Microtubule networks for plant cell division

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Abstract During cytokinesis the cytoplasm of a cell is divided to form two daughter cells. In animal cells, the existing plasma membrane is first constricted and then abscised to generate two individual plasma membranes. Plant cells on the other hand divide by forming an interior dividing wall, the so-called cell plate, which is constructed by localized deposition of membrane and cell wall material. Construction starts in the centre of the cell at the locus of the mitotic spindle and continues radially towards the existing plasma membrane. Finally the membrane of the cell plate and plasma membrane fuse to form two individual plasma membranes. Two microtubule-based cytoskeletal networks, the phragmoplast and the pre-prophase band (PPB), jointly control cytokinesis in plants. The bipolar microtubule array of the phragmoplast regulates cell plate deposition towards a cortical position that is templated by the ring-shaped microtubule array of the PPB. In contrast to most animal cells, plants do not use centrosomes as foci of microtubule growth initiation. Instead, plant microtubule networks are striking examples of self-organizing systems that emerge from physically constrained interactions of dispersed microtubules. Here we will discuss how microtubule-based activities including growth, shrinkage, severing, sliding, nucleation and bundling interrelate to jointly generate the required ordered structures. Evidence mounts that adapter proteins sense the local geometry of microtubules to locally modulate the

activity of proteins involved in microtubule growth regulation and severing. Many of the proteins and mechanisms involved have roles in other microtubule assemblies as well, bestowing broader relevance to insights gained from plants.

Keywords Microtubule · Phragmoplast · Cytoskeleton · Plant · Cortical array · Cytokinesis

Introduction

In the course of the cell cycle of higher plants, four microtubule networks in succession control plant cell growth and division (Wasteneys 2002) (Fig. 1). The location and orientation of the future division plane is established already before mitosis itself starts. First, microtubules associate to the plasma membrane and form an aligned cortical array (CA). Then, during the plant-specific pre-prophase a dense ring-like structure of microtubules develops out of the cortical array. The microtubule orientation of this pre-prophase band (PPB) is preserved from the CA (Cleary et al. 1992; Vos et al. 2004). The still intact nucleus is located at the ring's centre and dictates the construction of the mitotic spindle during the disassembly of the PPB (Vos et al. 2008; Masoud et al. 2013). Finally the bipolar microtubule array of the spindle is reused in the phragmoplast (Lee and Liu 2013). The phragmoplast deposits wall material containing vesicles to construct a new membrane enclosed cross-wall, termed the cell plate, in the cell centre. The phragmoplast gradually expands in the direction of the plasma membrane and becomes ring-shaped after microtubules at completed sections in the middle of the cell plate disassemble. The locus of the connection of the cell plate to the plasma membrane coincides with the position of the erstwhile PPB that has left

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Fig. 1 Spatial relations between microtubule networks in plant cell division. The cortical microtubule array **1** develops into the dense ring-like pre-prophase band **2** whose location coincides with the cell nucleus *blue*. Other microtubules that are initially formed around the nuclear

envelope develop into a mitotic spindle that segregates sister chromatids **3**. The phragmoplast **4** forms out of the spindle remnant and expands radially towards the location of the erstwhile pre-prophase band *black* leaving a finished cell plate in its wake *yellow*. (Color figure online)

cortical markers, making the PPB therefore a 100 % marker for the division plane (reviewed in Van Damme et al. 2007; Van Damme 2009; Müller et al. 2009; Rasmussen et al. 2013; Smith 2001). Communication between the cytoplasmic phragmoplast and cortical remnants of the PPB allow for control over symmetric and asymmetric cell divisions (Gallagher and Smith 1999; Rasmussen et al. 2013). How the four networks exchange relevant positional and orientational information is summarized in Fig. 1.

A striking feature of all microtubule networks in land plants, including the mitotic spindle, is that their organization occurs in absence of centrosome-like organelles (Murata et al. 2007). Most microtubules in animal cells first appear at centrosomes, typically associated with the nuclear envelope, and then grow outwards. In contrast, microtubule growth in plants is initiated exclusively at spatially dispersed sites. The initiation, or nucleation, is catalysed by specialized nucleation complexes, and is the first of a number of microtubule-associated activities that occur in almost all networks (Teixidó-Travesa et al. 2012). After nucleation, periods of microtubule growth and shrinkage succeed each other in a GTP-dependent process termed *dynamic instability* (Gardner et al. 2013). Switches between the two states are termed *catastrophes* and *rescues* respectively. Dynamic instability is most pronounced at the so-called plus-end of microtubules, but also the minus-end can have different states, being primarily static or shrinking (Ehrhardt and Shaw 2006). Throughout the eukaryotic kingdom, the dynamic state of microtubules is controlled by a host of conserved proteins (Subramanian and Kapoor 2012). Moreover, lateral associations between microtubules are facilitated by bundling proteins and specialized motor proteins. The latter activity can generate a force to

