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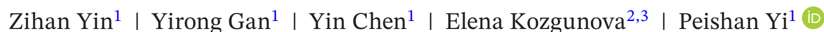
The Microtubule Cytoskeleton in Bryophytes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ABSTRACT

Microtubules (MTs) are essential cytoskeletal elements in all eukaryotes, playing critical roles in cell shape, intercellular organization, cell division, and cell motility. The organization of the MT network has undergone significant changes throughout plant evolution. Some MT structures, such as the preprophase band and phragmoplast, are innovations in plant lineages, while others, including the centriole and flagellum, have been lost over time. Bryophytes, consisting of mosses, liverworts, and hornworts, are the earliest land plants and occupy a key phylogenetic position in the evolution of MT organization. In the past two decades, advances in genomics, genetics, and cell imaging technologies have significantly enhanced our understanding of MT organization and function. Two representative species, *Physcomitrium patens* (moss) and *Marchantia polymorph* (liverwort), have become established model organisms, and new models for hornworts are emerging. In this review, we summarize the current knowledge of the MT cytoskeleton, drawing from early electron microscopy studies and recent advances in these emerging models. Our aim is to provide a comprehensive overview of the major MT array types and key factors involved in MT organization in bryophytes, offering insights into MT adaptation during plant evolution.

1 | Introduction

Microtubules (MTs) are linear polymers of αβ-tubulin dimers that play essential roles in cell division, cell motility, and intracellular transport in all eukaryotes (Pollard and Goldman 2018). Although MTs have been discovered around the same time in animals and plants (Ledbetter and Porter 1963; Slautterback 1963), their assembly and function remain less studied in plants than in animals (Akhmanova and Kapitein 2022; Hashimoto 2015; Liu and Lee 2022; Yagi et al. 2024). The organization of MTs in plants exhibits profound differences. One common feature is that MTs in most plant cells (termed cortical MTs) are confined to a thin layer of cytoplasm due to the presence of a large central vacuole (Elliott and Shaw 2018). These cortical MTs are required for cell expansion, cell division, and morphological adaptation; their arrangement relies on specialized mechanisms (Elliott and Shaw 2018; Yan et al. 2023). In addition, unique MT arrays involved in cell division, namely the preprophase band (PPB) and phragmoplast, emerged in the green lineage (Buschmann and Zachgo 2016). At the same time, the triplet MT-based organelle centriole, which serves as an MT-organizing center (MTOC) in flagella/cilia and the mitotic spindle, is absent from most plant cells (Hodges et al. 2012; Nabais et al. 2020). The emergence and loss of the MT-related structures have progressively transitioned from early eukaryotic lineages (Buschmann and Zachgo 2016; Hodges et al. 2012; Nabais et al. 2020; Yubuki and Leander 2013).

Bryophytes, comprising liverworts, hornworts, and mosses, are among the earliest land plants (Donoghue et al. 2021). They represent a pivotal evolutionary stage in the colonization of terrestrial

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environments. Unlike vascular plants, bryophytes lack specialized conducting tissues, relying on diffusion and capillary action for water and nutrient transport (Ohtani et al. 2017; Woudenberg et al. 2022). Their relatively simple yet highly adaptable body plans provide an excellent model for exploring how the cytoskeleton—particularly microtubules—has adapted to life on land (Szovenyi et al. 2019). Since 2008, the genome sequences of representative species in all three phyla of bryophytes have been made available (Bi et al. 2024; Bowman et al. 2017; Li et al. 2020; Rensing et al. 2008). Genetic, molecular, and imaging techniques are being continuously developed, especially for the moss *Physcomitrium patens* (*P. patens*), also known as *Physcomitrella patens*, and the liverwort *Marchantia polymorpha* (*M. polymorpha*) (Frangedakis et al. 2021; Ishizaki et al. 2016; Rensing et al. 2020). These advancements have led to the discovery of crucial roles of MTs and their regulators in development and the characterization of unique features of MT organization in bryophytes (Naramoto et al. 2022; Wu et al. 2018; Yamada and Goshima 2017; Yi and Goshima 2018). In this article, we review the current understanding of the MT cytoskeleton in bryophytes with a focus on *P. patens* and *M. polymorpha*. By comparing the organization and function of MTs in these organisms, we hope to provide insight into the adaptation of MTs for morphogenesis and physiology in early land plants.

