

Twisting to a Different Rhythm: How Plants Have Used Conserved Microtubules for Plant-Specific Functions

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That plant cells have a cytoskeleton whose components are highly conserved when compared with their animal cell homologues can surprise some scientists. And yet what truly are surprising are not the similarities between animal and plant cytoskeletons but the differences that have evolved despite the strong conservation of cytoskeletal proteins. This review of plant microtubules will contrast the conservation of tubulin with the evolution of plant-specific microtubule arrays and a diversification of microtubule-associated proteins (MAPs), notably kinesins. Furthermore, microtubules contribute to the formation of the plant cell wall through controlling the oriented deposition of cellulose. This relationship, with cellulose microfibrils formed parallel to cytoplasmic microtubules, was first observed almost 50 years ago, but it is only recently that molecular details of this relationship have begun to be dissected.

Plant Intermediate Filaments and Microfilaments

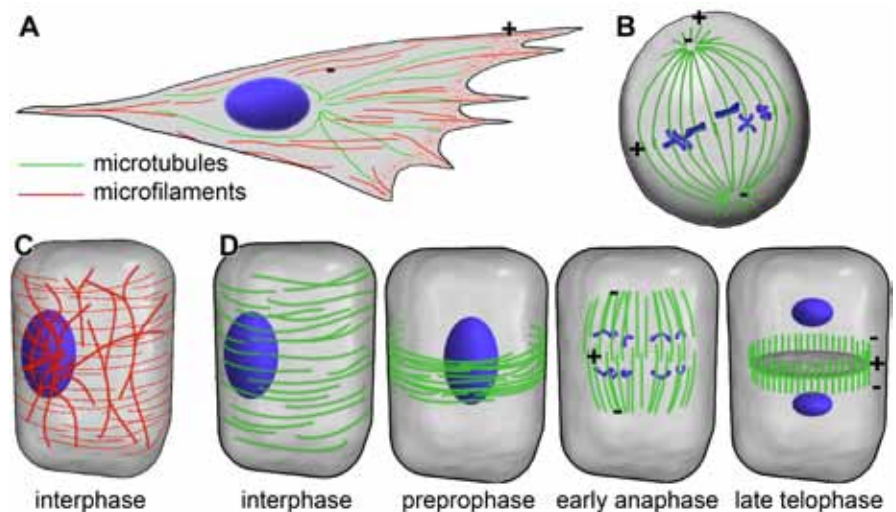
Before looking at microtubules, a detour via intermediate filaments and microfilaments is instructive, for understanding their loss and conservation can help in understanding plant microtubules. Classic intermediate filament proteins are absent from plant genomes and it is not difficult to suggest reasons for this loss as turgor

pressure and the cell wall provide the structural support to plant cells that intermediate filaments generate in animal cells. The exception to this loss of intermediate filaments is the retention in plants of a nucleoskeleton. Although the protein composition of this remains unknown, plant genomes contain several lamin-like proteins that show only limited conservation with their animal homologues.

Actin shows approximately 95% intraspecies sequence conservation between cytoplasmic and muscle actin isoforms in humans and *Drosophila*, but these isoforms maintain specific functions and localisations. The model plant *Arabidopsis* expresses seven actin isoforms whose 10% sequence variation is higher than in animal cells. These isoforms perform specific functions in growth and development (1), but it remains uncertain whether they polymerise into the different microfilament arrays present in a typical plant cell (Fig. 1C). Plant cells also contain numerous actin-binding proteins (ABPs) with homologies to animal ABPs. These include the motor protein myosin, and bundling and monomer-binding proteins. However, filament stabilisation proteins (e.g. tropomyosin, caldesmon) and membrane attachment proteins (e.g. spectrin) are absent from plant genomes (2). These absences, and the novel structures and functions of plant microfilaments, suggest that plant genomes contain unidentified, plant-specific ABPs (2).

