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Polar growth in pollen tubes is associated with spatially confined dynamic changes in cell mechanical properties

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ABSTRACT

Cellular morphogenesis involves changes to cellular size and shape which in the case of walled cells implies the mechanical deformation of the extracellular matrix. So far, technical challenges have made quantitative mechanical measurements of this process at subcellular scale impossible. We used micro-indentation to investigate the dynamic changes in the cellular mechanical properties during the onset of spatially confined growth activities in plant cells. Pollen tubes are cellular protuberances that have a strictly unidirectional growth pattern. Micro-indentation of these cells revealed that the initial formation of a cylindrical protuberance is preceded by a local reduction in cellular stiffness. Similar cellular softening was observed before the onset of a rapid growth phase in cells with oscillating growth pattern. These findings provide the first quantitative cytomechanical data that confirm the important role of the mechanical properties of the cell wall for local cellular growth processes. They are consistent with a conceptual model that explains pollen tube oscillatory growth based on the relationship between turgor pressure and tensile resistance in the apical cell wall. To further confirm the significance of cell mechanics, we artificially manipulated the mechanical cell wall properties as well as the turgor pressure. We observed that these changes affected the oscillation profile and were able to induce oscillatory behavior in steadily growing tubes.

Introduction

Plant development is the result of three fundamental processes: cell expansive growth, cell division, and cellular differentiation. Cellular growth in plants involves dramatic changes to the size and the shape of the cell. In most types of animal cells, the mechanical work required to achieve such changes is performed by the cytoskeleton. In plant cells, on the other hand, because of the presence of a more or less stiff extracellular matrix, these changes require the concerted action of two mechanical processes: the deformation (stretching) of the existing cell wall and the secretion and deposition of new cell wall material. The cytoskeleton is crucial for the precise targeting of the latter, but the former is thought to be driven by the turgor pressure (Cosgrove, 1993; Geitmann and Ortega, in press; Lockhart, 1965; Ortega, 2004; Schopfer, 2006).

To mechanically understand plant cell growth, it is helpful albeit simplistic to model the cell as a thin-walled pressure vessel. The liquid or gel-like contents is under pressure thus leading to tensile stress in the shell. Hydrostatic pressure is a non-vectorial force, however. Therefore, if the mechanical properties of the shell were uniform and isotropic, the result of a pressure-driven expansion would inevitably be a spherical body. However, differentiated plant cells come in all shapes and sizes ranging from simple cylindrical cells (e.g. palisade mesophyll) to star-shaped complex structures (e.g. astro-sclereids). The generation of geometries other than spheres necessitates local differences in mechanical properties of the shell to allow for its localized or uneven deformation. It is, therefore, the pattern of shell extensibility that determines the geometry of the growing body by controlling the spatial distribution of strain on its surface (Green, 1969). Two important mechanical parameters of the shell determine the local generation of strain by an applied tensile stress: (a) anisotropic extensibility causes the overall symmetry of the body to change by allowing preferential stretching along a particular axis. The plant cell wall is essentially a fiber reinforced composite material with the fibrous component consisting of cellulose microfibrils. Preferential orientation of the microfibrils conveys overall anisotropy to the cell wall (Baskin, 2005). (b) Non-uniform distribution of shell extensibility prioritizes expansion of certain regions of the body versus others leading to spatially confined growth (Geitmann and Ortega, in press). The result is typically a cellular protuberance. Localized secretion of "softer" cell wall material or of agents affecting the properties of the existing cell wall are considered necessary for the generation of such local differences (Smith and Oppenheimer, 2005).

The biomechanics of global cell wall extensibility has been investigated in much detail using tensile testing of plant tissues and organs,

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but the investigation of local growth events that are spatially confined to subcellular regions requires a single cell approach (Geitmann, 2006a). Regarding such spatially confined cellular growth events, significant advances have been made on the biochemical and biological front. Our knowledge of the agents controlling cell wall extensibility and the molecular mechanisms of cell wall yielding has increased exponentially in the past decade (Cosgrove, 2005). Intriguingly, experimental manipulation of cell wall mechanical properties is able to induce morphogenesis (Pien et al., 2001), but we lack quantitative experimental data that demonstrate the changes to cellular mechanics associated with this process. Cytomechanical approaches will help us understand the physical principles governing plant cell growth and thus might contribute to solving conceptual controversies (Schopfer et al., 2008).

The quantification of physico-mechanical properties at subcellular level is a challenge for micromanipulation and until recently had been thought to be impossible on fast-growing plant cells (Messerli and Robinson, 2003). However, the development of micro-indentation and atomic force microscopy have made the mechanical characterization of walls surrounding living cells possible (Geitmann, 2006a,b; Geitmann and Steer, 2006; Parre and Geitmann, 2005b; Zhao et al., 2005). To study the generation of spatially confined growth events, we chose pollen tubes which are long, cylindrical protrusions formed by germinating pollen grains (Chebli and Geitmann, 2007; Holdaway-Clarke and Hepler, 2003; Malhó, 2006). Their biological purpose is the transfer of the male gametes from the male gametophyte, the pollen grain, to the female gametophyte, located in the ovule of a flower. Pollen tubes exhibit rapid tip growth, a growth pattern that is also characteristic for root hairs and fungal hyphae (Geitmann et al., 2001; Geitmann and Emons, 2000; Heath, 1990). The growth process is unidirectional and confined to the apex of the cellular protrusion (Geitmann and Dumais, 2009; Geitmann and Steer, 2006).