1.1 | Life Cycle and Development of Bryophytes

The life cycle of liverworts, hornworts, and mosses is dominated by a haploid gametophyte and begins with the germination of a spore (Figure 1) (Frangedakis et al. 2021; Kohchi et al. 2021; Naramoto et al. 2022; Rensing et al. 2020). After germination, the spore develops into a filamentous protonema (2D development) which later transforms into a leafy gametophore in mosses or typically a thallus in liverworts and hornworts (3D development) (Frangedakis et al. 2021; Kohchi et al. 2021; Naramoto et al. 2022; Rensing et al. 2020). The protonemal stage is usually short, but in some moss species, such as *P. patens*, it can be maintained for a long period under laboratory conditions (Cove 2005). Interestingly, protonemal growth in liverworts and hornworts is highly adaptable and can be stimulated by low-intensity light and reverted back from the prothallus (O'Hanlon 1926; Wada et al. 1984). These facts indicate that protonemata are common

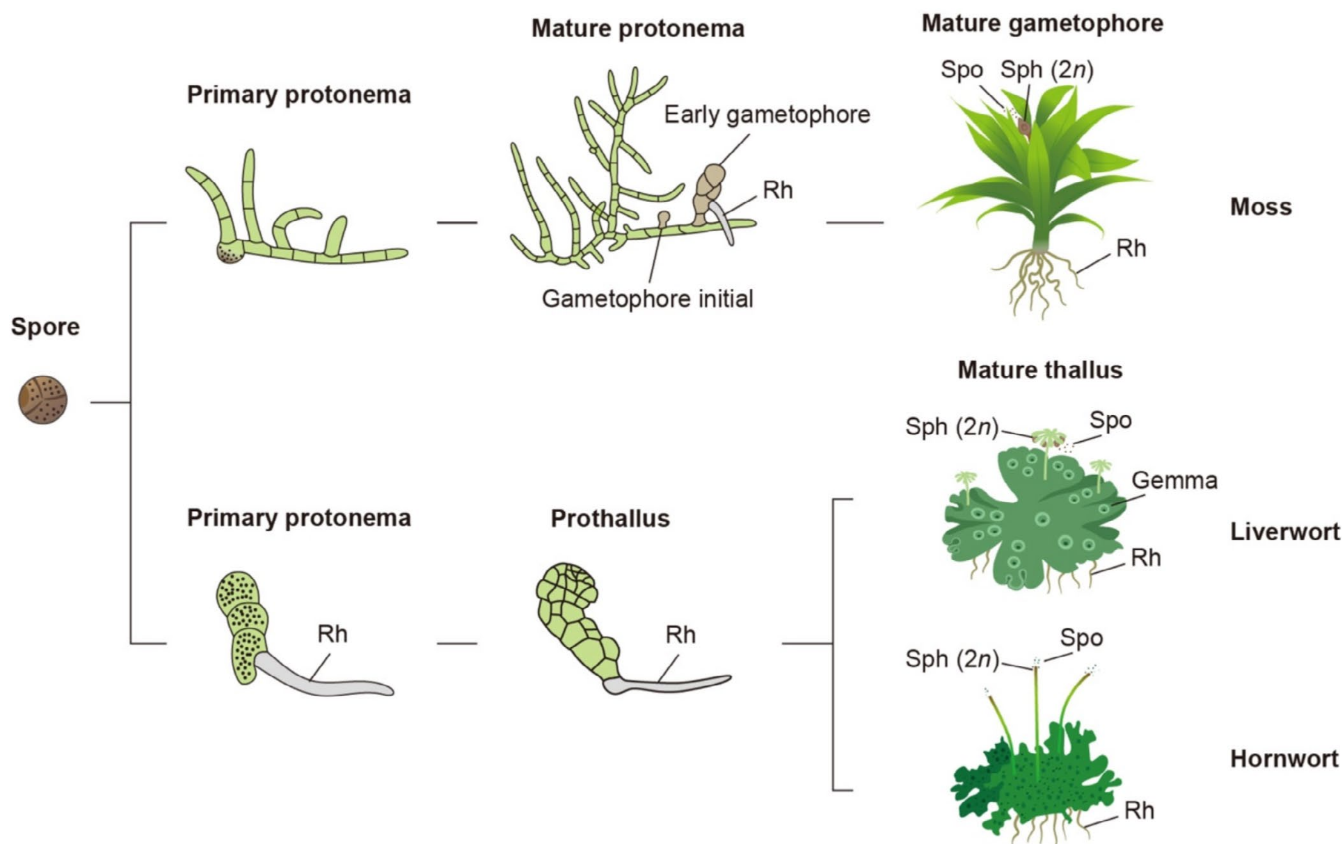

FIGURE 1 | The life cycle of mosses, liverworts, and hornworts. After germination, the spore of bryophytes develops into a primary protonema. The protonemal stage is usually short, but in mosses, it can be maintained for a long period under laboratory conditions. In liverworts and hornworts, the primary protonema transforms into a prothallus. Later, the apical cells of prothallus develop into a mature thallus. Some of the basal cells of primary protonema and mature thallus differentiate into rhizoids (Rh). Antheridia and archegonia are produced at the apical side of mature thallus and generate sperms and oocytes, respectively. After fertilization, the zygote develops into a diploid sporophyte (Sph), which is nourished by the thallus. The spores (Spo) are generated by meiotic division of sporocytes inside the sporophyte. In mosses, a leafy gametophore develops from a side branch cell called gametophore initial on the mature protonemata. After several rounds of oblique divisions, the gametophore initial is transformed into an early gametophore, of which the apical cells develop into a leafy gametophore and some of the basal cells differentiate into rhizoids. The reproduction process is similar to that in liverworts and hornworts, but occurs on the gametophore stem.

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transitional stages in bryophyte development and may represent an ancient yet simple multicellular structure in early land plants.

In liverworts and hornworts, the protonemata quickly transform into a prothallus, of which one or more basal cells differentiate into primary rhizoids, and an apical cell cluster generates the multilayered prothallus (O'Hanlon 1926; Wada et al. 1984). Thallose-type protonema and primary rhizoids are not common in mosses (Nishida 1978). Instead, multiple buds are generated on protonemata and develop into leafy gametophores (Nishida 1978). At a later stage, terminal rhizoids differentiate from the basal epidermis of thallus or gametophore, undergoing tip growth and mediating anchorage to soil and nutrient acquisition (Frangedakis et al. 2021; Kofuji and Hasebe 2014; Kohchi et al. 2021; Naramoto et al. 2022). The reproductive organs antheridia (male) and archegonia (female) are formed on the apical surface of thallus and gametophore in liverworts and mosses, respectively, but are embedded in the thallus in hornworts (Frangedakis et al. 2021; Renzaglia et