Fig. 1. 'Typical' microtubule and microfilament arrays in animal (A,B) and plant (C,D) cells. The plus- and minus-ends of polarised microtubules are indicated (+/-).

- A. An interphase animal cell, with polar microtubules radiating from sites of polymerisation at the nucleus, and with several microfilament arrays.
- B. Animal cell division, with microtubules in astral arrays focused on tight spindle poles.
- C. Microfilaments in an interphase plant cell undergoing elongation: bundled microfilaments are present throughout the cell that support rapid, myosin-dependent cytoplasmic streaming, while transversely aligned cortical microfilaments run parallel and adjacent to cortical microtubules.
- D. Microtubules during the plant cell cycle. Interphase and preprophase band microtubules are bipolar, running in both directions, while the mitotic spindle (lacking spindle poles and astral microtubules) and phragmoplast are polarised arrays.



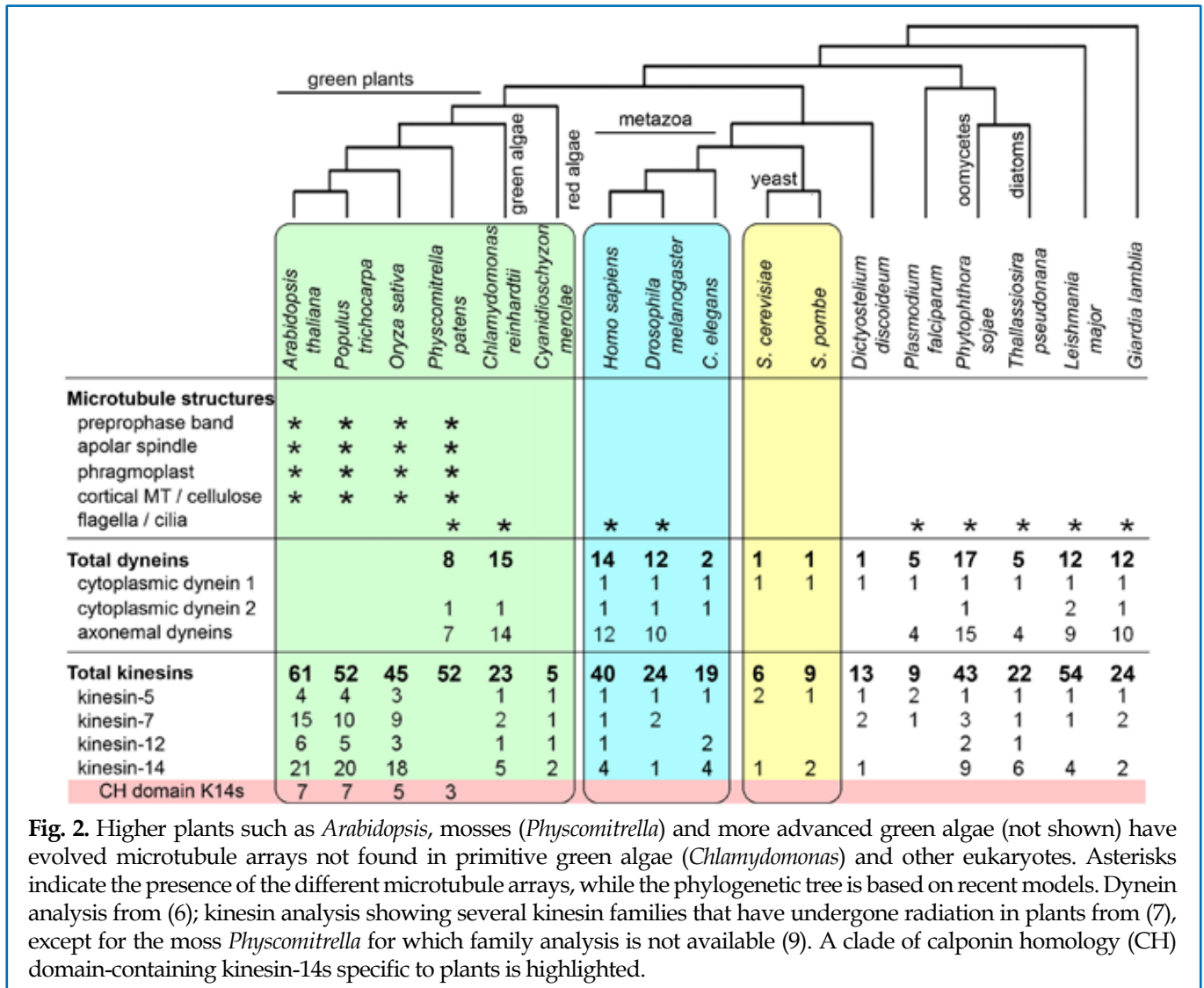


Fig. 2. Higher plants such as *Arabidopsis*, mosses (*Physcomitrella*) and more advanced green algae (not shown) have evolved microtubule arrays not found in primitive green algae (*Chlamydomonas*) and other eukaryotes. Asterisks indicate the presence of the different microtubule arrays, while the phylogenetic tree is based on recent models. Dynein analysis from (6); kinesin analysis showing several kinesin families that have undergone radiation in plants from (7), except for the moss *Physcomitrella* for which family analysis is not available (9). A clade of calponin homology (CH) domain-containing kinesin-14s specific to plants is highlighted.

Plant Tubulins, MAPs and Microtubules

Tubulins, like actins, are highly conserved in eukaryotes. However, unlike animal actin which cannot incorporate into plant microfilaments, animal and plant tubulins are functionally conserved. Fluorescently tagged animal tubulin microinjected into plant cells revealed microtubule turnover rates comparable to animal cells (3), which was subsequently confirmed by microtubule-labelling fluoroproteins (4). However, while plant and animal microtubules are identical, major differences exist in the organisation of microtubule arrays. Animal cells ‘typically’ have polarised microtubules radiating from the