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Review

Insights on the mechanics of hyphal growth

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ABSTRACT

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This article reviews recent progress in understanding the mechanics of hyphal growth. The pressurization of hyphae by osmosis is often considered an important feature of the tip growth process. But although the hydrostatic pressure within the cytoplasm smooths the expanding surface of these cells, there is little evidence that high levels of turgor are necessary for growth until the hypha encounters friction from its surroundings. Research on movement and growth processes in other kinds of cells, including amoebae and pollen tubes, has done a great deal to inform recent studies on hyphae. Experiments on pressure waves and rhythmic or pulsatile growth are particularly significant and are discussed in relation to the erratic extension rate of hyphae. Significant findings have also come from research on the hyphal cytoskeleton and on the biomechanics of invasive growth. It has been clear for some time that turgor powers the propulsion of hyphae through solid materials and experiments using new techniques have quantified the relevant forces.

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1. Introduction

Hyphae were first illustrated in the seventeenth century by Marcello Malpighi in his *Anatome Plantarum* (Malpighi 1675–1679; Fig 1). The term ‘hypha’ was coined more than a century later by German botanist Carl Ludwig Willdenow (1810), who defined it as ‘a more or less filamentous, watery, or fibrous stem, which is formed by repeated branching’. Since Willdenow’s time we have learned a great deal about hyphal structure and development. Contemporary research on the growth mechanism is following three complementary approaches: (i) molecular analysis of hyphal components and their assembly; (ii) observation and manipulation of whole cells, and (iii) the development of mathematical models to create virtual hyphae that mimic and inform us about the real things. This brief article reviews recent progress in these areas with an emphasis on experiments on whole cells.

2. The illusory nature of the hypha’s ‘driving force’

It is difficult to get very far in understanding hyphal growth without considering that hyphae are highly pressurized structures. The idea that turgor acts as the driving force for hyphal extension is often expressed in a matter-of-fact way by mycologists, but says more about our loyalty to catchy phrases than it reflects any clear understanding of the growth mechanism. The idea originates with the observed disruption of growth that occurs when hyphae are deflated by treatment with hyperosmotic media. But the fact that turgor and growth are sensitive to changes in water availability offers little support for the driving force concept. Irrespective of the loss of turgor, reversal of the normal osmotic gradient between the cell and its surroundings will perturb expansion because the aqueous cytoplasm cannot enlarge without absorbing more water. So

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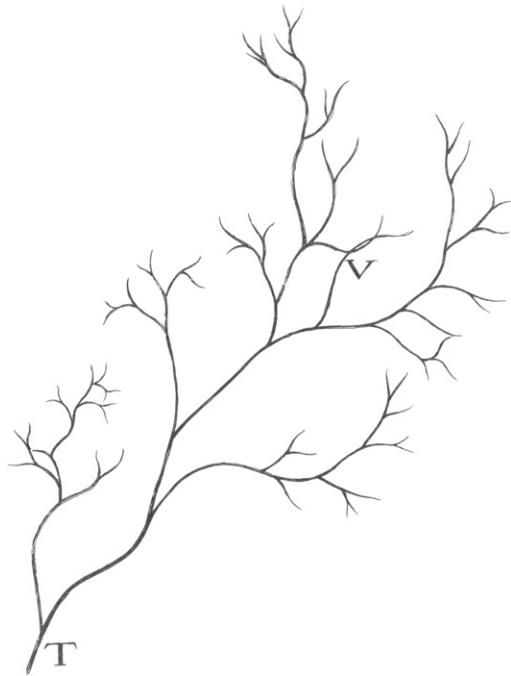


Fig. 1 – The first illustration of hyphae from Marcello Malpighi’s *Anatome Plantarum* (1675–1679). Malpighi described the illustration as follows: ‘Here in general stems T are drawn upwards, and thereupon branchlets are sent forth on this side and that, which gather together in a projection; many of which mutually entwine to make interconnections V, and occasionally sport little spheres around branches at the end.’ Translation from Latin by Mike Vincent, Miami University, Oxford, Ohio. Image courtesy of the Lloyd Library, Cincinnati, Ohio.

what do we really know about the function of hyphal turgor pressure?

Turgor is an unavoidable consequence of the walled, semi-aquatic lifestyle of fungi, and hyphae are usually pressurized to a few atmospheres by osmosis. This hydrostatic pressure holds the wall under tension, which is evident from the relaxation of the wall if the cell is punctured. The fact that the growing hypha accommodates this pressure, elongating rapidly without ripping its apical wall apart, is one of the remarkable features of this cell type.

