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Cytokinesis series

Midbodies and phragmoplasts: analogous structures involved in cytokinesis

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Cytokinesis is an event common to all organisms that involves the precise coordination of independent pathways involved in cell-cycle regulation and microtubule, membrane, actin and organelle dynamics. In animal cells, the spindle midzone/midbody with associated endo-membrane system are required for late cytokinesis events, including furrow ingression and scission. In plants, cytokinesis is mediated by the phragmoplast, an array of microtubules, actin filaments and associated molecules that act as a framework for the future cell wall. In this article (which is part of the *Cytokinesis* series), we discuss recent studies that highlight the increasing number of similarities in the components and function of the spindle midzone/midbody in animals and the phragmoplast in plants, suggesting that they might be analogous structures.

Introduction

Cell division is required for the propagation of all living things. Of central importance is the equal partitioning of chromosomes between daughter cells, which in all eukaryotes is mediated by the mitotic apparatus or spindle. The spindle comprises microtubules (MTs) and associated proteins that function to physically segregate sister chromatids to opposite ends of the cell. In animals, the spindle also specifies the position of an actin- and myosin-based contractile ring, which constricts and drives the physical separation of the two daughter cells [1]. In plants, cytokinesis is mediated by the phragmoplast (see Glossary), an array of microtubules, actin filaments and associated molecules that act as a framework for formation of a new cell wall. A large number of proteins involved in plant, yeast and animal cytokinesis have been identified [2–8], which appear to share functional homology (Table 1). In this review, we focus on advances that have added significantly to our understanding of the late steps in cytokinesis in both animal and plant cells. We will highlight key insights into assembly of the midbody and phragmoplast and growing similarities between these two structures.

The midbody

In animal cells, after sister chromosomes have separated, the remaining non-kinetochore, overlapping MTs form a structure called the spindle midzone (Glossary). At this time, the actomyosin ring begins to assemble and contract. The remnant of the spindle midzone persists as a structure known as the residual body or midbody (Glossary) that has been compressed by the ingressing cleavage furrow (Figure 1a) and which will eventually reside within the intercellular canal (Glossary) that connects the two cells [9]. The midbody consists of a compact, dense matrix of proteins embedded in the region of microtubule overlap. In the early 1970s, the midbody had been proposed to play a role in preserving spindle bipolarity during anaphase and maintaining the division of the cytoplasm, originally established by the cleavage furrow. The resistance of the midbody structure to chemical or physical disruption suggested that the midbody MTs were associated with a dense

Glossary

Midzone: a structure consisting of bundled anti-parallel microtubules and associated proteins that forms between separating chromosomes during anaphase. It can have roles in spindle elongation and cytokinesis in various organisms. The microtubules are most likely remnants of the mitotic spindle that were not attached to chromosomes.
Midbody: a compact, dense matrix of proteins embedded in the region of microtubule overlap that is formed from the spindle midzone and cleavage furrow and fills the intercellular channel connecting daughter cells at the completion of cytokinesis in animal cells.
Intercellular canal: the channel connecting two daughter cells that remains after the contractile ring pinches in the plasma membrane. This channel must be sealed in a process that is distinct from furrow ingression and might be similar to plant cytokinesis.
Cell plate: flattened membrane-bound structure that forms from fusing vesicles in the cytoplasm of a dividing plant cell and is the precursor of the new cell wall.
Phragmoplast: a cylindrical structure containing two opposing arrays of actin filaments and microtubules, with their plus (+) ends facing the plane of division. Its main function is to transport Golgi-derived vesicles to the site of cell plate assembly.
Preprophase band: a circumferential array of parallel microtubules that appears in G2 and marks where the cell plate will fuse with the existing cell walls. It disappears before prophase but leaves some, as yet unknown, mark behind.

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Table 1. Conserved components of the spindle midzone/midbody and phragmoplast[a]

C. elegans	*D. melanogaster*	Mammals	*S. pombe*	*S. cerevisiae*	Plants	Refs
SPD-1	**Fasceto/FEO**	**PRC1**	**Ase1p**	**Ase1p**	**MAP-65**	[28,29,31–34,37]
CYK-4	**RacGAP50C/ Tumbleweed**	**MgcRacGAP**	Rga7p	Rgd1p	At4g24580	[1,93]
ZEN-4	**Pavarotti/PAV**	**MKLP1/CHO1, RabK6/MKLP2**	– – –	– – –	At1g20060 (AtT20H2.17)	[1,7,95]
AIR-2	**DmAuroraB**	**AuroraB/AIM-1**	**Ark1p**	**Ipl1p**	**At-Aurora 1, -2**	[7,96,97]
ICP-1	**DmINCENP**	**INCENP**	**Pic1p**	**Sli15p**	– – –	[1,96]
BIR-1	Deterin	**Survivin**	**Bir1p/Cut17p**	**Bir1p**	– – –	[1,96]
CSC-1	DmBorealin	**Borealin/CDCa8**	– – –	– – –	– – –	[1,96]
CDC-14	CG7134	**Cdc14a**	*Cdc14p*, **Clp1p/Flp1p**	**Cdc14p**	– – –	[98–100]
KLP-12, **KLP-19**	**KLP3A**	**KIF4**	– – –	– – –	*FRA1/At5g47820*, At3g50170, At5g60930	[7,49,101,102]
BMK-1	**KLP61F**	**BimC**	**Cut7p**	**Kip1p, Cin8p**	**TKRP125, DcKRP120–2**	[53,103–107]
PLK-1, -2, -3	**Polo**	**PLK1–4**	*Plo1p*	*Cdc5p*	At4g24400	[108]
TAC-1	*d-tacc*	**TACC1**	– – –	– – –	– – –	[109–111]
ZYG-9	**Msps**	**ch-TOG/XMAP215 homolog**	*Dis1p*, **Alp14p**	**Stu2p**	**MOR1/GEM1**	[7,46,112,113]
KLP-3, *-15*, *-16*, *-17*	***Ncd***	***KIFC1*/*CHO2***, KIFC2, KIFC3	**Pkl1p, Klp2p**	**Kar3p**	**AtK1/AtKatA**, AtK2/AtKatB, AtK3/AtKAtC, AtK4/AtKatD, **AtK5**, AtKCBP	[7,55,57,114]
EBP-1, VW02B12L.3	**DmEB1**	**EB1**	**Mal3p**	**Bim1p**	**AtEB1**	[7,115–118]
Y48G8AL.1	CG9135	**TD-60**	**Pim1p**	– – –	At1g19880	[119,120]
C33D9.8	*dCLP-190*	**CLIP-170/Restin**	**Tip1p**	Bik1p	**MCLIP-170**	[7,115,121]
DYN-1	**Shibire**	**Dynamin II**	*Dnm1p*	*Dnm1p*	**DRP2A**[b], **AtDRP1/Phragmoplastin, ADL1A, ADL1E**	[7,76,122,123]
CDK-1	Cdc-2	**p34cdc2/CDK1**	Cdc2p	Cdc28p	**CDC2**	[7,124–126]
SYN-4*	**Syntaxin 5***	**Syntaxin 2***	– – –	– – –	**KNOLLE/AtSYP111***[c]	[66–69]
CYK-1	**Diaphanous/DIA1**	**Formin/mDia**	**Bni1p**	**Cdc12p**	**AtFH5**	[25,127–130]