slide microtubules along each other (Kapitein et al. 2005; Janson et al. 2007; Peterman and Scholey 2009). Finally, existing microtubules can become severed to form two new microtubules (Roll-Mecak and McNally 2010). To mechanistically understand the formation of ordered microtubule networks for plant cell division it will be key to investigate microtubule nucleation, dynamics, bundling, sliding, and severing in an integral manner. Here, we will focus on recent progress on two plant-specific networks, the CA and the phragmoplast and will discuss how feedback between different microtubule activities is organized.

Division plane selection

As already mentioned, the orientation of the division plane is determined by the PPB, which in turn inherits its orientation from the CA. The CA consists of microtubules associated to the inner face of the plasma membrane and is linked to the cell wall by an as yet unidentified molecular component. It is by now well established that microtubules of the CA are able to self-organize into a highly aligned state, whose default orientation is transverse to the growth direction of the cell (Ehrhardt and Shaw 2006). The driving force for this ordering process are collisions between growing plus-ends and other obstructing microtubules (Dixit and Cyr 2004). In such a collision the incoming growing microtubule can either bend and continue to grow alongside the obstructing microtubule—an event called *zippering* or *entrainment*—, switch to a shrinking state—an event called *induced catastrophe* or *catastrophic collision*—, or slip over the obstruction and continue to grow in the same direction—an event called *cross-over*—. A

number of modelling studies have shown that, provided there are enough of these collisions, requiring e.g. a high enough rate of nucleation of new microtubules, spontaneous alignment of microtubules will occur (Allard et al. 2010b; Eren et al. 2010; Tindemans et al. 2010; Hawkins et al. 2010). There is still some debate on which of these collision outcomes is necessary and/or sufficient to explain the ordering process, but it is clear that induced catastrophes by themselves are a sufficient cause (Deinum and Mulder 2013). The relative probabilities of collision outcomes were shown to be dependent on the interaction angle and differ between cell types (Dixit and Cyr 2004; Wightman and Turner 2007). Control over collision outcomes may well involve microtubule bundling proteins and physical forces that are generated during collisions of cortically adhered microtubules (Allard et al. 2010a; Portran et al. 2013). There is also increasing evidence that selective severing of microtubules at cross-over sites by the severing protein katanin, in conjunction with adaptor proteins such as SPIRAL2, plays an important role in establishing and maintaining proper organization of the CA (Wightman and Turner 2007; Wightman et al. 2013; Zhang et al. 2013; Lindeboom et al. 2013).

The mechanism of relative alignment in itself is not sufficient to explain the lining up of microtubules in a particular direction for division plane selection. Here it turns out there is a key role for cell shape, and more specifically for the “edges” between the different cell faces in coaxing alignment into a particular direction. A notable physical difference between these edges is their radius of curvature. Cell edges that are generated when a new cell wall is constructed during cytokinesis are relatively sharp. Older edges are more rounded. Ambrose et al. (Ambrose et al. 2011) have shown that microtubules impinging on sharp edges have a very high probability ($\sim 90\%$) of suffering a catastrophe. For the remaining edges these edge-induced catastrophes are more rare, occurring roughly in 25 % per cent of all cases. This difference is sufficient to ensure that the preferred orientation of the CA becomes parallel to the sharp edges (Deinum 2013). Clearly, to form a division plane in a perpendicular direction, as observed for cell patterning during plant development, this strong selection must be overridden. Here there appears to be a role for the conserved microtubule associated protein CLASP that promotes the microtubule growth phase. In this case CLASP is seen to be localized to specific edges and to decorate microtubule bundles that cross-over from one cell face onto an adjacent cell face (Ambrose et al. 2011). CLASP may therefore guide microtubule growth through hard-to-take corners and enable the formation of CAs that are perpendicular to sharp cell edges.

The orientation-maintaining transition from the ordered CA to the PPB is as yet still poorly understood. One

possibility that has been suggested is a “search-and-capture” mechanism, by which a local propensity to bundle and stabilize microtubules at the locus of the PPB, possibly under control of the nucleus, serves to concentrate the microtubules to this region, either by depleting the free tubulin pool in the rest of the cortex, or by incorporating non-transverse microtubules through the zippering mechanism (Vos et al. 2004).

