OPINION PAPER

Plant microtubule cytoskeleton complexity: microtubule arrays as fractals

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Abstract

Biological systems are by nature complex and this complexity has been shown to be important in maintaining homeostasis. The plant microtubule cytoskeleton is a highly complex system, with contributing factors through interactions with microtubule-associated proteins (MAPs), expression of multiple tubulin isoforms, and post-translational modification of tubulin and MAPs. Some of this complexity is specific to microtubules, such as a redundancy in factors that regulate microtubule depolymerization. Plant microtubules form partial helical fractals that play a key role in development. It is suggested that, under certain cellular conditions, other categories of microtubule fractals may form including isotropic fractals, triangular fractals, and branched fractals. Helical fractal proteins including coiled-coil and armadillo/beta-catenin repeat proteins and the actin cytoskeleton are important here too. Either alone, or in combination, these fractals may drive much of plant development.

Key words: Arabidopsis thaliana, fractal, microtubules, root hair, trichome, vascular bundle, xylem.

Introduction

Classically, healthy biological systems self-regulate to reduce variability and maintain constancy. However, the outputs of many systems fluctuate in a complex manner. Often, statistical methods indicate the presence of long-range, power-law correlations, suggestive of multifractal cascades operating over a wide range of time scales. In ill-health, this fractal organization breaks down, showing that complexity is essential for the maintenance of healthy biological processes (Goldberger et al., 2002).

The plant microtubule cytoskeleton plays a vital role in growth and development, with cellulose microfibrils laid down on the outside of the plasma membrane in patterns reflecting the underlying microtubule arrangement along the inside of the plasma membrane. As such, complexity of microtubule function is very important for the maintenance of homeostasis in plants. This complexity is built through various means including the presence of multiple tubulin isoforms, multiple microtubule-associated proteins (MAPs), post-translational modification of these components, and pairwise association of MAPs. The evolution of complexity in plant microtubule function has been driven by various processes, including the dynamic properties of microtubules themselves and the co-opting of proteins with other functions for use as MAPs.

Having shown that complexity is an inherent and important facet of the plant microtubule cytoskeleton, it is then suggested that fractal processes grant additional layers of complexity to plant growth and development while maintaining coherence. Plant microtubule arrays appear to have a tendency to form different fractal patterns depending upon the milieu in which they find themselves, that is, the MAPs, post-translational modifications, and tubulin isoforms within the cellular environment. By altering this environment the cell can bring different fractal patterns to the fore and thus modulate cellular development.
Building complexity into the plant microtubule cytoskeleton

Tubulin isoforms

The multi-tubulin hypothesis states that diversity in tubulin structure mediates diversity in microtubule organization (Wilson and Borisy, 1997) and specialized microtubule functions (Tischfield and Engle, 2010). However, even Saccharomyces cerevisiae, a single celled organism, has two alpha-tubulin genes, TUB1 and TUB3. These two tubulins show dramatically different dynamics, with TUB3 (representing approximately 10% of alpha-tubulin) alone forming microtubules far less dynamic than wild-type microtubules (Bode et al., 2003). This suggests that diversity in tubulin isoforms may act to contribute to the complexity, and thus robustness of microtubule function. Arabidopsis has at least nine expressed beta-tubulin genes (Snustad et al., 1992) and six expressed alpha-tubulin genes (Kopczak et al., 1992), allowing a great deal of scope for multiple tubulin isoforms to be present in a single microtubule. Indeed the two gamma-tubulin genes of Arabidopsis have redundant functions (Pastuglia et al., 2006).

Multiple MAP isoforms

A wide variety of MAPs interact with, and regulate, the plant microtubule cytoskeleton. These include katanins, MOR1, the MAP65 family of MAPs, the MAP70 family of MAPs, phospholipase D, and others (Buschmann and Lloyd, 2008). Some of these MAPs are from highly diversified protein families, for example, there are 61 kinesins present in the Arabidopsis genome (Lee and Liu, 2004). This is likely to increase the complexity of plant microtubule function in a similar way to that of multiple tubulin isoforms.