As new materials are added to the plasma membrane and the cell wall, work must be done to expand the cell surface. Logic demands that the power for this process must come from turgor because this is the only known source of mechanical stress within the wall that can cause its polymers to slide apart from one another. But here’s a crucial point: the reason that hyphae have lots of turgor pressure has nothing to do with the energetic requirements for prying microfibrils apart. Experiments on oomycete water moulds (Kingdom Straminipila) showing that hyphal growth can occur at greatly diminished turgor prove that the multi-atmospheric pressures that are usually measured from hyphae are not essential for growing them (Money & Harold 1993; Harold 2002). Unfortunately, the same experiments fail when applied to species

from Kingdom Fungi because turgor is not easily manipulated in these organisms by changing medium osmolality (Lew *et al.* 2004; Lew & Levina 2007). Nevertheless, the significance of turgor as a driving force is not at all apparent from the observation that homeostatic mechanisms maintain relatively constant levels of pressure in fungi like *Neurospora crassa* over a wide range of environmental conditions.

In his pioneering work on hyphal and root hair growth, Max Otto Reinhardt (1892) documented an orthogonal pattern of tip expansion consistent with the action of an isotropic force against the inner surface of the cell wall. He argued, however, that turgor was necessary only to maintain contact between the plasma membrane and the inner surface of the cell wall. More recent mathematical models suggest that turgor does more than this, serving as the isotropic force that continually smooths the expanding hyphal surface (Bartnicki-Garcia *et al.* 2000). But none of the models specify the magnitude of this pressure. Cytoskeletal contractions in the protist *Amoeba proteus* and the reactive force from its cell surface pressurize its cytoplasm to around 6 kPa (= 1/100th the pressure of a hypha; Yanai *et al.* 1996). The amoeba’s rounded contours reflect the pressure of their fluid filling in the same way that the profile of a hypha emerges from the exertion of cytoplasmic turgor against the inner surface of its wall. We don’t refer to non-walled amoebae as turgid cells, but I’m not convinced that cytoplasmic pressure is any more or less important as a determinant of shape for an amoeba than it is for a hypha. In other words, the concept of turgor as the driving force is a red-herring, because to many mycologists it implies that the high pressures measured in hyphae are part of some radically different way of growing a eukaryote. This is not supported by the available evidence.

In wrestling with the relationship between turgor and hyphal growth it is also important to consider the environment in which the fungus is growing, because hyphae developing on surfaces and hyphae penetrating solid materials experience very different mechanical challenges. Indeed, the shape of the hyphal apex changes as it encounters friction from its surroundings (Goriely & Tabor, 2008), and while a hypha growing over an agar surface may not need much turgor, it cannot push through anything without it. These issues are addressed in Section 6.

3. Contractions, pressure waves, wall compliance, and pulsatile growth

A handful of recent studies have gone a long way toward clarifying the mechanics of tip growth. Charras *et al.* (2005) demonstrated that localized yielding of the surface of animal cells results in a pressure decrease in the subtending cytoplasm and that non-equilibrium pressures can persist for 10 s or so over a distance of 10 μm . These observations are important because they question the long-held assumption that any changes in pressure are transmitted almost instantaneously throughout the fluid within a cell. For a hypha, this means that pressure gradients might be maintained on time scales that can have significant consequences for tip growth. The cytoplasmic contractions documented within hyphae by Reynaga-Pena and Bartnicki-Garcia (2005) seem likely to

generate pressure waves along hyphae. These contractions were observed in a number of ascomycetes and have a duration of about 1 s. In addition, Lew (2005) demonstrated pressure gradients along hyphae of *Neurospora crassa* and suggested that they drive mass flow of cytoplasm. These pressure gradients are probably very small, no more than 10 kPa per mm (approx. 0.1 atm mm^{-1}), predicting a maximum pressure differential of 2 % of turgor over a 1 mm length of hypha (based on turgor of 500 kPa). Earlier researchers estimated pressure gradients within mycelia and suggested that pressure differentials of many atmospheres could be generated between basal and apical compartments of septate mycelia (Robertson & Rizvi 1968). Inaccurate measurements of cytoplasmic osmolality using plasmolytic methods were probably responsible for these conclusions.

Research on pollen tube extension is also pertinent to the present discussion (Geitmann 2006; Zonia et al. 2006). Apical growth of pollen tubes occurs in an oscillatory fashion and it has been proposed that pulses of fast extension follow wall loosening in response to ion fluxes (see citations in Zonia et al. 2006). The relationship between growth pulses and wall loosening is contradicted, however, by the fact that although the physical properties of the wall vary along the length of the pollen tube, the stiffness of cell wall at any single location appears to be rather constant (Geitmann & Parre 2004). In addition, changes in extracellular osmolality induce immediate changes in cell volume, which suggests that the wall behaves as a constitutively-responsive fabric rather than a resistive structure whose properties are modulated to control the rate of expansion (Zonia et al. 2002).