In previous studies, we showed that the growing region of pollen tubes spatially coincides with a region of lower cellular stiffness which in turn is a result of differences in the biochemical composition of the cell wall (Bolduc et al., 2006; Geitmann et al., 2004; Parre and Geitmann, 2005a,b). In the present study, we investigated the temporal aspect of the relationship between cellular stiffness and growth activity. To this end, we studied the mechanics of two phenomena—the re-initiation of a tubular outgrowth from a sphere-shaped swelling, and the oscillatory change of the pollen tube growth rate.

Materials and methods

Pollen tube growth

In this study, pollen from different plant species was used since the differences in size and growth dynamics allowed for optimal use in each particular experimental setup. *Papaver rhoeas* pollen was used for experiments involving apical swelling and recovery upon cytochalasin D treatment. *Nicotiana tabacum* and *Petunia hybrida* pollen tubes show oscillatory growth with periods in the range of 1 to several minutes. These species were used for experiments addressing the role of turgor and cell wall properties on the growth rate (sucrose, auxin, pectin methyl esterase data sets). There was no difference in behavior between these two species. *Lilium longiflorum* pollen tubes have a very large diameter and are thus easier to handle in micromanipulation experiments. This species was used for the quantification of cellular stiffness during growth rate oscillations. The typical oscillation period of this species is between 20 and 50 s; *Lilium* was also used for investigating role of turgor on oscillation frequency (mannitol data set).

Papaver rhoeas pollen was obtained from plants grown in the greenhouses of the Montreal Botanical Garden. *Nicotiana tabacum* and *Petunia hybrida* pollen were obtained from plants grown in the Botanical Garden of Siena, Italy. *Lilium longiflorum* flowers were obtained from a local Montreal florist. After collection, pollen was dehydrated in gelatin capsules on anhydrous calcium sulfate overnight and stored at −20 °C. Pollen was rehydrated in humid atmosphere for 30 min before cultivation. The growth medium for *Lilium* contained 0.16 mM H_3BO_3, 0.13 mM $Ca(NO_3)_2$, 1 mM KNO_3, 5 mM MES, 100 mg mL^{-1} sucrose, pH 5.5. Unless specified otherwise, the medium for all other species was composed of 100 μg mL^{-1} H_3BO_3, 300 μg mL^{-1} $Ca(NO_3)_2$ H_2O, 100 μg mL^{-1} KNO_3, 200 μg mL^{-1} $MgSO_4$ $7H_2O$, 50 mg mL^{-1} (*Papaver*) or 120 mg mL^{-1} (*Nicotiana, Petunia*) sucrose (modified after (Brewbaker and Kwack, 1963)). For the mannitol data set, the medium contained 70 mg mL^{-1} sucrose and was complemented with 16 or 32 mg mL^{-1} mannitol to reach the same osmolarity as that of media containing a total of 100 or 130 mg mL^{-1} sucrose, respectively.

For micro-indentation, pollen was brushed on poly-lysin or gelatin-coated cover slips, re-hydrated, and covered with drops of liquid growth medium. For cytochalasin D treatment, the liquid growth medium was changed for medium containing 10 μM of the drug once germination had occurred. After 10 min, the slide was submerged into the medium filled sample chamber of the micro-indenter (described below) which effectively diluted the drug to subeffective levels (approximately 0.5 nM).

Time lapse imaging

Time lapse imaging for growth rate measurements were carried out on pollen growing on the surface of a thin layer of agarose as described previously (Geitmann et al., 1996). Auxin, pectin methyl esterase, and altered sucrose and mannitol concentrations were administered replacing the liquid layer of medium with medium containing the substance in question. Quantitative analysis of the growth rate was carried out as described previously (Geitmann et al., 1996) or using image acquisition with a Roper fx-cooled CCD camera and the tracking function of the ImagePro software (Media Cybernetics, Bethesda, MD, USA).

Micro-indentation

Pollen was incubated as described and after germination had occurred, cover slips were submerged in the growth medium containing experimental chamber of the micro-indenter. The design and principles of operation of the micro-indenter have been described previously (Elson et al., 1983; Petersen et al., 1982). The micro-indentation assemblies used here were mounted on either a Zeiss IM 35 inverted light microscope or a Nikon TE2000 inverted microscope. In the experiments reported in this paper, the motor was programmed to execute a single triangular waveform with a velocity of 4 μm s^{-1} and a total amplitude of 10 μm. Time lapse imaging was performed with a Nikon TE2000 inverted microscope equipped with a Roper fx cooled CCD camera. Image acquisition and tracking was done with ImagePro (Media Cybernetics).

Fourier analysis

The Fourier analysis technique is based on the fact that a signal can be decomposed into a sum of simple cosine or sine functions with periods that are a multiple of the total recording time. A certain periodicity in the recording is reflected by the large weight (Fourier coefficient) that multiplies the cosine or sine function with the respective periodicity in the Fourier decomposition (Fourier series). The value of these Fourier coefficients as a function of their period (or frequency) is called the Fourier transform. Since the Fourier transform is a complex quantity, we calculate the power of the Fourier series which is a real quantity.