nucleus during interphase (Fig. 1A), form a focussed mitotic spindle with astral microtubules radiating from the spindle poles (Fig. 1B), and use a cleavage furrow to complete cytokinesis. In contrast, interphase plant cells have cortical microtubules that lack polarity, and have evolved several, novel microtubule arrangements during cell division (Fig. 1D). These include the preprophase band marking the division plane prior to mitosis, acentriolar and apolar mitotic spindles, and the phragmoplast, an interdigitated array of microtubules that completes cytokinesis. Most higher plants, however, lack microtubule-containing flagella.

As microtubules themselves are conserved, plants use a wide diversity of MAPs different to those found in animal cells (5) to generate their novel microtubule arrays. While

examples of bundling proteins such as MAP1 or tau do not occur in plant genomes, homologues of pan-eukaryotic MAPs including katanin, XMAP215 and kinesin exist, and the MAP65 family of bundling proteins has undergone extensive radiation. Plants also contain a collection of novel, plant-specific MAPs such as Spiral1 (5).

The molecular motors (dynein and kinesin) have had different fates in plants (Fig. 2). Dyneins are absent from angiosperm genomes with cytoplasmic dynein 1, which functions in organelle movement and the mitotic spindle, being lost early in plant and algal evolution. Axonemal dyneins and cytoplasmic dynein 2, which function in vesicle trafficking required to construct flagella, were lost with the loss of motile sperm cells. While they are present in the moss *Physcomitrella* (6), and are probably present in ferns and gymnosperms (cycads and *Ginkgo*) that also retain motile sperm, dyneins are absent from flowering plant genomes. While dyneins were lost during plant evolution, kinesins proliferated with their number and diversity being exceptional (Fig. 2). Not only do plant genomes contain the most kinesins with *Arabidopsis* having 61 (7), but their distribution among the 14 kinesin families is skewed compared to other eukaryotes. Two families, kinesin-7 and kinesin-14, account for more than half of the total (Fig. 2). Did kinesin proliferation occur because of the specific functions of plant microtubules during interphase and division?