[a]Proteins in bold have been shown to localize to and/or were isolated from the spindle midzone/midbody or phragmoplast. Proteins in italics have been localized to other structures but not shown to localize to the spindle midzone/midbody or phragmoplast. Proteins underlined have not been localized. Dashed lines indicate the lack of a clear ortholog.

*These proteins might not be homologs, but syntaxins are involved in cytokinesis in many organisms.

[b]DRP2A is related to conventional animal dynamins, which are involved in clathrin-coated vesicle formation and membrane fission during endocytosis. Some members of the DRP1 family appear to constrict cell-plate membranous tubules without leading to membrane scission.

[c]KNOLLE, a cytokinesis-specific SNARE, is also a plant-specific member of the *Arabidopsis* SYP1 family.

population or 'glue' of microtubule-associated proteins (MAPs) that would maintain the structure [10]. In addition to MAPs, Golgi membranes are also found along the MTs of the midbody and in regions just outside the midbody [7] (Figure 1a). The membranes are often Golgi derived [11], but, in *Drosophila* and *Caenorhabditis elegans*, extensive ER structures have also been shown to be associated with the spindle [12,13]. The extent of Golgi membranes in these systems has not been explored and might predominantly be associated with the ER during this time. In mammalian tissue-culture cells, spindle-associated Golgi has been suggested to function in the transport of proteins and membrane to seal and complete division [7,11,14]. The midbody structure and midzone MTs must be maintained until new membrane insertion has occurred. In *Drosophila*, the final pinching off of membrane is not always complete, and here persisting intercellular canals or ring canals are seen between developing germ cells [15]. Recent work in both *C. elegans* and *Drosophila* has shown that cytokinesis, whether complete or incomplete, is sensitive to Brefeldin A (BFA) [2,16], a drug that affects the assembly of vesicles from the Golgi [17].

The phragmoplast

The phragmoplast, the structure that plays an essential role during cytokinesis in plants, appears, at first glance, to be very distinct from the midbody in animal cells, but an interesting number of molecular and functional similarities are beginning to appear. The phragmoplast can be differentiated topographically into two areas, the midline that includes the central plane where some of the plus-ends of both anti-parallel sets of microtubules (MTs) interdigitate (as in the midbody matrix), and the distal regions at both sides of the midline (Figure 1b). When the cell plate grows and matures between the two sets of MTs, the phragmoplast midline is lost and the MTs quickly disassemble. New MTs polymerize close to the cell plate growing edge, establishing a new midline region. The phragmoplast midline is therefore a crucial area because it is, first, where most of the membrane fusion takes place and, second, where the two sets of anti-parallel MTs are held together. The discovery of an important variety of molecules that localize to the phragmoplast midline is shedding light on the complex processes operating in this phragmoplast region (Table 1; Figure 2b).

A filamentous matrix, the CPAM (cell-plate assembly matrix), is located at the phragmoplast midline, enclosing the cell plate growing edges, fusing vesicles, and most of the MT plus-ends (Figures 1b and 2b) [18]. Although the composition of this matrix is not known, it has been postulated to play an important role in both stabilization

