, these have to provide quantitative information on the mechanical deformation of existing structures or the addition of structural components over time. In plant cells, growth processes have generally been modeled either by considering the cell wall as deformable or by adding additional cell wall material. Even though knowledge about the molecular interaction between the cytoskeleton and the cell wall is increasingly available (Wasteneys and Galway 2003), none of the mechanical models of plant cell growth have made a real effort to integrate both. In fact, the cytoskeleton has been largely ignored in mathematical models describing plant or hyphal cells and focus has hitherto been on the interplay between turgor and wall properties.

There are two main approaches to the formation of a dynamic model: (i) the force balance method, which is based on the classical theory of Newtonian mechanics, and (ii) the energy balance approach, which combines an energy balance statement with a constitutive strain relation. The force balance method identifies forces that perform work on a system and result in motion and deformation. The drawback of this model is that all forces need to be known. In a complex system, the energy balance model, which uses the change of thermodynamic potential energy as the process performing the work, can therefore be an advantage. A more detailed analysis of the usefulness of these approaches is given in Bruce (2003).

The complexity of a theoretical model and the associated calculations strongly depend on the complexity of the geometry of the investigated structure. Therefore, simple three-di-

mensional configurations have significant advantages, such as tip-growing cells. In addition to their simple cylindrical geometry and unidirectional growth, these cells typically exhibit impressive growth rates and thus allow observations of visible changes in real time. Tip growth is a widespread mode of cellular morphogenesis found in prokaryotes (streptomycetes), fungi (e.g., hyphae and budding yeasts), plants (e.g., root hairs and pollen tubes), and animals (e.g., neurons). Typically, these cells do not form a tissue but individually invade other tissues or substrates. The defining feature of tip growth is that cell expansion proceeds from one extremity of the cell. Consequently, all of the key processes that control morphogenesis are confined to a small region of the cell where they can be observed concomitantly. In plant and hyphal cells, two basic principles characterize the mechanics of tip growth: a combination of stretching (or deformation) of the existing cell wall and the continuous addition of new cell wall material by exocytosis. Both processes are confined to the region of the apical dome, contrary to the principle realized in diffusely growing cells. This makes good sense energetically, since this mode of growth is more efficient than having to overcome the extra friction with the growth substrate that would be generated if the growth occurred throughout the length of the cell. The driving force that causes the stretching of the cell wall is mainly attributed to the turgor-controlled increase of cellular volume through water uptake, but a mechanical role for the cytoskeletal elements has also been proposed (Money 1997; Steer 1990), especially for situations when turgor is low (Heath and Steinberg 1999). It remains to be shown experimentally whether forces established by cytoskeletal dynamics are actually able to contribute an appreciable propulsion force in fully turgid cells. In certain situations, the cytoskeleton might actually act on the opposite side of the equation by reinforcing the growing tip and protecting it against the propulsion force exerted by the turgor pressure (Jackson and Heath 1993).

The striking features of tip growth morphogenesis have inspired many attempts to model this process, most of which were applied to hyphae (Bartnicki-Garcia 2002). Most of the early models put forward equations that approximate the shape of these cells and therefore have their merit. However, many of them are basically geometric exercises that formulate equations from artificial coordinates and reference points for which there are no clearly identified corresponding subcellular structures underlying these markers (Reinhardt 1892; Da Riva Ricci and Kendrick 1972; Trinci and Saunders 1977; Prosser and Trinci 1979; Koch 1982; Prosser 1994; Denet 1996). The vesicle supply centre model by Bartnicki-Garcia et al. (1989) was an attempt to develop a mathematical model based on a subcellular structure involved in the production of additional cell wall material: secretory vesicles. The original two-dimensional mathematical formulation was based on the concept that tip growth is produced by wall-building vesicles emanating randomly, and in all directions, from a vesicle supply centre that advances moving along a straight path. The position of the theoretical vesicle supply centre corresponds to that of the Spitzkörper found in many hyphae, a cytoplasmic aggregation of secretory vesicles and cytoskeletal elements close to the growing apex. The subsequent three-dimensional derivation