Post-translational modification of tubulin and MAPs

Post-translational modification of tubulins and MAPs is another means by which organisms can increase their complexity and thus robustness of the microtubule cytoskeleton. A proteomic analysis of mammalian brain MAPs revealed 573 tubulin-binding proteins, with many post-translational modifications, amino acid changes, and alternative splice variants (Kozieliski et al., 2010). It is likely that plant microtubules are similarly complex. Post-translational modification of microtubules also regulates microtubule motors and factors that affect the organization and dynamics of microtubules (Wloga and Gaertig, 2010). Post-translational modification of MAPs can affect their binding to microtubules. For example, phosphorylation of sites in the NH2-terminal part the microtubule-binding domain of CLASPs by glycen synthase kinase 3β likely regulates the affinity of CLASPs for microtubule lattices (Wittmann and Waterman-Storer, 2005).

Several studies underline the possible importance of post-translation modifications to the plant microtubule cytoskeleton. Inhibitors of protein kinases and phosphatases alter root morphology and disorganize cortical microtubules (Baskin and Wilson, 1997). Acetylated alpha-tubulin is found in Zea mays pollen and leaves, with different isoforms acetylated in each. Polyglycylated, detyrosinated, and polyglutamylated alpha-tubulins were also detected (Wang et al., 2004). Tyrosinated alpha-tubulin is essentially found in latent Vitis vinifera buds and in bursting buds (Parrotta et al., 2010). These studies demonstrate the probable importance of tubulin post-translational modifications in plant development.

Evolution of complexity

Dynamic instability of microtubules leads to increased complexity

Organisms carefully regulate microtubule destabilizing complexity, since the dynamic instability of microtubules means that they are particularly prone to rapid depolymerization. The MEI-1/katanin microtubule severing complex is required for meiosis in Caenorhabditis elegans but must be inactivated prior to mitosis. Two protein degradation pathways, the CUL-3/MEL-26 ubiquitin ligase pathway and the MBK-2/DYRK kinase pathway, act redundantly to degrade MEI-1 microtubule severing activity after meiosis (Lu and Mains, 2007). In interphase Xenopus extracts, katanin is inhibited by at least four separable components, one of which contains the MAP4 homologue XMAP230 (McNally et al., 2002). One of these components may be LAPSER1, which binds p80 katanin and regulates cell motility through inhibiting katanin’s microtubule severing action (Sudo and Maru, 2008). Similar redundancy in the regulation of microtubule destabilizing factors is likely to be present in plant cells.

Co-opting of enzymes as MAPs increases complexity

In various organisms it has been shown that proteins with pre-existing non-microtubule-related functions have been co-opted for use as MAPs. This adds an additional layer of complexity to microtubule function. Calpains are a family of Ca2+-dependent cysteine proteases. Calpains can cleave tubulin and MAPs in vitro (Goll et al., 2003). Interestingly, a non-active member of the calpain family, calpain 6, binds and stabilizes microtubules (Tonami et al., 2007). Protein kinase CK2 is a ubiquitous protein kinase involved in diverse cellular functions. CK2 binds and stabilizes microtubules and the kinase activity of CK2 is not required for this function (Lim et al., 2004). Similar processes are at work in plants. Four enzymes have been identified as microtubule-actin crosslinking proteins in tobacco pollen. These proteins are homocysteine methytransferase, phosphofructokinase, pyruvate decarboxylase, and glucan protein synthase (Romagnoli et al., 2010).

Plants are ‘partial fractals’

A fractal is ‘a rough or fragmented geometric shape that can be split into parts, each of which is (at least approximately) a reduced-size copy of the whole’ (Mandelbrot, 1982), a property called self-similarity. Fractals were defined by Mandelbrot (the discoverer of the Mandelbrot set) to be...
a set with Hausdorff-Besicovitch dimension $D_H$ strictly exceeding the topological dimension $D_T$. No plant exactly matches this definition since they consist of a finite number of primitives (lines or polygons) and $D_H > D_T$. However, this all changes if the term ‘fractal’ is used in a broader sense (Prusinkiewicz and Lindenmayer, 1996). A large number of fractal mathematical constructs have been described and, surprisingly, some of these have been found in nature, leading to their use in medicine, soil mechanics, seismology, and technical analysis (Gleick, 1987). Mandelbrot also wrote that ‘trees may be called fractals in part’ and it has been suggested that the cognitive recognition of self-similarity in plant structures makes it possible to render them using algorithms developed for fractals (Prusinkiewicz and Lindenmayer, 1996). This gave rise to L-system (or Lindenmayer system) mathematics whereby formal grammar is used to replicate plant-like structures. Whereas L-systems are concerned only with organismal shape and form, the analysis is extended here to the subcellular level.