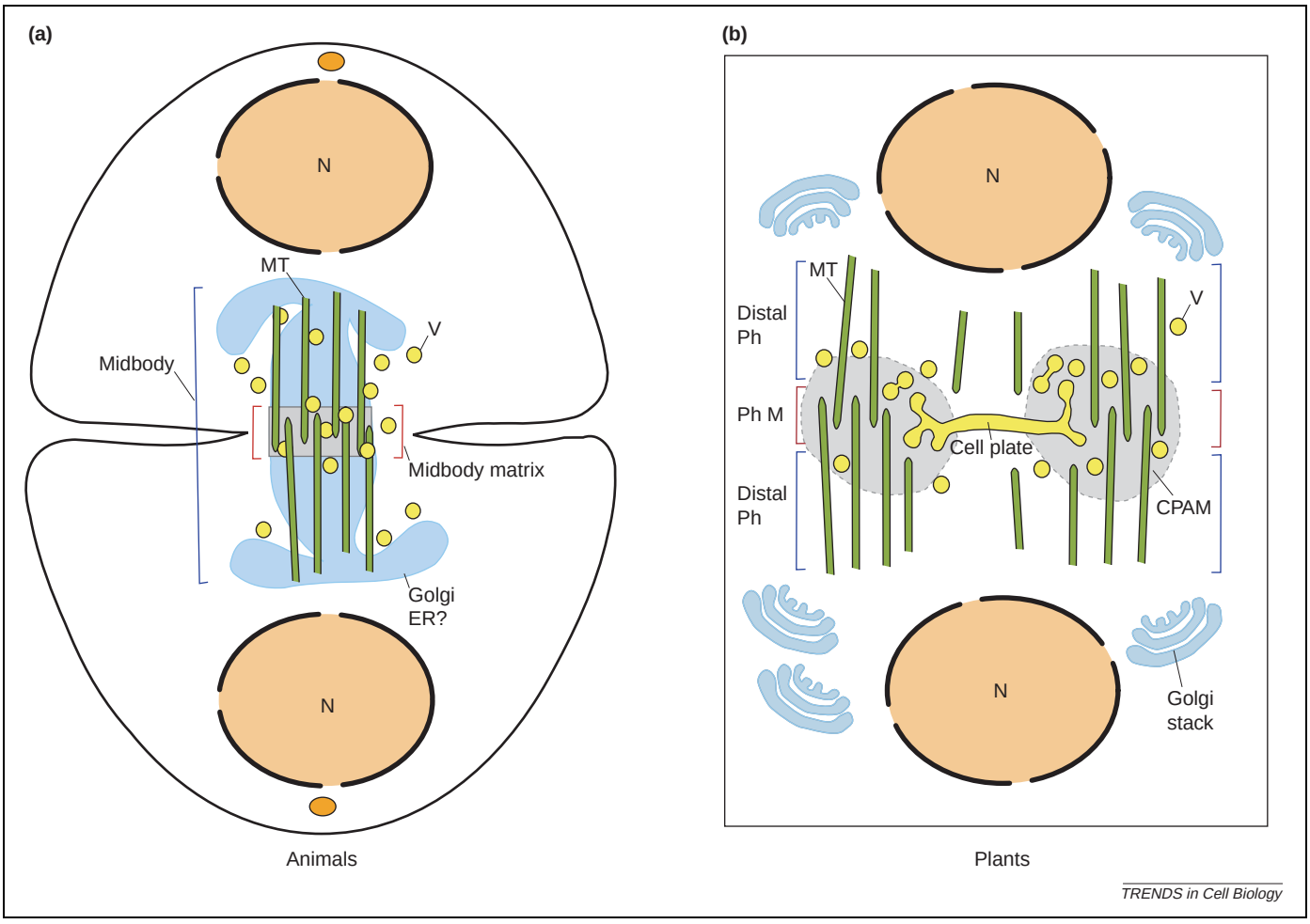

Figure 1. Overview of animal and plant cytokinesis. **(a)** Cytokinesis in animal cells. The spindle midzone/midbody forms when microtubules (MTs) from opposite poles overlap. It consists of the overlapping microtubules as well as associated proteins that bundle these MTs and other proteins that together form a dense protein matrix. This matrix excludes antibodies against MTs, giving a stereotypical region devoid of staining. As the furrow ingresses, the midzone is swept into one larger structure called the midbody. The Golgi and endoplasmic reticulum (ER) membranes are also found in the midbody during telophase to cytokinesis. It is proposed that vesicles (V) traffic along the midbody microtubules toward the ingressing furrow. **(b)** Cytokinesis in somatic plant cells. The forming cell plate is assisted by the phragmoplast at the future site of the new cell wall. Two topographic regions can be distinguished in the phragmoplast: the phragmoplast midline (Ph M), where the opposing set of microtubules interdigitate, and the distal phragmoplast (distal Ph), at both sides of the phragmoplast midline. A filamentous cell-plate assembly matrix (CPAM) accumulates at the phragmoplast midline. Key: MT, microtubule (green); N, nucleus (tan); V, vesicle (yellow); Golgi (pale blue); midbody matrix (gray box); CPAM (gray circles).

of phragmoplast MT plus-ends and membrane fusion [19]. Many of the proteins mentioned in this review colocalize with the CPAM and, therefore, might be recruited by the CPAM or be CPAM components. Although the CPAM has recently been identified in plants, a similar structure, called the telophase disc, was described several years ago in animal cells [20]. The telophase disc is a dense region proposed to bisect the cell in telophase and precedes the formation of the cleavage furrow. It will be interesting to see whether the CPAM and telophase disc share similar protein composition.

The cell plate is thought to be directed to the cortical division site by an acto-myosin-mediated mechanism [21]. Besides the two bipolar, anti-parallel sets of phragmoplast actin filaments, there is a set of actin filaments that assemble on the growing domains of the cell plate [22]. Some of these actin filaments connect the edge of the cell plate to the maternal plasma membrane [23], directing the growing cell plate to the cortical division site. In addition, the actin cytoskeleton is required for phragmoplast expansion as the inhibition of myosin motors with 2,3-butanedione monoxime and ML-7 results in altered phragmoplast expansion [21]. Recently, AtFH5, one of the 20 formin homologs found in *Arabidopsis* has been shown to localize to the cell plate [24]. Formins, including AtFH5, have actin nucleation activity and interact with fast-growing barbed ends of actin filaments [24] and are an essential component of the contractile ring in animal cells, modulating the growth of actin filaments [25].