Compared to other kinesins, kinesin-14s are unusual (8). Whereas most kinesins are plus-end directed motors, kinesin-14s are minus-end directed. Further, a subgroup of kinesin-14s with an N-terminal calponin homology (CH) domain and a microfilament-binding motif present in some ABPs occurs in higher plants (7) including the moss *Physcomitrella* (9), but is absent from green algae and other eukaryotes (7) (Fig. 2). These kinesins are the only known plant proteins containing *bonafide* actin- and microtubule-binding domains. Biochemical assays show microtubule- and microfilament-binding *in vitro*, and colocalisation with both elements of the cytoskeleton *in vivo* (10). However, the function of these C14-kinesins, and whether they actually link microtubules to microfilaments, remains to be determined. Kinesin-7s have also undergone rapid expansion in plants. The classical representative of this family is CENP-E, a component of the kinetochore complex responsible for chromatid aggregation at the metaphase plate. Most eukaryotes contain only a few kinesin-7s, but with *Arabidopsis* containing 15 kinesin-7s (Fig. 2), it seems likely that the role(s) played by kinesin-7s have also expanded. Two *Arabidopsis* kinesin-7s expressed only during cell division are essential for cytokinesis, being required for microtubule depolymerisation during phragmoplast expansion (11).

Of the 61 kinesins in *Arabidopsis*, fewer than 20 have been studied to any degree. However, almost all studied kinesins function during cell division and not interphase. This is consistent with analysis of kinesin expression in tobacco culture cells with synchronised cell divisions, where only two of 10 kinesins identified were expressed during interphase (12), with organelle motility in plants being actin / myosin-based, and with microtubule movement in interphase cells being limited to microtubule treadmilling (4). Of the three *Arabidopsis* kinesins known to function during interphase, two play roles in trichome (leaf hair) morphogenesis and only FRA1 (fragile fiber 1), an *Arabidopsis* kinesin-4, is known to function in cell wall development. Loss of this protein results in a significant loss of secondary cell wall strength without modifying cell wall composition (13), but how this occurs remains unknown. Considering the major role played by microtubules in cell wall formation, the minimal involvement of kinesins in this process is intriguing.

Plant Microtubules and Cellulose

Unlike animal cells, which can readily change shape, plant cells surrounded by rigid cellulose walls cannot. This difference has major implications for plant development. Plant growth occurs through the ordered expansion of cells in a single direction in a process driven by turgor pressure. This conversion of isotropic turgor pressure into anisotropic growth depends on variations in the biophysical properties of the cell wall. In an elongating cell, cellulose microfibrils are oriented transversely, perpendicular to elongation, where they prevent radial expansion but allow longitudinal growth. Furthermore, as the pattern of transverse microfibrils follows the pattern of transverse cortical microtubules (Fig. 3A), the physical properties of cell walls, and of cell wall-based materials such as wood, paper and cotton, depend on the properties of plant microtubules. However, this fundamental, long-standing and economically important question concerning the link between plant microtubules

and oriented cellulose deposition remains largely unsolved.