Cell plate formation

At the onset of mitosis a basket of microtubules appears around the still intact nuclear envelope and develops into a bipolar, noncentrosomal spindle after the nuclear envelope is broken down (Jensen and Bajer 1973; De Mey et al. 1982; Vos et al. 2008; Nakaoka et al. 2012; Masoud et al. 2013). The mechanism of centrosome-independent spindle formation in plants is very relevant to other eukaryotic cells since their spindles more generally contain a subset of microtubules that are generated independently of centrosomes (Wainman et al. 2009; Uehara and Goshima 2010). Spindles comprise two mirrored sets of polarized microtubules with plus-ends pointing towards the centre (Dhonukshe et al. 2006). Microtubule plus-ends either connect to their counterparts of the other spindle half or link each chromatid to the spindle apparatus via the multiprotein kinetochore complex. Whereas the latter microtubules shorten during sister chromatid segregation (Jensen and Bajer 1973), the former persist and form the template for the phragmoplast (Fig. 2). As such, phragmoplast position, orientation and bipolarity are inherited from the spindle apparatus. These events are very much reminiscent of the animal cytokinetic microtubule array which also develops from remaining spindle microtubules (Glotzer 2009). During cellularization of certain multinucleated plant cells, spindles do not precede phragmoplast assembly. However, here another bipolar microtubule array is reported to form between nuclear surfaces to act as a precursor for the phragmoplast (Van Lammeren et al. 1985; reviewed in Otegui and Staehelin 2000; Pickett-heaps et al. 1999; De Storme and Geelen 2013). Thus, in general, the phragmoplast maintains the bipolarity that is established by a preceding microtubule structure.

A new cell wall section is constructed at the equatorial plane of the phragmoplast (Fig. 2), but how are the factors involved in cell wall assembly targeted to this site? Since microtubule plus-ends point towards the phragmoplast centre, an attractive targeting mechanism is directed transport of vesicular cargo along microtubules towards the division plane (Euteneuer and McIntosh 1980; Lee et al. 2001; reviewed in McMichael and Bednarek 2013). A subset of microtubules not only terminate near the

Fig. 2 Cytokinesis visualized by live cell imaging in the moss *Physcomitrella patens*. Microtubules are shown in green and chromatin and cell-plate material in red. Chromatids are segregated towards spindle poles and the remaining microtubules of the mitotic spindle (*top*) are used as template for the initial phragmoplast (*middle*; 6 min later). The reforming daughter nuclei are seen at the phragmoplast poles while accumulation of cell plate material is visible in the central plane of the phragmoplast where microtubules of opposite poles form antiparallel overlaps (*arrowheads*). Fifteen minutes later the central region of the cell plate has matured and the phragmoplast has expanded laterally (*bottom*). Microtubules were visualized using GFP fused to α -tubulin (Hiwatashi et al. 2008), chromatin using mRFP fused to histone2B (Nakaoka et al. 2012) and the membranous material delivered to the cell plate with the lipophilic styryl dye FM4-64. The scale bar indicates 10 μ m

phragmoplast centre, but extend across the division plane and meet with the opposing set to form short, antiparallel overlaps that coincide with the site of cell plate assembly. A key player for proper formation of these overlaps is the microtubule cross-linker MAP65, without which the two opposing sets of microtubules are not properly engaged, ultimately leading to cytokinesis failure (Müller et al. 2004; Ho et al. 2011a; Kosetsu et al. 2013). Besides aiding in maintenance of the overall bipolar structure of the phragmoplast, these cross-linked antiparallel overlaps may

provide a very localized scaffold for directing cytokinetic processes, much akin to the stem body/Flemming body in animal cells (Otegui et al. 2005; Fuller et al. 2008; Glotzer 2009). Such a notion is supported by the delayed cell plate build-up when MAP65 is down-regulated even though a seemingly normal, bipolar phragmoplast is present (Kosetsu et al. 2013). Microtubule overlaps therefore seem to be important for both the structural integrity of the phragmoplast and the proper recruitment of cell wall material.