Midbody and phragmoplast assembly

The midbody is derived from the spindle midzone microtubules and associated proteins found there. There are two major components known to be widely required for the formation of the spindle midzone in all animals, Centralspindlin and the chromosomal passenger proteins. Centralspindlin, which contains the kinesin-like protein MKLP1/ZEN-4/PAVAROTTI, and the GTPase-activating protein MgcRacGAP/CYK-4, localize to the midzone/midbody and can bundle microtubules in a cell-cycle-dependent manner. The chromosomal passengers include INCENP/ICP-1, the kinase AuroraB/AIR-2, Survivin/BIR-1 and Borealin/CSC-1 (Table 1), localize to chromosomes at

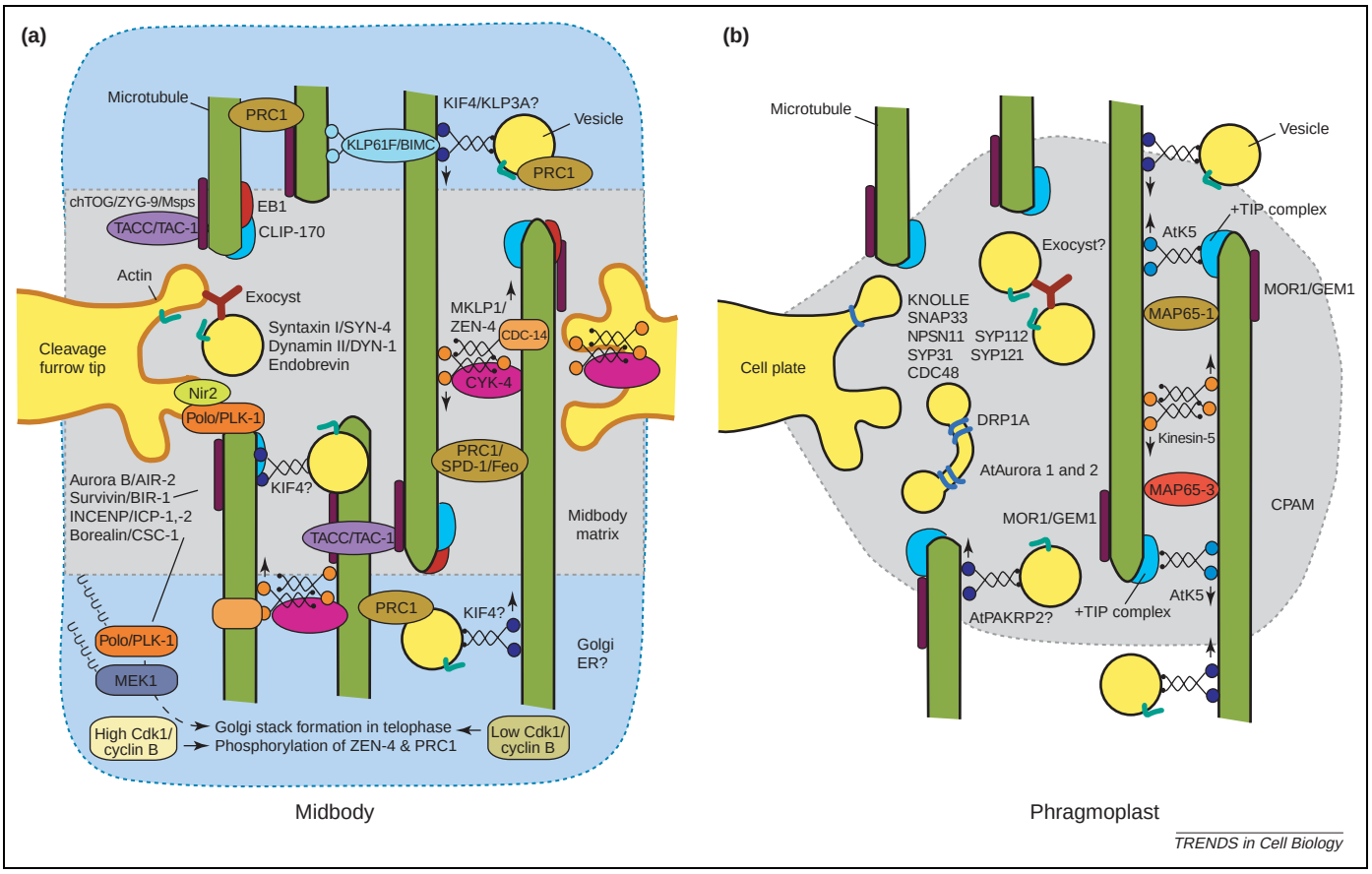

Figure 2. Models for assembly of the midbody and phragmoplast. **(a)** Model depicting midbody organization. Overlapping microtubule plus-ends are embedded within the midbody matrix. The midbody microtubules are stabilized and crosslinked by several kinesins, microtubule-associated proteins (MAPs), kinases and other proteins, such as MKLP1/ZEN-4/PAV, MgcRACGAP/CYK-4, PRC1/SPD-1/FEO/Ase1p, chTOG/ZYG-9/Msps, TACC/TAC-1, CLIP-170, EB1, KLP61F/BimC, and the chromosomal passengers Aurora B/AIR-2, Survivin/BIR-1, INCENP/ICP-1, -2 and Borealin/CSC-1. PRC1 is possibly transported by KIF4 along the midzone microtubules (MTs). It is possible that KIF4 also transports Golgi-derived vesicles along midbody MTs where they fuse with the ingressing furrow. ZEN-4 and PRC1 are phosphorylated by Cdk1. Golgi assembly occurs when Cdk1 activity is low in telophase. Golgi stack assembly is also promoted by the degradation of Polo kinase and MEK1 in telophase (U: ubiquitin moiety). NIR2, a Golgi-associated protein, is found more prominently on the cleavage furrow as well as the spindle midzone in telophase to cytokinesis in a complex with Polo kinase. Membrane fusion occurs at the tips and along the ingressing furrow and involves a variety of proteins such as the exocyst complex, SNAREs, dynamin/DYN-1 and their interactors. Note: to simplify the figure, we have omitted several homologs/orthologs of several proteins. **(b)** Model depicting phragmoplast organization in plant cells. Microtubule plus-ends enclosed inside the cell-plate assembly matrix (CPAM) are stabilized and crosslinked by several MAPs, such as MAP55-1, MAP65-3, MOR1/GEM1, and by kinesin motor proteins. Golgi-derived vesicles are transported to the division plane along MTs by a kinesin motor protein, where they fuse with others while enclosed by the CPAM. Membrane fusion at the cell plate involves a variety of proteins such as tethering factors, SNAREs and their interactors. Formation of tubular domains in the developing cell plate appears to be mediated by DRP1A.

metaphase and effect chromosome separation and then switch to the spindle midzone where they interact with Centralspindlin to form the midzone. Both complexes function in furrow progression and completion of cytokinesis. More recently, a third component, the spindle midzone-specific microtubule bundling protein Ase1p/MAP65/PRC1/SPD-1/FEO has been identified (Table 1; Figure 2). We will focus more on Ase1p/MAP65/PRC1/SPD-1/FEO in this review as the chromosomal passengers and Centralspindlin have been described in recent reviews [26,27] (Box 1) and functional homology has been described in both plants and animals.