In common with pollen tubes, wall compliance differs along the length of the hypha as most models of growth have anticipated. Atomic force microscopy shows greater wall compliance at the hyphal tip versus sub-apical regions in *Aspergillus nidulans* (Ma et al. 2006), and these data are consistent with pressure probe studies on oomycete water moulds (Money & Hill 1997; Walker et al. 2006). While there is some uncertainty about the temporal variability of wall extensibility in pollen tubes, it seems very likely that secreted enzymes are involved in wall-loosening in both plants and fungi. It is less clear whether the family of pH-dependent proteins called expansins implicated in wall enlargement in plants (Cosgrove 2005) plays a similar role in fungi. Li et al. (2002) reported related genes in fungi but their function is unknown.

Like pollen tubes, hyphae extend in a discontinuous fashion, showing bursts of fast growth, lasting a few seconds, punctuated by refractory periods of similar duration in which extension proceeds at a more sedate pace (López-Franco et al. 1994). Studies on *N. crassa* and *Saprolengia ferax* (an oomycete water mould) show that fluctuations in the hyphal growth rate do not occur with the kind of regularity characteristic of pollen tubes (Sampson et al. 2003). Sampson et al. (2003) limit the term 'pulsatile tip growth' to growth with a regular alternation of fast and slow rates of extension. It is not clear whether or not this is an important distinction; whether, for example, the random signature of the growth pulses in hyphae reflects functional differences between the tip growth mechanisms in plants and fungi.

The discontinuous nature of tip growth in plant and fungal cells makes a lot of intuitive sense when we consider the

demands upon the cell in maintaining the supply of new cell wall polymers during fast extension, and in controlling turgor and cytoplasmic osmolality. Because water is almost incompressible, even very tiny changes in cytoplasmic volume can result in tremendous swings in turgor pressure. Consider a hypha with a diameter of $10 \mu\text{m}$ and length of $200 \mu\text{m}$; this cell has a volume of approx. $16,000 \mu\text{m}^3$. If this hypha extends at a velocity of $0.08 \mu\text{m s}^{-1}$ ($5 \mu\text{m min}^{-1}$) it will increase in volume by 0.04 \% s^{-1} (this percentage will decrease, of course, as the cell enlarges). If this hypha is growing under dry conditions that preclude water influx, this rate of expansion would cause complete loss of turgor in a few milliseconds. No changes in wall compliance, however swift, can compensate for this kind of violent deflation. Loss of turgidity doesn't occur under wet conditions because the hypertonicity of the cytoplasm induces a continuous net influx of water. But without compensatory osmolyte production, the dilution of the hyphal contents caused by a 0.04 \% s^{-1} increase in volume will be matched by a 0.2 kPa s^{-1} drop in turgor (from an initial turgor of 500 kPa; Fig 2). At this rate, 75 % of the pressure in the hypha will be lost within 2 h. Osmoregulatory mechanisms prevent pressure loss, or at least serve to dampen it, driving ion uptake from the environment and synthesis of compatible organic osmolytes (Lew & Levina 2007).

4. What about the cytoskeleton?

The cytoskeleton is a major player in the hyphal growth process (Steinberg 2007a, b; Fischer 2007). Actin filaments, microtubules, and associated motors constitute a polarized transport system that delivers thousands of vesicles to the cell surface every minute. These vesicles are destined for

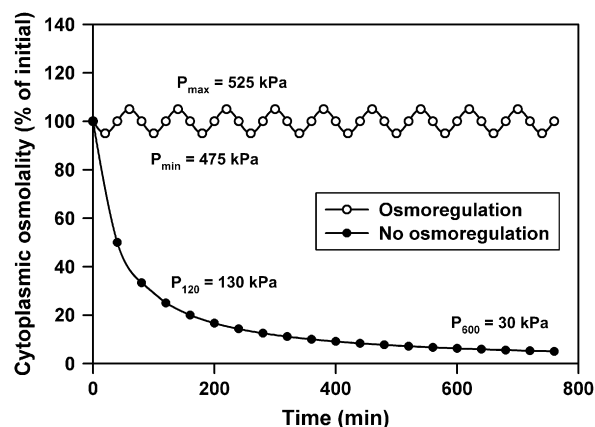


Fig. 2 – Changes in hyphal osmolality and turgor pressure in the presence and absence of an osmoregulatory mechanism. Data calculated for hypha with initial volume of $16,000 \mu\text{m}^3$, osmolality of 200 mmol kg^{-1} , turgor pressure of 500 kPa, and extension rate of $5 \mu\text{m min}^{-1}$. Osmolality of osmoregulating cell based on slow response of extending hypha to cytoplasmic dilution and concentration that allows turgor to vary by $\pm 5 \text{ \%}$. In reality, osmotic adjustment may maintain turgor within a smaller range of values and occur too swiftly to be visible with the scaling used for this plot.