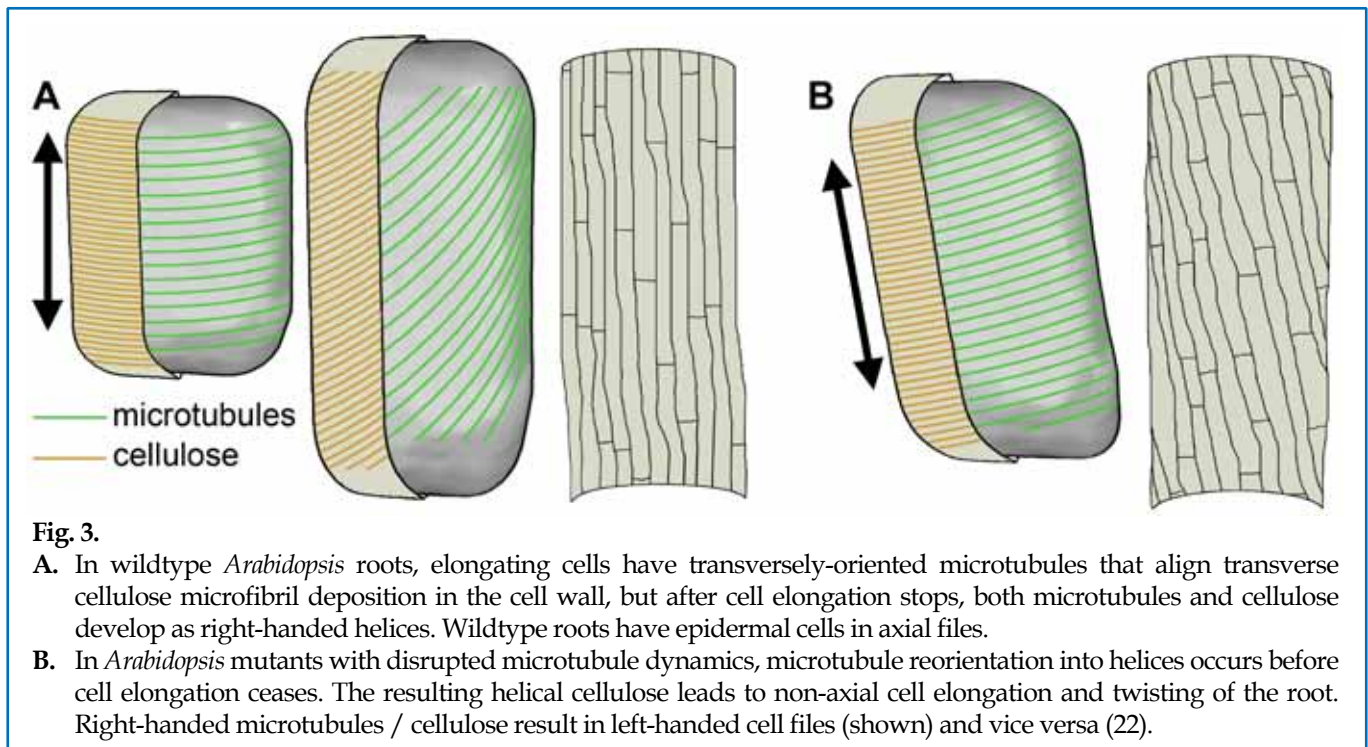
Even before microtubules were discovered, Green's 1962 experiments demonstrated that they were important for plant growth (14). Colchicine treatments, known at the time to disassemble mitotic spindles and now known to depolymerise microtubules, caused radial swelling and a loss of anisotropic growth. Green realised that the same proteins that form spindle fibres must be responsible for cellulose organisation. The subsequent discovery of cortical plant microtubules in patterns that mirror cellulose microfibril orientation (15) was immediately recognised as important. Only limited progress, however, was made in the following 40 years in identifying molecular mechanisms involved in this co-alignment (16).

A significant advance came in 2006 when GFP-tagged cellulose synthase complexes were shown to move through the *Arabidopsis* plasma membrane parallel to RFP-tagged microtubules, and to reorient following microtubule realignment (17). Cellulose synthase movement does not, however, require kinesin as the extrusion of cellulose itself provides the motive force. Although the molecular mechanism that allows interaction between cellulose synthesis and microtubules remains elusive, several cellulose synthase-deficient mutants are hypersensitive to microtubule disruption (18), showing that microtubules and cellulose synthesis are closely linked.