Ase1p/MAP65/PRC1/FEO/SPD-1-microtubule bundling proteins

First identified in budding yeast as Ase1p ('anaphase spindle elongation 1 protein') [28] and subsequently in plants as MAP65 [29], this family of proteins now includes members in mammals (PRC1, 'protein regulating cytokinesis 1') [30], flies (FASCETTO) [31], nematodes (SPD-1) [32] and fission yeast (Ase1p) [33,34]. These proteins share weak sequence homology (~20–25% identity) and a more conserved 16 amino acid domain in the C-terminus, which is within the region of PRC1 known to bind to MTs. They also encode predicted coiled-coil domains at the N-terminus [35]. Budding yeast Ase1p, mammalian PRC1 and plant MAP65 have been shown to bind to and bundle MTs *in vitro* [38,40], and fission yeast Ase1p, *Drosophila* FEO and mammalian PRC1 bundle MTs when overexpressed ectopically *in vivo* [30,35]. All proteins localize to the spindle midzone, and, in most organisms (except the yeasts), they are involved in cytokinesis (Figure 2a). In addition, fission yeast Ase1p and *C. elegans* SPD-1 both concentrate around centrosomes [32,34]. Mammalian PRC1 was first identified as a substrate for cyclin-dependent kinase (CDK) phosphorylation, and this phosphorylation controls its MT bundling activity in a cell-cycle-dependent manner [30]. Cells treated with siRNA against PRC1 lack midzone MTs [36] and fail late in cytokinesis during scission [36]. In *C. elegans*,

Box 1. Assembly of the spindle midzone

The spindle midzone mainly comprises tightly bundled, overlapping microtubules (MTs) from the spindle poles. Two of the molecules that localize to the midzone – the complex Centralspindlin (the kinesin MKLP1/ZEN-4 and the GTPase-activating protein MgcRacGAP/CYK-4) and the coiled-coil protein PRC1 – can independently bundle MTs *in vitro,* but depletion of either one of these proteins disrupts bundling of MTs in the spindle midzone *in vivo* and therefore mislocalizes the other components [30,35,93]. This makes it impossible to determine whether there is a hierarchy of assembly for these molecules. One exception is that *Caenorhabditis elegans* SPD-1–GFP still localizes to MTs in the absence of other components but appears to be more broadly localized [32]. This suggests that, in *C. elegans* embryos, SPD-1 first binds to and bundles MTs and that Centralspindlin and chromosomal passenger proteins might rearrange the bundled MTs into higher-order structures that function as the spindle midzone. By contrast, PAV, the *Drosophila* ortholog of MKLP1/ZEN-4, localizes along longer stretches of MTs and is not restricted to the spindle midzone in Feo-depleted cells. Additionally, ASP ('abnormal spindle protein'), a microtubule minus-end-binding protein, becomes mislocalized in the central spindle of Feo-depleted cells, suggesting that the MTs are not properly organized [31]. These differing results suggest that the midzone might form differently in these two organisms. Orthologs of these proteins (MAP65 and At1 g20060) also exist in plants (see main text), suggesting that these MT-bundling functions might both be conserved; however, how they interact to form the midzone *in vivo* is still not clear.

Importantly, some of the spindle midzone components also localize to the equatorial cortex or ingressing furrow. In *C. elegans,* the Centralspindlin components ZEN-4 and CYK-4 localize to the ingressing furrow, and this does not require SPD-1 [32,36]. In mammalian cells, the orthologs MKLP1 and MgcRacGAP do not localize to the furrow, but the chromosomal passenger proteins localize to the equatorial cortex before and during furrow ingression [36]. This localization also does not require PRC1 [32,36]. Cells depleted of ZEN-4, CYK-4 and chromosomal passengers often fail while the furrow is ingressing [32,36]. By contrast, both SPD-1 and PRC1 depletion results in very late defects in cytokinesis after the furrow appears to complete. The ability of furrows to ingress and cytokinesis to complete in *spd-1* mutant embryos, therefore, appears to be due to the presence of furrow-localized ZEN-4 and CYK-4. How do these molecules reach the cortex, especially in large cells such as those of the *C. elegans* embryo, where the midzone is far from the cortex? Studies in a neuronal cell line suggest that a cytoplasmic pool of the chromosomal passenger protein Aurora B requires astral MTs to reach the cortex, whereas centromeric Aurora B moves to the midzone [94]. This might be especially important in larger embryos where the midzone is far from the equatorial cortex.

spd-1 ('spindle defective 1') temperature-sensitive mutants or RNAi embryos lack midzone microtubule bundles so that the spindle appears to break in two during all cell divisions. Defects in scission occur similarly to mammalian PRC1 but only in a specific subset of cells, suggesting that, although the midzone is important for cytokinesis, it is not required. The *Drosophila* member of the family, Feo/Fascetto, localizes to the spindle midzone but only during late anaphase and telophase [31]. The central spindle appears to form correctly in mutant cells during anaphase but is much thinner during telophase. In addition, the normal exclusion of staining by antibodies against microtubules caused by the spindle midzone matrix is absent. As with other *Drosophila* mutants that disrupt the spindle midzone, the contractile ring was also disrupted in Feo-depleted cells.