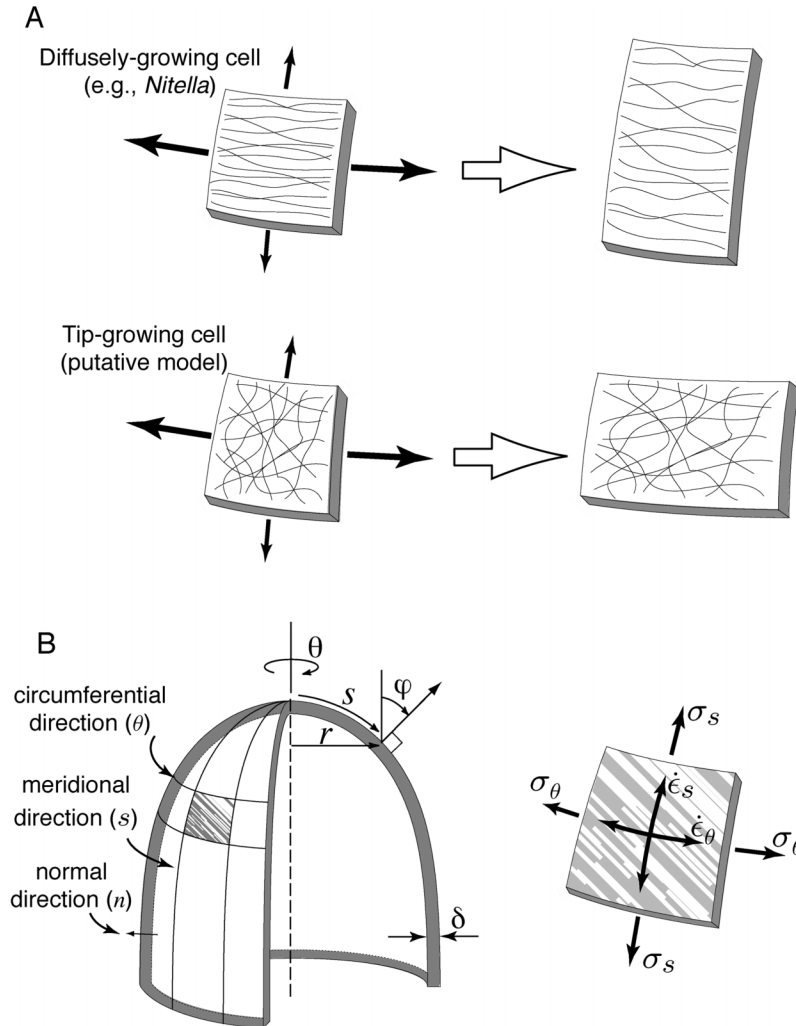
of this model led to a mathematical indeterminacy whose solution required defining a priori the pattern of expansion of the wall (Bartnicki-Garcia et al. 2000; Gierz and Bartnicki-Garcia 2001). The authors therefore measured wall expansion by tracing the movement of marker points on the cell surface. They thus added the parameter describing the mechanical deformation of the wall to their model. Another recent improvement of the model concerned the mechanisms of vesicle delivery and vesicle fusion (Tindemans et al. 2006). While being able to explain the formation of a cylindrical tube very elegantly, the vesicle supply center model fails to include parameters important for the functioning of the living cell such as the turgor pressure.

Turgor was included in the approach by Goriely and Tabor (2003a, 2003b) who modeled the cell wall as a stretchable and growing elastic membrane with geometry-dependent elastic properties using large-deformation elasticity theory and combining its elastic response with surface reparameterization to simulate wall rebuilding. The model demonstrates a simple mechanism for hyphal swelling and beading that is observed in the presence of cell wall lysing agents, thus demonstrating its usefulness for the prediction of cellular behaviour under certain experimental conditions (Fig. 4C).

This and other models for tip growth imply that the expansion at the apex of a tubular cell is only possible if the apical cell wall is softer than that of the shank (Green 1963). If the physical properties of the cell wall were uniform and isotropic around the entire cell, pressure should cause a ballooning or bursting of the cylindrical part instead of forcing the apical end to expand. It is well known that this gradient of biomechanical properties is based on the particular distribution pattern of cell wall components along the longitudinal axis of the growing cell, but its quantification has only been achieved recently. The local cellular stiffness and viscoelasticity of growing pollen tubes were probed using microindentation revealing that cellular stiffness is considerably lower at the growing apex (Bolduc et al. 2006; Geitmann and Parre 2004). Similar observations were made in fungal hyphae using atomic force microscopy (Ma et al. 2005). In pollen tubes, this axial gradient of physical properties is based on the nonuniform distribution of the cell wall polymer callose (Parre and Geitmann 2005b) and the changing degree of methyl esterification in the pectin layer surrounding the cell (Parre and Geitmann 2005a).

These data are consistent with findings in the giant tip-growing cells of the alga *Vaucheria terrestris* in which the application of internal pressure by inflation of the cell by mineral oil revealed that the only part of the cell wall that is extensible is the narrow region around the growing tip (Mine and Okuda 2003). A detailed study of the growth process in the tip of *Medicago truncatula* root hairs has furthermore revealed a strong meridional gradient of wall expansion (Dumais et al. 2004). The studies on these cells also indicated that in the growing apex, stress anisotropy can account for most of the observed wall expansion anisotropy but not for all (Fig. 5). Further structural studies of the cell wall as well as the distribution of wall secretion events are therefore warranted to understand the mechanical aspect of tip growth and to establish a theoretical model that incorporates all mechanical aspects.