In mathematics, a self-similar structure is exactly, or approximately, similar to a part of itself. Statistical self-similarity implies a similarity of statistical properties at different scales. It is suggested here that, over limited ranges of magnification, microtubules (and other proteins) can form different partially self-similar, or partially fractal, structures. These structures are not exactly self-similar, suggesting statistical self-similarity rather than absolute self-similarity but, on the other hand, there are elements of absolute self-similarity as well, for example, the helical structure of microtubules, cells, and organs in plants (see ‘Microtubules form fractal helices’ below). Thus it is suggested, as Mandelbrot did, that the best description of these structures is ‘partially fractal’. This makes the attribution of a numerical fractal dimension difficult, as any such attribution will necessarily require the loss of information. Thus, a descriptive, rather than a numerical, approach is best in describing these structures at the current time.

Fractals enable the transmission of information across differing scales and thus, in biological systems, may play a role in development. It is suggested here that plant microtubules have intrinsic fractal-based organizing tendencies and that different cell types may be based on different fractals derived from microtubules, with MAPs possibly modulating which fractal is displayed in any given cell. In plants, microtubules are largely associated with the cytoplasmic face of the plasma membrane during interphase. Encounters between microtubules are thus important drivers of self-organization of the microtubule array, with encounters modulated by cortical attachment, bundling, severing, and various MAPs playing key roles (Wasteneys and Ambrose, 2008). The two-dimensional nature of the plant cortical microtubule array means that both two- and three-dimensional fractals may be of use in describing plant microtubule partial fractals do not have functional consequences. For example, it could be argued that the branched fractal structure of arrays seen by Wasteneys and Williamson (1989) in regenerating Nitella cortical arrays following depolymerization do not have organismal consequences, yet Nitella does display a branching phenotype that may be microtubule-dependent.

Actin filaments may, under certain circumstances (see ‘Fractal triangles’ below), play a part in microtubule partial fractal development. As the actin and microtubule cytoskeletons interact closely, this follows. It appears likely that microtubules are more prone to forming fractal arrays than actin, since mutations in actin proteins do not lead to gross helical phenotypes similar to those seen with tubulin mutations. However, it could also be argued that elongated actin filaments within elongated cells form a type of fractal and, indeed, mutations in actin and actin-binding proteins often lead to isotropic growth of plant cells, data which are supported by drug studies (Mathur, 2004).

Cell division is another important process reliant on microtubules. However, it appears unlikely that cytoskeletal fractals are involved in preprophase band site choice as actin filaments are not necessary in this process (Mineyuki and Palevitz, 1990).

### Fractal helices

#### Microtubules form fractal helices

Microtubules are imperfect helices of $\alpha\beta$-tubulin dimers. In plants, in elongating organs such as the root and hypocotyl they form helices beneath the plasma membrane that, in turn, regulate the deposition of cellulose in the cell wall and hence the direction of expansion of the elongating cells. Various mutations in tubulin genes cause yet another level of helix (Ishida et al., 2007), with affected plants showing helical twisting of roots and other organs. Low doses of anti-microtubule drugs (Furutani et al., 2000), cause a left-handed helical growth form in roots. Defects in cell division do not appear to play a role here and the defect of growth seen in the $tor2$ alpha-tubulin 4 mutant appears to be based on cell expansion only (Buschmann et al., 2009). Thus, elongating plant organs are helical fractuals of at least four degrees—helical tubulin subunit arrangement (Fig. 1A), helical cortical microtubule arrays, helical cell shape, and helical organ shape (Fig. 1B).