The MAP-65 proteins in plants are considered homologs of the mammalian PRC1 and yeast Ase1p proteins [37,38] (Figure 2b). In *Arabidopsis*, the MAP-65 family consists of nine members named AtMAP65-1 to AtMAP65-9. AtMAP65-1 localizes to all MT arrays in plants: interphase cortical MTs, preprophase band (Glossary), mitotic spindle midzone and phragmoplast midline [39]. AtMAP65-1 and its close homologs carrot 65-kDa protein [40] and tobacco MAP65 [38] induce MT bundling *in vitro* by forming 25-nm cross-bridges between MTs [39]. Another member of the MAP65 family, AtMAP65-3/PLEIADE, exclusively localizes to the midzone of anaphase spindles and to the phragmoplast midline. In the *atmap65-3/ple* mutant roots, nuclear division is not affected but cells become multinucleate owing to serious alterations in progression through cytokinesis [41]. The phragmoplast midline in the *atmap65-3/ple* cells is abnormally broader and the overall phragmoplast is wider. This mutant phenotype is consistent with MAP65-3/PLE playing a role in crosslinking and stabilizing the interdigitating MTs at the midline and/or regulating the flux of tubulin through the plus-ends of MTs, probably by interacting with MOR1/GEM1 (see below) [41]. In any case, the two sets of phragmoplast MTs remain together in the *atmap65-3/ple* mutant, indicating that other proteins besides MAP65-3/PLE maintain the integrity of the phragmoplast.

While mostly structural roles have been ascribed to these proteins, there is some evidence that they might have other functions during mitosis. Fission yeast Ase1p is involved in the cytokinesis checkpoint [33], and PRC1 binds to MgcRacGAP and inhibits its activity towards Cdc42 during metaphase. This regulation might have a role in spindle formation [42]. Interestingly, the chromosomal passenger Aurora B has also been shown to regulate MgcRacGAP such that it is more active towards RhoA during cytokinesis [43]. This suggests that the regulation of this protein is very important for its function and raises the question of whether PRC1 also has an effect on MgcRacGAP during cytokinesis.

Other MAPs involved in cytokinesis

The *Arabidopsis* MOR1/GEM1 ('microtubule organization 1/gemini pollen 1') protein is homologous to *Xenopus* XMAP215 and human ch-TOG, which are key components in regulation of MT dynamics [44]. Although ch-TOG has been identified from isolated midbodies [7], and a *Dictyostelium* homolog, DdCP244, has been shown to be required during cytokinesis [45], little is known regarding the involvement of ch-TOG or XMAP215 in spindle midzone assembly or cytokinesis. We will focus on role of MOR1/GEM1 in plants as this protein plays a key role during assembly of the phragmoplast.

The MOR1/GEM1 protein localizes to interphase cortical MTs, the preprophase band and mitotic spindle midzone and phragmoplast midline [46] (Figure 2b). In the *mor1* mutant roots, cell plates are misaligned and branched and phragmoplast MTs are spatially disorganized [47]. Two other mutant alleles, *gem1–1* and *gem1–2*, result in a truncated protein missing the C-terminal region. The *gem1* cytokinesis-defect phenotype is visible during pollen grain development (male gametophyte) [46]. The protein region missing in the *gem1* mutants contains

a putative MT binding site, five potential phosphorylation sites, and two and eight HEAT repeats in the *gem1-1* and *gem1-2*, respectively. Therefore, the cytokinesis-defect phenotype observed in the *gem1* mutants might be due to reduced MT binding activity and the lack of regulation by reversible phosphorylation or protein–protein interaction through the HEAT motifs [46]. In addition, MAP200, the tobacco homolog of *Arabidopsis* MOR1/GEM1, promotes tubulin polymerization *in vitro* [48]. A putative role for MOR1/GEM1 during plant cytokinesis could be to stabilize the MT plus-ends at the phragmoplast midline and promote the tubulin dynamics at the MT ends [46].

Kinesins in cytokinesis

Besides the kinesin-like protein MKLP1/ZEN-4/PAVAROTTI that forms part of the Centralspindlin complex, other kinesins are also important in the successful completion of animal cytokinesis. The Kinesin-4 family members mammalian KIF4 and its *Drosophila* homolog KLP3A have been shown to localize to the midbody [7,49] and are required for midzone formation and cytokinesis [7,50]. PRC1 binds to KIF4 late in mitosis during periods of low CDK1 activity [50]. In KIF4-deficient cells, PRC1 localizes along MTs throughout the central spindle, suggesting that the kinesin is required to transport PRC1 to the microtubule plus-ends in the midbody [51]. (Figure 2a). It will be interesting to determine whether this interaction is conserved in other organisms. The *Arabidopsis* genome appears to encode 61 kinesins [52]. Although a putative homolog of MKLP1/ZEN-4/PAV exists in plants (At1 g20060), it has not been characterized.

Tobacco TKRP125 and carrot DcKRP120-2 belong to the kinesin-5 family (previously referred to as BimC kinesins) and localize to the midzone of mitotic anaphase spindles and to the phragmoplast midline [53,54]. In *Drosophila,* kinesin-5-like motors also localize to the midzone of the anaphase spindle and animal midbodies where they crosslink anti-parallel MTs and slide them apart [55] (Figure 2b). This suggests a conserved function for these motor proteins in maintaining bipolar arrays of MTs.