exocytosis to supply new membrane, cell wall components and synthases, and to release digestive enzymes into the immediate environment. The polarity of the exocytotic machinery is regulated by the positioning of the actin-rich Spitzenkörper whose placement is, in turn, determined by a protein complex called the polarisome (Harris *et al.* 2005). While little is known about the interactions between the actin cytoskeleton and microtubules, there is reasonable support for the idea that microtubules and microtubule-associated kinesins and other proteins form a longer-range transport system for vesicle delivery toward the actin-rich apex (Evans & Sugden 2007; Steinberg 2007a, b; Sugden *et al.* 2007). The actin cytoskeleton is also essential for endocytosis, which plays a significant role in controlling hyphal morphology (Bourett *et al.* 2007; Steinberg 2007a, b).

The available evidence suggests that none of the cytoskeletal components plays a mechanical role in expanding the tip. Theoretical considerations indicate that the forces exerted by microfilament and microtubule elongation, and motor-driven sliding of these elements, are simply swamped by the relentless force of turgor pushing against the apical membrane (Money 2007). Recent experiments strengthen this conclusion by suggesting that the cytoskeleton acts as an internal brace that actually limits expansion (Walker *et al.* 2006; Mathur 2006). Under conditions of reduced turgor, cytoskeletal forces may become more significant in cell expansion (Steinberg 2007a, b).

5. The challenge of deviant morphology

A paper by Bowen *et al.* (2007) offers a challenge to everyone interested in modeling hyphal growth. In addition to the rounded profiles of hyphae that have been described by mycologists since Reinhardt (1892), we must now consider how tips can produce fine projections. Bowen and colleagues show that a ‘microtip’ can form at the apex of *Aspergillus niger* hyphae under conditions of nutrient stress, so that the cell resembles the shape of a dolphin’s head (Fig 3). The function of this morphology is not known, though Bowen *et al.* (2007) speculate that it may play some role in sensing the topography of the surfaces over which the cells are growing. There are other cases of hyphal morphology that do not conform to the available models for tip growth. For example, the hyphae of water moulds extending from seeds or dead insects appear to form as cones rather than cylinders and can expand to a basal diameter in excess of 0.1 mm. This morphology requires a growth mechanism that coordinates continuous radial expansion of the entire hyphal wall with the processes that extend the tip. The effectiveness of future models of hyphal growth will be determined by their ability to incorporate alterations in tip symmetry, diversions from the usual cylindrical form, and other variations in morphology that are apparent every time we examine a living colony in a culture dish.

6. Invasive growth: the principal function of hyphal turgor

Invasive growth is a significant topic from a practical perspective because hyphae are, primarily, invasive cells and this