#### Other protein fractal helices are important in plant development

Coiled-coil proteins possess a structural motif with two to seven alpha helices coiled together like the strands of a rope, for example, the GCN4 leucine zipper dimer (Fig. 2A). A coiled coil is a form of three-dimensional fractal and indeed coiled-coil proteins have been employed in nanotechnology to create multi-length scale fractal structures (Zhao and Zhang, 2007). Coiled-coil cytoskeletal proteins play key roles in plant development. The AtMAP70 family
of proteins is likely to be related to lamin intermediate filament proteins (Gardiner et al., 2011) and act as plant microtubule associated proteins. AtMAP70-5 is involved in patterning the xylem secondary cell wall (Pesquet et al., 2011) and regulates cell polarity, organ twisting, and growth (Korolev et al., 2007). A family of large coiled-coil Arabidopsis proteins is also related to lamins (Gardiner et al., 2011) and probably fulfils structural roles in the plant nucleus (Gindullis et al., 2002).

Another form of protein fractal is the armadillo (also known as beta-catenin) repeat. This structure is a super-helix of alpha helices (Fig. 2B; Huber et al., 1997). Again, this form of protein fractal plays key roles in plant cytoskeleton regulation and plant development. Arabidillo-1 and -2 promote lateral root development (Coates et al., 2006) while cellulose synthase-interactive protein 1 contains armadillo repeats and regulates CesA complex distribution and movement in the plasma membrane (Gu et al., 2010). Armadillo repeat-containing kinesins regulate epidermal-cell morphogenesis in Arabidopsis, possibly limiting the assembly and distribution of endoplasmic microtubules (Sakai et al., 2008). Both coiled-coil proteins and armadillo repeat proteins are likely to impart a helical twist to any macromolecular structures they form a part of, such as cellulose synthase complexes, thus extending the range of their fractals.

Isotropic fractals

The default growth pattern for plant cells is isotropic. This is seen clearly when microtubules are depolymerized by drugs, leading to organ swelling in roots due to isotropic cell expansion. This in itself is a form of fractal. If a two-dimensional cross-section is taken through a plant organ it reveals an isotropic fractal organization. Circular xylem vessels give rise to circular vascular bundles which are, in turn, part of a circular stem or root (Fig. 3). The degree of expansion of any plant cell is under the control of the
microtubule cytoskeleton, thus microtubules may play a role in the development of these vascular two-dimensional fractals. This possibility is highlighted by the regulation of vascular bundle patterning by the microtubule-associated protein AtMAP70-5 (Pesquet et al., 2010).

Fractal triangles
Tensile integrity (tensegrity) architecture has been proposed to be a means by which cells stabilize their shape and sense mechanical signals from the nanoscale to the macroscale (Fig. 4A; Ingber, 2010). Importantly, these networks are in a state of isometric tension (i.e. experience a tensile pre-stress), ensuring that molecular-scale mechanoochemical transduction mechanisms proceed simultaneously (Ingber, 2008). In cells, it is suggested that microtubules provide compression-resistant components and actin-filaments tensional elements in a unified tensegral architecture whole (Fig. 4A). There is evidence to support this theory, with living cells behaving like discrete structures composed of an interconnected network of actin filaments and microtubules when mechanical stresses are applied to cell surface integrin receptors (Wang et al., 2001). Protein folding may operate as a function of tensegrity, with repulsive van der Waals or electrostatic forces acting as compression-resistant components and attractive forces as tensional elements (Zanotti and Guerra, 2003).

The triangle is the basis of all tensegral structures, as it is the only polygon that cannot be deformed by force. Thus there is a fractal cascade from amino acid folding (and possibly even smaller scale) through to cytoskeletal organization. This is represented in fractal terms by the Sierpinski triangle fractal (Fig. 4B). In Arabidopsis, the organization of epidermal pavement cell microtubules in the cotyledon (Fig. 4C; Zhang et al., 2011) is suggestive of the Sierpinski triangle and microtubule–actin interactions are particularly important in the formation of interdigitating epidermal cells (Fu et al., 2005). While tensegrity in plants is not an established doctrine, there are certainly links between the actin and microtubule cytoskeletons, with mor1, botero, and spiral1 mutants being hypersensitive to latrunculin B (Collings et al., 2006).