To act as a robust scaffold and hence to facilitate building of a well-defined cell plate, the position and size of overlaps would require strict control. Recent work using various model systems has provided insight into possible mechanisms for length regulation of overlaps in bipolar microtubule structures (Subramanian and Kapoor 2012). A common concept encountered in these cases is that the dynamic parameters of microtubules engaged in an overlap are locally altered by an effector protein recruited to the overlap by microtubule cross-linkers of the Ase1/PRC1/MAP65 family. For instance, in yeast and human spindles the microtubule rescue factor CLASP is recruited (Bratman and Chang 2007; Liu et al. 2009; Al-Bassam et al. 2010) and in case of the animal midzone the growth-inhibiting kinesin KIF4 is recruited by PRC1 (Bieling et al. 2010; Hu et al. 2011; Nunes Bastos et al. 2013). Large fluctuations in microtubule length are thus generally suppressed in overlaps. Plus-end-directed motors at overlaps, some of which are possibly also recruited by cross-linkers, may furthermore contribute to length control by sliding microtubules apart (Nislow et al. 1992; Kapitein et al. 2005; Fu et al. 2009). In plants, microtubule growth in overlaps in combination with sliding by unknown motor proteins forms a plausible explanation for the continuous outward-directed flux of microtubule mass observed in both spindles and phragmoplasts (Asada et al. 1991; Dhonukshe et al. 2006; Murata et al. 2013). In the phragmoplast several kinesin motors have been identified that reside at the overlap zone and function in phragmoplast assembly and maintenance (Lee and Liu 2000, 2013; Lee et al. 2007; Hiwatashi et al. 2008; Ho et al. 2011a). Yet, how they contribute to the forces at play in the antiparallel overlaps of the phragmoplast is still not as well conceived as in other systems. It will be intriguing to learn how microtubule sliding, bundling and dynamics jointly control overlap length. The manner in which multiple overlaps are aligned in space is yet another intriguing and open question.

What drives the radial expansion of the phragmoplast towards the maternal cell wall? Phragmoplast microtubules themselves exhibit no or very little lateral movement (Yasuhara et al. 1993). Instead, existing microtubules are thought to disassemble at completed cell plate sections while newly nucleated microtubules form new overlaps at the leading edge, propagating the bipolar configuration

laterally (Murata et al. 2013). In agreement, genetic or antibody interference of components of the microtubule-nucleation pathway severely inhibits phragmoplast expansion (Ho et al. 2011b; Hotta et al. 2012; Nakaoka et al. 2012; Murata et al. 2013). Temporal control over phragmoplast expansion likely incorporates feedback from the state of cell plate assembly (Yasuhara and Shibaoka 2000) and may be achieved by a MAPK pathway that post-translationally modifies MAP65 (Sasabe et al. 2006a, b; Sasabe and Machida 2012).

Aforementioned microtubule nucleation factors localize throughout the phragmoplast with the exception of the equatorial region (Liu et al. 1993; Zeng et al. 2009; Ho et al. 2011b; Nakaoka et al. 2012; Murata et al. 2013). Recent observations indicate that they may primarily reside on microtubule bundles that run perpendicular to the division plane (Murata et al. 2013). In the plant cortical array microtubule-dependent nucleation occurs at approximately 40° with respect to the template microtubule (Murata et al. 2005; Murata and Hasebe 2007; Chan et al. 2009). Though not yet directly shown, it is likely that microtubule branching angles in the phragmoplast also occur within a certain regime to reinforce its bipolar organization. Using meiotic frog extracts such a mechanism in which existing microtubules form a template for the ordered branching of new microtubules was elegantly demonstrated to operate in animal spindles (Petry et al. 2013). The ordered branching of microtubules may therefore be an important factor for setting up and maintaining network polarity.

Ultimately, the direction of expansion of the phragmoplast should be towards the sites on the parental cell membrane, occupied earlier by the PPB (reviewed in Van Damme et al. 2007; Van Damme 2009; Müller et al. 2009; Rasmussen et al. 2013; Smith 2001). Early centrifugation experiments have demonstrated that dislocated phragmoplasts are able to move towards the PPB-marked division zone, yet the nature of the driving forces remains elusive to date (Ota 1961; Mineyuki and Gunning 1990). To induce changes in phragmoplast position, the marked cortical area could function as a signaling beacon, producing a gradient of a certain signaling molecule that affects phragmoplast dynamics. Alternatively, cytoskeletal filaments could form a physical link between the marked cortex and the phragmoplast (Lloyd and Traas 1988; Sano et al. 2005; Van Damme et al. 2007). The selective presence or absence of various cytoskeletal-related proteins at the PPB-marked cortex is in support of such a linking mechanism. Proteins that are differentially located at the PPB-marked cortex include kinesin motors and actin itself (Cleary et al. 1992; Vanstraelen et al. 2006; Müller et al. 2006; Van Damme et al. 2007; Miki et al. 2014). The identification of various other molecules that tune phragmoplast expansion will help