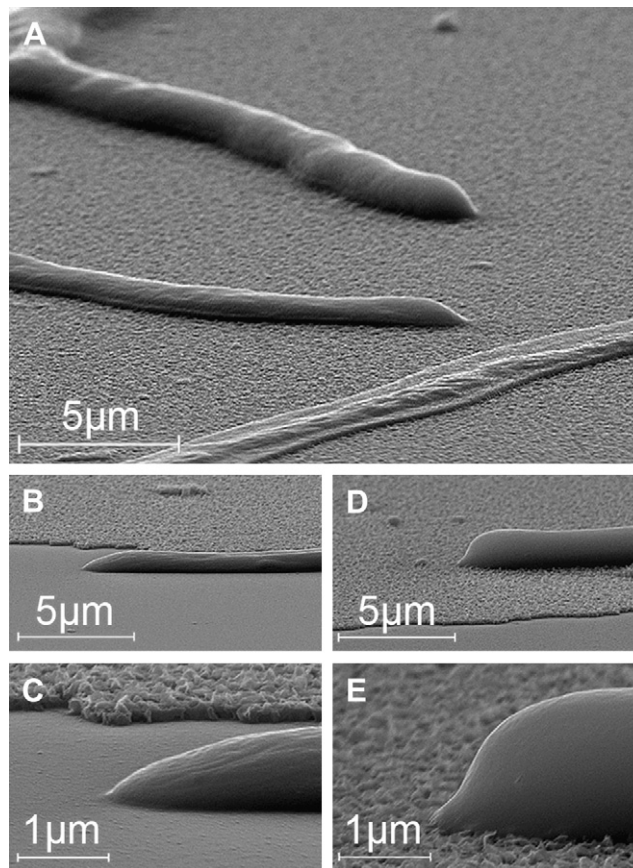


Fig. 3 – Hyphal tips of *Aspergillus niger* growing under low-nutrient conditions on glass. (A) shows two hyphae with unusual morphology. The tip of the hypha in the foreground has a smooth taper; this shape is also shown in (B) and (C). The hypha in the background in (A) has a more pronounced microtip that is also shown in (D) and (E). From Bowen *et al.* (2007) with permission.

feature of their behaviour is essential to understanding the filamentous growth form and its mechanical properties. Detailed consideration of this topic is provided by Money (2007), whose main points can be summarized as follows: (i) turgor pressure powers the penetration of solid materials; (ii) without turgor, hyphae are non-invasive; (iii) the pressure exerted by the tips of hyphae appears to be controlled by loosening of the apical wall, so that a hypha with a looser apical wall applies more of its internal pressure against its surroundings; (iv) invasive growth involves a combination of enzyme-catalyzed digestion of the substrate and pressure-driven penetration of residual mechanical obstacles, and (v) the relative significance of enzymes and turgor in invasive growth differs from species-to-species and between substrates of varying resistance.

Because the pressure exerted by hyphal tips is not the same as the turgor pressure within the hypha (see point (iii) above), new experimental approaches are needed to measure the amount of the turgor pressure that is applied by the extending apices. Miniature strain gauges have been used in the author’s lab (Money *et al.* 2004), and Nick Read and

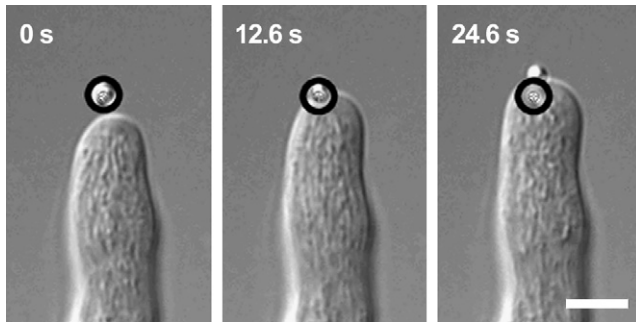


Fig. 4 – Displacement of 1 μm polystyrene bead from a 20 mW laser trap by hypha of *Neurospora crassa*. The black circle represents the focus of the laser. Bar = 5 μm . From Wright et al. (2005) with permission.

colleagues have been working with optical tweezers (Wright et al. 2005, 2007; Fig 4). Experiments show that individual hyphae of a variety of fungi and oomycete water moulds apply pressures of up to 200 kPa or 2 atmospheres (Money et al. 2004). These applied pressures represent a maximum of 54 % of the available turgor pressure inside the hypha, suggesting that somewhat greater pressures may be unleashed by enhanced wall yielding.

The similarity between the mechanical behavior of hyphae in Fungi and water moulds is especially interesting as an illustration of evolutionary convergence (Money et al. 2004). The formation of a highly-branched mycelium makes a great deal of adaptive sense for nutrient prospecting and absorption, serving as an efficient strategy for searching a given volume of substrate for nutrients (Bebber et al. 2007; Hanson et al. 2006; Fricker et al. 2007; Tlalka et al. 2007). The ability of each of the filaments to probe and penetrate solid surfaces with some degree of independence allows fungi to exploit a vast range of energy sources that would otherwise be unavailable. The utility of the invasive lifestyle probably explains the evolutionary origin of the hyphal form and its growth mechanism.

7. Conclusions

This paper provides an overview of current progress toward a satisfactory understanding of the way that fungi produce their tubular cells. Continuing elucidation of the hyphal growth mechanism has involved characterization and manipulation of the molecular components of the hypha, cell biological experiments on whole living cells, and fresh approaches to mathematical modeling. There have been some important breakthroughs in the last decade, but because few investigators are addressing mechanistic questions there are marvelous opportunities for advancing the field. The following questions highlight areas for fruitful inquiry: (i) How does the hypha establish and maintain polarity? (ii) What mechanisms underlie changes in the velocity of hyphal extension? (iii) What pushes the hyphal cytoplasm forward as the cell elongates? (iv) How does a hyphae regulate the force exerted by its tip without bursting? Because the majority of the fungi are hyphal, answers to these questions promise broad

implications for agriculture, biotechnology, cell biology, ecology, genetics, medicine, and plant pathology.

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