Branched fractals
Microtubules in plant cells are nucleated via the recruitment of gamma-tubulin to extant cortical microtubules, giving rise to branched microtubules (Murata et al., 2005). Ethylene receptor ETR2 controls trichome branching through regulation of microtubule assembly (Plett et al., 2009). Branching of trichomes is regulated by a kinesin, with knockout plants having four as opposed to three branches (Lu et al., 2005). ANGUSTIFOLIA is another microtubule-associated protein required for trichome branching, and interacts with ZWICHEL, a second kinesin involved in the branching process (Folkers et al., 2002).

Taxol causes trichome branching in mutants, again suggesting a role for microtubules in trichome development (Mathur and Chua, 2000). Interestingly, spatial information for branch formation may be derived from mechanisms employed in cell divisions (Schnittger and Hülskamp, 2002), and gamma-tubulin is known to play a key role in mitosis in Arabidopsis (Binarová et al., 2006). Thus, under certain environmental conditions such as those seen in trichomes, microtubules may form a branched fractal, perhaps similar to that of the Julia dendrite (Fig. 5).

There are many similarities between the development of root hairs and trichomes (Ishida et al., 2008). Defects in the armadillo repeat-containing kinesin MRH2 cause a branching root hair phenotype (Yang et al., 2007). Reduced expression of alpha-tubulin genes points to a role for microtubules in the development of root hair branching as well as that in trichomes (Bao et al., 2001). Both oryzalin and taxol cause root hair branching suggesting that
microtubule dynamics are important in determining the directionality of growth (Foreman and Dolan, 2001) and mutations in a katanin microtubule-severing ATPase cause ectopic root hairs (Webb et al., 2002). Further evidence from drug studies in *Nitella* demonstrate that plant microtubules may form fractal arrays. Here, regenerating microtubule arrays following oryzalin drug treatment show a branching phenotype which is gradually obscured by a parallel microtubule organization as time progresses (Wasteneys, 2002).

**MAPs may play a key role in determining which microtubule fractal is present**

The environment of the cell may determine which microtubule fractal is able to express itself. This will include the tubulin isoforms present, the MAPs present, and the post-translational modifications of tubulin and MAPs. There is some evidence for this suggestion. Heat shock protein 90 acts as a phenotypic buffer, suppressing natural variations in plant growth and development (Sangster et al., 2008) and associates mainly with microtubules in plants (Petrášek et al., 1998) where it may affect fractal expression. Microtubules in CLASP knockout leaf epidermal cells are hyper-parallel, suggesting a role in modulating microtubules between helical and triangular fractal arrangements (Ambrose and Wasteneys, 2008).

**Conclusions**

The plant microtubule cytoskeleton shares in the multilayered complexity that is typical of biological systems. This complexity has various aspects that are particular to microtubules, such as probable redundancy in the regulation of microtubule destabilizing factors and aspects that are unique to plants, such as a large number of kinesin microtubule motors. Possibly unique to plants, microtubules appear to be able to switch between different modes of organization based around fractal properties of the microtubule cytoskeleton. It is proposed that much of plant development is achieved through these fractal modes, or combinations of these modes. This insight may assist in future studies of plant growth and development, for example, computational studies on the role of the plant microtubule cytoskeleton. There has been good recent progress in the use of computers to mimic plant microtubule dynamics (Allard et al., 2010) and the use of fractals in simulation may enable the extension of this work to the molecular level on one hand, and the organismal level on the other. Such an approach will have to take into account the ‘partial fractal’ nature of cytoskeletal arrays, however, with slightly different algorithms required at each iteration. It would be interesting to examine the expression of different MAPs in a time-course as microtubules recover from depolymerization for an insight into the role of the MAPs in modulating the switch from a branched microtubule fractal to a helical fractal, as seen by Wasteneys and Williamson (1989).

**References**