al. 2000). Fertilization is completed by the fusion of a motile sperm produced by antheridia with an egg inside the archegonia (Haig 2016; Renzaglia et al. 2000). The fertilized zygote develops into a diploid sporophyte nourished by the gametophyte (Goffinet and Buck 2013). Inside the sporophyte sac, spores are generated through meiotic division of sporocytes (Brown and Lemmon 2013). The origin of initial cells for 3D thallus development in liverworts and hornworts is not well defined (O'Hanlon 1926; Wada et al. 1984). However, the development of the moss gametophore can trace back to a single cell that emerged from the protonemata (Moody 2020; Nishida 1978).

1.2 | A Glance at Tubulin Isotypes and Microtubule Regulators in Bryophytes

1.2.1 | Tubulin Isotypes

With the availability of sequenced genomes (Bi et al. 2024; Bowman et al. 2017; Li et al. 2020; Rensing et al. 2008), we could now systematically compare the tubulin isotypes and MT regulators between bryophytes and other plants; therefore, obtaining a first glance at the divergence of MT structures and MT-related processes in land plants. As representatives, genes in the moss *P. patens*, liverwort *M. polymorpha*, and flowering plant *Arabidopsis* are analyzed. By searching the genomes with keywords tubulin and microtubule in Phytozome (https://phytozome-next.jgi.doe.gov) and manual inspection through BLAST-driven homolog verification (Goodstein et al. 2012), we obtained 68 subgroups of MT-related genes belonging to 11 functional categories (Table 1 and Table S1). As shown in Table 1, most of the characterized MT-related genes are present in all three genomes. Tubulins in bryophytes are highly conserved and closely related to those in other land plants, as plant-specific antibodies can recognize α-tubulins in mosses, ferns, and flowering plants but not in animals (Mizuno et al. 1985). Ectopic expression of liverwort β-tubulin can label MTs in tobacco BY-2 cells (Buschmann et al. 2016). In *P. patens*, β-tubulins exhibit less variability in their carboxyl termini, which usually end with an alanine (Jost et al. 2004); all α-tubulin isotypes terminate with a tyrosine, which may be subject to a detyrosination-tyrosination regulatory cycle. By contrast, three out of seven α-tubulins in *M. polymorpha* lack a terminal tyrosine. In addition to α-, β-, and γ-tubulins, the genomes of *P. patens* and *M. polymorpha* encode δ- and ε-tubulins, which are missing in *Arabidopsis*. As centriole-derived flagella/cilia are lost in flowering plants (Nabais et al. 2020; Yubuki and Leander 2013), this fact suggests that δ- and ε-tubulins may play a major role in flagella/cilia assembly, as they do in mammals (Stathatos et al. 2021). To evaluate a possible correlation of the evolutionary loss between δ−/ε-tubulins and centriole, we searched homologs in representative species of lycophytes, ferns, and gymnosperms in Phytozome and the GinkgoDB (https://ginkgo.zju.edu.cn/) (Goodstein et al. 2012; Gu et al. 2022). Indeed, δ- and ε-tubulins are also present in the lycophytes *Diphasiastrum complanatum* and *Selaginella moellendorffii*, and fern *Ceratopteris richardii*, but not in the gymnosperm *Thuja plicata* (Table 2). Interestingly, δ- and ε-tubulins are also absent in *Ginkgo biloba*, a representative gymnosperm that has preserved centrioles and flagella (Hodges et al. 2012) (Table 2). Therefore, the loss of δ- and ε-tubulins may occur before the degeneration of centrioles.