Members of the kinesin-14 family (previously referred as C-terminal kinesins) have also been implicated in mitotic spindle assembly in plants and other eukaryotes. The kinesin-14 family members, such as *Drosophila* Ncd, *Xenopus* XCTK2, *S. cerevisiae* Kar3 and *Arabidopsis* AtK5, are a unique group of kinesins that translocate exclusively toward the MT minus-ends [55,56]. Some members of this family of kinesins can bind to MTs by their tail regions and move MTs in relation to one another. During cell division, AtK5 localizes to the spindle midzone and to the phragmoplast midline, which suggests that AtK5 is a minus-end-directed motor protein as well as a plus-end tracking protein [57]. As is proposed for other members of this family, AtK5 could also contribute to stabilizing the interdigitating anti-parallel arrays of MTs in spindles and phragmoplasts.

Members of the kinesin-12 family have also been shown to play a role in plant cytokinesis. AtPAKRP1, AtPAKRP1L and AtPAKRP1L′ associate with plus-ends of phragmoplast MTs and are predicted to be plus-end-directed motor proteins. Loss-of-function mutations in each gene do not cause cytokinesis defects in somatic cells [58], although an *atpakrp1 atpakrp1L* double mutant shows defects in post-meiotic cytokinesis during pollen grain formation [52]. Another phragmoplast-associated kinesin, AtPAKRP2, is associated with cell-plate-forming vesicles in the phragmoplast and it could be responsible for the transport of vesicles along MTs [59]. In fact, kinesin-like linkers connecting vesicles and phragmoplast MTs have been visualized in electron tomograms of *Arabidopsis* endosperm cell plates [60].

Membrane dynamics during plant cytokinesis

The plant homologs of some of the main molecular players that mediate membrane trafficking at the cleavage furrow in animal cells, such as dynamins, SNAREs and the exocyst complex, also appear to be involved in cell plate assembly during plant cytokinesis. It is not the intention of this review to cover in detail the literature on membrane trafficking during cytokinesis but to provide a general overview that compares the components involved in plant membrane fusion during cell division with those involved in animal cytokinesis. Detailed reviews on membrane trafficking during plant [61] and animal cytokinesis [62] have been published recently.

In plants, Golgi-derived vesicles are transported to the phragmoplast midline, where they fuse with each other and with the early cell-plate domains. Electron-tomographic studies have shown that vesicles are tethered by linkers before fusion, and it has also been speculated that these linkers could be exocyst complexes [18,19] (Figure 2b). The exocyst is a multiprotein complex that localizes to site of polarized secretion and is required for membrane fusion [63]. Similarly, in animal cells, membrane is also added to the cleavage furrow by vesicle fusion. In fact, the exocyst complex is required for cytokinesis in a variety of eukaryotic cells, including the fission yeast [64]. Several exocyst subunits have been cloned in *Arabidopsis* [65], but their exact role during cytokinesis has not been elucidated.

Syntaxins have been also shown to localize to the cleavage furrow during animal cytokinesis [66–68]. They have been proposed to mediate the fusion of individual vesicles to the invaginating plasma membrane [68]. In plants, vesicles appear to fuse with one another before being incorporated to the growing cell plate, but these fusion events are also mediated by syntaxins. In *Arabidopsis*, numerous SNAREs and some of their interactors, such as the syntaxins KNOLLE/AtSYP111, AtSYP31, AtSYP112, AtSYP121, the SNAP25 homolog SNAP33, the SNARE NPSN11 and the AAA-type ATPase CDC48, have been shown to localize to the phragmoplast midline and mediate membrane fusion [69–72] (Table 1) (Figure 2b).

Membrane removal by endocytosis is also necessary for cytokinesis. In fact, 75% of the total membrane surface is removed from growing cell plates in some plant tissues [60]. In *Dictyostelium*, *Drosophila*, *C. elegans* and many other organisms, molecules involved in the formation of endocytic vesicles, such as clathrin and dynamin, are also required for completion of cytokinesis [73–76]. Plant cytokinesis also requires dynamin-related proteins (DRPs) that appear to mediate the formation of membranous tubular structures from fusing vesicles [19,60] (Figure 2b).

In contrast to the dynamins involved in endocytosis, these plant cytokinesis dynamin-related proteins do not lead to membrane fission but to stabilization of the membranous tubules that assemble in the growing edge of the cell plate [60].

Membrane dynamics during spindle midzone and midbody formation in animal cells

The function of the Golgi in formation of the cell wall during plant cytokinesis is well documented [77] (described above). In animal cells, however, the extent the Golgi plays in the targeting of new membrane during cytokinesis is unclear. Several membrane and Golgi-associated proteins have been identified from isolated midbodies that also play roles in cytokinesis [7]. These recent findings suggest that, even though the Golgi undergoes fragmentation during mitosis in mammalian cells, the Golgi membrane appears to cluster in the midbody in a manner similar to that of the Golgi-derived vesicles that accumulate in the phragmoplast. Furthermore, as in plants, cell separation in animal cells also requires specific SNARE-mediated membrane fusion events to complete division [68,78].

Is the mitotic Golgi, at least during late stages of cytokinesis, poised for targeting and fusion events required for daughter cell separation like that of the plant mitotic Golgi stacks? In plants, AtPAKRP2, an ungrouped N-terminal motor kinesin, has been suggested to maintain the phragmoplast MT array and/or transport Golgi-derived vesicles along the phragmoplast MTs [59]. In animal cells, KIF4, a kinesin originally identified in neurons for anterograde organelle trafficking [79], is required to maintain the spindle midzone by binding to the microtubule-bundling protein PRC1 [51]. These divergent kinesins might function similarly by moving or organizing Golgi-derived membrane material along bundled or organized MTs to the site of cell separation in both systems. The Golgi undergoes fragmentation during prophase in animal cells and remains so through anaphase, yet during telophase and cytokinesis, Golgi membranes undergo extensive remodeling to re-form stacks, and intercellular protein traffic resumes [14,80,81]. Lippencott-Schwartz and colleagues have suggested that the Golgi fuses with the ER in mitosis [82]. In *Drosophila* and *C. elegans* embryos, ER material has been shown to concentrate in the spindle midzone in telophase [12,13,16].