Twisted Research

One system for investigating microtubule / cellulose links is *Arabidopsis* root elongation. In wild-type plants, transverse microtubules and cellulose drive cell and root elongation without radial expansion or root twisting. As cell elongation ceases, microtubules re-orient from transverse into right-handed helices (Fig. 3A) (19). Certain *Arabidopsis* mutants with disrupted microtubules twist or spiral as they elongate. These mutants include the *lefty* mutants in α -tubulin that show a left-handed twist (20) (Fig. 3B), a large collection of left- and right-handed mutations in α - and β -tubulin (21), and numerous different MAPs (22). Significantly, kinesins have not yet been identified from the twisting mutants, confirming that most plant kinesins are division-specific rather than associated with interphase wall formation.

Twisting mutants contain microtubules that prematurely reorient into helices while cells are still elongating (Fig. 3B). Helical microtubules cause helical cellulose deposition, which results in non-axial cell expansion and spiralling of roots, with right-handed microtubules associated with left-handed twisting and vice versa (22). From analyses of mutants, and because low doses of the microtubule-disrupting drug propyzamide also generate left-handed twisting (20), it has been suggested that microtubule destabilisation leads to left-handed twisting and stabilisation to right-handed twisting (22). However, this is likely to be an oversimplification. Testing a broader range of microtubule disrupting compounds shows that while microtubule disruption with propyzamide causes left-handed twisting, related depolymerisers such as terbutool and pendimethalin cause right-handed twisting. In contrast, microtubule stabilisation with taxol also causes left-handed twisting. Further, a novel, semi-dominant *Arabidopsis* β -tubulin mutant shows left-handed twisting as a heterozygote, but right-handed twisting as a homozygote



(Collings, unpublished data). Nevertheless, a careful analysis of microtubule dynamics in mutants and following drug treatments may disentangle the links between microtubule organisation and cellulose deposition.

Spiralling is a common phenomenon in plants, and not limited to *Arabidopsis* roots. Climbing plants grow with a strong preference for right-handed twisting (23), and a role for microtubules in this has been suggested (19,23). The grain of many trees can also spiral. This is economically important in the timber industry as spiral grain can lead to sawn planks warping after they are cut. The similarities between twisting in *Arabidopsis* and spiral grain in trees has led my laboratory to explore what role(s) microtubules might play in the development of spiral grain in radiata pine through a combination of proteomic, molecular and cell culture experiments.

A Role for Microfilaments in Cell Elongation?

While this review has focussed on plant microtubules to the exclusion of actin microfilaments, the fundamental role played by microtubules in cell elongation does not preclude a role for microfilaments. Considerable evidence exists for cross-talk between transverse cortical microfilaments and microtubules (Fig. 1C,D) at microscopic and molecular levels (8). Microtubules can stabilise cortical microfilaments (24), and many microtubule mutants are hypersensitive to microfilament disruption with latrunculin (25). Indeed, an *Arabidopsis* mutant identified through hypersensitivity to microfilament disruption with latrunculin contains a mutation in β -tubulin (Collings, unpublished data). How such interactions might occur between microfilaments and microtubules remains uncertain, although CH domain-containing kinesin-14s (10) remain the most likely possibility. When *Arabidopsis* microtubule mutants showing the latrunculin hypersensitivity response (25) were analysed with gene chips, several CH-kinesin-14s were down-regulated (Collings and McCurdy, unpublished data).

Directions

Plants have used the conserved structure of the microtubule in numerous, novel ways. The presence of division-specific arrays such as the preprophase band and phragmoplast were likely imposed on plants by the presence of the rigid cellulose wall, itself a product of microtubule function during interphase. The evolution of these novel arrays led to a diversification of plant MAPs, notably kinesins. Although most plant kinesins remain to be characterised, to date there is remarkably little evidence for kinesins functioning in anything but cell division. Instead, the organisation of interphase microtubules, necessary for oriented cellulose deposition and thus of significant economic importance, occurs through as yet unknown mechanisms. The plant microtubule cytoskeleton would seem to contain enough mysteries to keep biologists entertained for some years to come.

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