Fig. 3 Feedback between microtubule organization and microtubule activities **a** Augmin-mediated branching of new microtubules from the sides of existing microtubules. **b** Points of microtubule crossover in plants are sites of microtubule severing by katanin, possibly regulated by SPIRAL2. **c** Crosslinking proteins in between overlapping microtubules recruit proteins that locally regulate dynamic instability or drive microtubule sliding. **d** Crosslinkers confined in between sliding microtubules are driven together by converging microtubule ends. **e** Existing microtubules obstruct the growth of incoming microtubules triggering shrinkage in the near two-dimensional geometry of the plant cortex

to unravel the signaling network through which a flagged cortical domain can guide cell plate formation (Cleary and Smith 1998; Müller et al. 2006; Walker et al. 2007; Xu et al. 2008).

Outlook

Microtubules in eukaryotic cells exhibit very diverse network geometries all involving a recurring set of activities: nucleation, dynamic instability, bundling, severing and sliding. Recent work on microtubule networks has provided insight into how feedback between these activities is organized through adapter proteins (Fig. 3a–c). Existing microtubules for example trigger nucleation of new microtubules through augmin-mediated recruitment of microtubule nucleation complexes leading to local microtubule amplification (Clausen and Ribbeck 2007; Petry et al. 2013). Directionally biased microtubule-dependent nucleation can reinforce an existing polarity (Smertenko et al. 2011; Kamasaki et al. 2013). Another example of feedback involves the dynamic instability of microtubules in bundles, which is adapted locally through recruitment of CLASP or KIF4 by bundling proteins (Bratman and Chang 2007; Bieling et al. 2010). Bundling proteins were also reported to bind motors, generating crosstalk between bundling and sliding action (Fu et al. 2009). Microtubule cross-overs may furthermore trigger microtubule severing through SPIRAL2-mediated modulation of katanin activity

(Wightman et al. 2013). In general, adapter proteins may sense the local configuration of microtubules to provide feedback on microtubule network layout. Examples are MAP65/Ase1/PRC1, which bind to antiparallel-oriented microtubules, and SPIRAL2, which is recruited to sites of microtubule cross-over (Janson et al. 2007; Subramanian et al. 2013; Wightman et al. 2013). The use of such adapter proteins may enable a conserved set of proteins to be efficiently used in various network layouts and may facilitate fast switching between network architectures as observed in plant cell division. Other forms of feedback may be more physical in origin (Fig. 3d, e), such as the packing of bundling proteins within the overlaps of sliding microtubules or the induction of catastrophes through collisions with neighbouring microtubules (Allard et al. 2010a; Braun et al. 2011). Cell geometry plays an additional role as shown by local cell wall curvature which may invoke microtubule catastrophes (Ambrose and Wasteneys 2012). Work on diverse microtubule networks in animal, yeast, and plant cells, and insights gained from reconstituted in vitro systems highlight that the organization of networks can only be understood if multiple microtubule associated activities are integrated into our models.

Future insights into the organization and functioning of plant microtubule networks for cell division will be aided by new developments on model systems and light microscopy. In particular the continuing development of plane-illumination microscopy will allow for the long-term visualization of 3 dimensional microtubule networks within developing plant tissues (Maizel et al. 2011). Cell division in higher plants occurs in complex meristematic tissues in which microscopic observations of cell division are often hindered by several layers of overlaying cells. Lower plants exhibit simpler body plans and model organisms like the liverwort *Marchantia polymorpha* and the moss *Physcomitrella patens* enable the observation of cell division without interference of other cells. Moreover, these organisms have a prominent haploid phase in their life cycle and efficient homologous recombination, which both greatly simplify the generation of controlled gene knockouts, gene alterations, and fluorescent reporter gene fusions (Ishizaki et al. 2013; Miki et al. 2014). These assets allow for large-scale studies into gene functioning and gene product localization that will greatly facilitate mechanistic studies. Moreover, they allow for an evolutionary view on the conservation and diversification of microtubule activities used for network construction. It emerges that some protein functions cannot be generalized from metazoans to plants, possibly reflecting the different challenges faced during cell division (Miki et al. 2014).

Microtubule arrays organized throughout cell division allow plants to organize a fascinating variety of tissues, which are intensely utilized by mankind as food,

construction material, and source of renewable energy. Moreover, plants provide us with an intriguing diversity of microtubule network layouts that may teach us fundamental lessons about self-organization in cell biology.

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