Plk1, MEK1 and CDK1 have been implicated in Golgi fragmentation and organelle inheritance during mitosis [83,84] in addition to cytokinesis [85,86]. Is there a link between these events? The peripheral Golgi protein Nir2 has been shown to be required for cytokinesis [87]. During mitosis, Cdk1 heavily phosphorylates Nir2 and facilitates its dissociation from the Golgi apparatus [11]. Cdk1/Cdc2 can also directly phosphorylate the Golgi matrix protein GM130, thus leading to fragmentation of the Golgi [88]. Phosphorylated Nir2 then interacts and colocalizes with Plk1 in the cleavage furrow and midbody. In addition, Cdk1 coordinates Golgi dispersion and the localization of Golgi-associated proteins and several mitotic kinases to the cleavage furrow and midbody. Although many questions remain, the link between Golgi inheritance and cytokinesis is a very interesting aspect of cell division.

Ultimately, the reassembly of the Golgi is dependent on cytoplasmic MTs [81]. Polymerization of MTs initially drives the relocation of the Golgi stacks toward the centrosomes [81,89]. Golgi membrane also accumulates in the midbody [7], but whether it has reformed into stacks and is then highly active in membrane targeting events is unknown. Alternatively, Golgi fragmentation mechanisms might be directly linked to the completion of cytokinesis by the need for equal partitioning of the organelle to each of the daughter cells. Interfering with the fragmentation prevents cells from entering mitosis [90]. The position of the Golgi might act as a sensor to regulate entry into the next mitosis [91]. Whether the microtubule bundles that form during telophase actually promote active Golgi formation in the midbody or whether they are required to target the Golgi to the site of scission is unknown. Further characterization of these structures will shed light upon these intriguing questions.

Concluding remarks

The acts of plant and animal cytokinesis have always been described as different processes, but it is becoming apparent that the late stages of animal cytokinesis (after furrow ingression) are more similar to plant cytokinesis than first realized. The phragmoplast and midzone/midbody, as the cytoskeletal array mediating cytokinesis in both organisms, share many structural and molecular features. Are the phragmoplast and midbody homologous structures? Have they evolved distinct features to fulfil their specific functions in different types of eukaryotic cells? One important function of the phragmoplast seems to be to direct the trafficking of Golgi-derived membrane to the newly forming cell plate. Is that also the case in animal cells? Do Centralspindlin and/or PRC1/KIF4 have a role in transporting vesicles along the midbody or are they required for organizing this structure? Do they have other roles such as physically linking the MT-based midzone and actin-based contractile ring or coordinating the disassembly of these structures with membrane-fusion events so that the channel connecting daughter cells can be cleared and sealed? As cytokinesis must occur precisely every cell cycle, it is quite possible that the midbody and phragmoplast have multiple redundant functions, some of which are conserved.

Although not covered in depth in this review, another intriguing similarity between plant and animal cytokinesis is the acto-myosin-based mechanism that drives daughter cell separation in both systems. Besides actin, myosins and formins, are there more proteins that localize to both the acto-myosin contractile ring and the cell plate? Might these similarities indicate some degree of conservation between the actin-mediated mechanisms driving furrow ingression in animal cells and the expanding forces that guide the cell plate to the division site in plants?

Several genetic and biochemical screens for proteins required for cytokinesis have been performed recently in *Arabidopsis, Drosophila*, mammalian cell, *S. pombe* and *C. elegans* systems [4–7,29,92] and have provided the field a large initial 'parts list' for proteins that control this event. It will be interesting to see the progress this field

will make in the next decade with the new hypotheses that have been obtained from this wealth of data. The complexity of cell division indicates that many proteins involved in this process remain to be identified. In addition, analysis of the similarities and differences in different organisms will continue to help further our understanding of this fundamental process.

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Update

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complex in thymocyte development. In contrast to B cells, however, there appears to be no defect in the ability to properly rearrange the TCR [11]. To further complicate matters, recent evidence has also suggested that Vav1 can translocate to the nucleus and participate in transcriptional regulation of NF-AT and NFκB target genes [16], thus raising the possibility that Vav1 also participates in the modulation of gene transcription following cellular activation, through the associated Ezh2 methyltransferase activity. However, the ability of the Vav triple knockout (Vav1–Vav3) mouse to develop normally disputes a role for Vav proteins in the coordination of histone-directed methylation by the Ezh2 complex [17]. However, the question can be raised as to whether this interaction is important for gene regulation in developing thymocytes or in other cell types following receptor engagement.

Concluding remarks

In their recent article, Su *et al.* have discovered a role for Ezh2 as a crucial regulator of actin cytoskeletal dynamics and proliferative responses downstream of activated cell-surface receptors, suggesting an essential role for lysine methylation in receptor-mediated signal transduction events. This exciting finding bolsters the notion that protein methylation is commonplace in signal transduction, perhaps resulting in distinct functional consequences, and adds to the complexity of protein regulatory mechanisms. Su *et al.* provide a novel means by which receptor-proximal protein methylation could occur during ligand-induced cellular responses. Although the exact significance of the Vav1–Ezh2 interaction is not entirely clear, and potential substrates of the cytosolic Ezh2 complex have yet to be identified, the idea that protein methylation plays a role in signal transduction is attractive. Therefore, identification of the methylated targets of the Ezh2 complex following receptor stimulation will enhance our understanding of such novel modes of protein regulation and will further illuminate the subtle complexities of protein structure and function.

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Erratum

Erratum: Midbodies and phragmoplasts: analogous structures involved in cytokinesis

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In this article by Ahna R. Skop and colleagues, which was published in the August 2005 issue of *TCB*, there was a production error that resulted in the omission of a footnote indicating that Marisa S. Otegui and Koen J. Verbrugghe contributed equally to the article and were therefore co-first-authors. We apologize to the authors for this error.

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