Fig. 5. (A) Two alternatives for anisotropic wall expansion. In diffusely growing cylindrical cells, stresses (solid arrows) favour transverse extension, but high mechanical anisotropy of the cell wall leads ultimately to axial extension. For tip-growing cells, the authors postulate that the cell wall has no intrinsic mechanical anisotropy in its plane so that expansion anisotropy reflects solely the bias in the wall stresses. (B) Geometry of a tip-growing cell. The plane stresses (σ) acting on a small wall element are shown along with the strain rates they produce. (From Dumais et al. (2004), reproduced with permission of Plant Physiol., Vol. 136, p. 3267, © 2004 American Society of Plant Biologists.)



Cellular exertion of forces

Cellular growth often has to occur against mechanical resistance by the surrounding substrate. In no cell type is this more obvious than in tip-growing cells, since their biological purpose is to invade more or less rigid substrates: the soil in the case of root hairs, the transmitting tissue in the case of pollen tubes, and any nutrient providing matrix in the case of hyphae. While the cells often use chemical means such as secreted enzymes to soften the growth substrate, unless this results in complete liquefaction, they are likely to meet substantial mechanical resistance that has to be overcome.

In liquid medium, the requirements for the amount of turgor pressure present to be able to perform cellular morphogenesis vary and can be almost immeasurable (Money and Harold 1993), but growth against mechanical obstacles other than the cell's own cell wall most certainly requires considerable internal pressure (Money 2004). This performance is

particularly impressive in hyphae that are able to penetrate epidermal plant tissue covered by thick cuticles, mammalian skin tissue, and even rocks (MacDonald et al. 2002; Ravishankar et al. 2001). The initial penetration is in many cases carried out by special structures, the appressoria. These structures are dome-shaped or cylindrical cells that differentiate from the end of fungal germ tubes. They adhere to the surface of the organ to be infected and subsequently form an infection peg that penetrates it. While the infection process by appressoria can involve enzymatic action to help soften the surface tissue, these structures are able to establish extremely high turgor pressures to physically break their way through mechanical barriers (Talbot 1999). As mentioned in the second section, turgor pressures up to 8 MPa have been measured in *M. grisea*, the causal agent of rice blast disease (Howard and Valent 1996). The biomechanical design of this highly pressurized structure has been modeled in terms of a bioelastic shell glued to a surface (Tongen et al. 2006). The actual invasive force exerted by a penetration peg

formed from an appressorium has been measured to be around 16.8 μN in *Colletotrichum graminicola* (Bechinger et al. 1999). The authors used an elegant method to assess this force. They grew appressoria on an optical waveguide and detected the deformation caused by the penetration peg pushing against it through a change in the intensity of the reflected light. Unfortunately, this technique necessitates tight adherence of an appressorium to the waveguide and is thus not useful for other types of tip-growing cells.

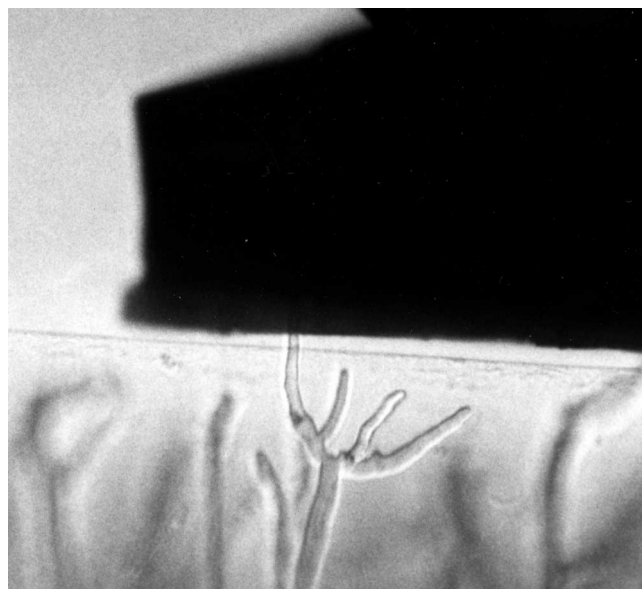
A different method was therefore used to quantify the invasive force of vegetative hyphae, i.e., hyphae that do not form penetration pegs. Money and his colleagues placed a miniature strain gauge in front of a growing hyphal apex and recorded the force exerted by the advancing cell (Fig. 6). When the hypha contacts the beam, the electrical output from the strain gauge immediately changes in proportion to the applied force, and the pressure exerted by the cell is derived by dividing the measured force by the contact area with the beam. It is important to be aware of the fact that the actual force exerted on the strain gauge or growth substrate does not directly relate to the amount of internal pressure but depends on the compliance of the cell wall at the apex of the invading cell, since the turgor has to push against it before being able to push against the substrate (Money 2001). The concept of the yield threshold is useful for grasping the fundamental mechanics that must operate during invasive growth. Money (1998, 2001) formalized a simple mathematical model:

$$\begin{aligned} \text{Invasive force } (\mu\text{N}) &= F_{inv} \\ &= [(\Psi_P + P_{CS}) - Y] \times A \end{aligned}$$

where Ψ_P is the turgor pressure, P_{CS} is any pressure exerted by the cytoskeleton against the inner surface of the apical cell wall, Y is the yield threshold of the apical cell wall, and A is the surface area of the growing tip. According to this model, the force exerted by the growing cell does not necessarily parallel changes in turgor but can increase or decrease in response to changes in any of the other variables in the equation. This is consistent with the finding that the amount of turgor pressure does not correlate with the velocity of growth in pollen tubes (Benkert et al. 1997) albeit it being a *conditio sine qua non* for cellular expansion. It is rather likely that the tensile strength of the apical cell wall (which is proportional to the yield threshold) plays an important role in the control of growth velocity and in the regulation of invasive force of the cell. For the oomycetes, the secretion of endoglucanases has been held responsible for the cell wall compliance and thus the fine tuning of the invasive force of the hypha (Money and Hill 1997). In pollen tubes, a changing composition of the cell wall depending on the stiffness of the surrounding growth substrate (Parre and Geitmann 2005b) is consistent with the concept stating that the cell adapts the cell wall compliance to adjust the available force exerted by the turgor pressure to meet the degree of mechanical resistance of its growth substrate.

An interesting phenomenon from various cell biological points of view is the oscillating or pulsatory growth occurring in many types of tip-growing cells. Pollen tubes of many species exhibit oscillating growth velocities, and in some, the alterations in growth rate resemble bursts of

Fig. 6. Hypha of *Mucor hiemalis* growing against the silicon beam of a miniature strain gauge. This setup allows recordings of forces exerted by a single tip-growing cell. (From Money (2004), reproduced with permission of Mycologist, Vol. 18, p. 73, © 2004 Cambridge University Press.)



growth during which growth rate increases more than an order of magnitude. While the regulation mechanism of this phenomenon involves ion fluxes across the membrane, the mechanical principle behind this oscillatory behaviour is based on the changing equilibrium between the turgor pressure and the apical cell wall over short periods of time (oscillations generally have a period between the tens and hundreds of seconds). The question is what is the mechanical oscillator in this system? A rhythmic fluctuation of turgor pressure has been proposed based on measurements with a miniature strain gauge on hyphae of the oomycete *Achlya bisexualis* (Johns et al. 1999). However, if turgor was the mechanical oscillator, two growing ends emanating from a single branched cell should show the same frequency of the growth fluctuations, since the cell can be considered as a single volume in which turgor pressure is identical everywhere. Observations of branched pollen tubes have revealed that this is not the case in this cell type (Geitmann 1997). It is therefore suggested that the tensile strength of the apical cell wall and not the internal pressure varies during the oscillation cycles in these cells. This alternation between softening and hardening might, for example, be caused by the secretion of new cell wall material with high plasticity that allows rapid expansion and subsequently hardens because of either strain hardening or enzymatic activity (Geitmann 1999).

The hypothesis that the cell wall properties do play a role in the control of the oscillations is corroborated by the application of agents that change these properties, such as pectin methylesterase, boron, or auxin, which are able to change pulsation frequencies or induce pulsations in steadily growing pollen tubes (Geitmann 1997). Despite these data, the final answer to the question as to what drives growth pulsations in apically growing cells still remains to be found.

Another question that remains is that of the biological purpose behind these growth oscillations. Strikingly, pollen tube oscillations with the highest amplitudes occur in plant species with solid styles in which pollen tubes have to grow intercellularly and invade the apoplast of the transmitting tissue. Pollen tubes of species exhibiting hollow styles, on the other hand, exhibit only small and more rapid oscillations. This correlation seems to suggest that the pulsations might have a mechanical function in the invasion of the transmitting tissue. The comparison with the mechanical principle of a hydraulic sledge hammer is maybe not too far-fetched.

Conclusions

The mechanical behaviour of an organism depends on the physical properties of the individual cells that compose it. In plant and hyphal cells, these are largely determined by the interaction between the turgor pressure and the cell wall. However, knowledge about the structural function of the cytoskeleton in these cells is scanty at best and warrants further investigation. The availability of more structural and mechanical data will allow the establishment of new and more realistic biomechanical models. The challenge will be to integrate all mechanical components and thus contribute to the understanding of cellular architecture and dynamics from a mechanical point of view.

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