1.2.2 | Posttranslational Modification Enzymes of Tubulins

In green algae and animals, tubulins undergo extensive posttranslational modifications, and most of them are evolutionarily linked to flagella/cilia (Janke and Magiera 2020; McKenna et al. 2023). Acetylation of α-tubulin is one of the best-known modifications identified to date. Acetylated MTs are abundant in the flagella axoneme and exhibit high stability (L'Hernault and Rosenbaum 1985; McKenna et al. 2023). The acetylation process is catalyzed by α-tubulin N-acetyltransferase (ATAT) which mainly acts on lysine 40 of α-tubulin (Akella et al. 2010; Shida et al. 2010). Among the land plants, the ATAT is present only in bryophytes, lycophytes, and ferns, suggesting a co-evolution with centriole loss (Table 2). The majority of eukaryotic α-tubulins contain a C-terminal tyrosine. In animals, this tyrosine is removed from polymerized MTs by tubulin carboxypeptidase (Aillaud et al. 2017; Kumar and Flavin 1981; Nieuwenhuis et al. 2017) and re-ligated to free tubulin dimers by tubulin-tyrosine ligase (TTL) (Ersfeld et al. 1993; Szyk et al. 2011). Detyrosinated α-tubulins as well as native β-tubulins can extend their C-termini by adding one or more glutamates and glycines, a process termed glutamylation and glycylation, respectively (Janke and Magiera 2020; McKenna et al. 2023). The ligation process is catalyzed by tubulin tyrosine ligase-like proteins (TTLLs) (Ersfeld et al. 1993; Janke et al. 2005; Wloga et al. 2009). Land plants appear to lack a canonical TTL. However, three types of TTLLs related to TTLL6, TTLL9, and TTLL12 in mammals, respectively, are present. The TTLL6- and TTLL9-type TTLLs are found in organisms ranging from green algae to ferns but are absent in seed plants (Table 2). The TTLL12-type is found in all land plants and green alga *Botryococcus braunii* but not in *Chlamydomonas reinhardtii* (*C. reinhardtii*). The divergence of TTLLs in plants suggests that TTLL6- and TTLL9-type TTLLs may be involved in flagella/cilia biogenesis, while the TTLL12-type is an innovation in green algae and land plants, which may participate in modifying non-axonemal

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TABLE 1 | Gene numbers of tubulin isotypes and microtubule regulators in moss *Physcomitrium patens*, liverwort *Marchantia polymorpha*, and *Arabidopsis*.

Category	Protein	Abbreviation	*P. patens*	*M. polymorpha*	*Arabidopsis*
Tubulin family	α-Tubulin	TUBA	13	7	6
	β-Tubulin	TUBB	11	5	9
	γ-Tubulin	TUBG	2	1	2
	δ-Tubulin	TUBD	1	1	0
	ε-Tubulin	TUBE	1	1	0
	FtsZ family	FTSZ	5	3	3
Nucleation factor	Gamma-tubulin complex component 2	GCP2	2	1	1
	Gamma-tubulin complex component 3	GCP3	2	1	1
	Gamma-tubulin complex component 4	GCP4	1	1	1
	Gamma-tubulin complex component 5	GCP5	1	1	2
	Gamma-tubulin complex component 6	GCP6	1	1	1
	Gamma-tubulin complex, DGRIP84/SPC97	DGRIP84/SPC97	1	0	0
	Gamma-tubulin ring complex targeting factor NEDD1	NEDD1	4	1	1
Branching factor	HAUS augmin-like complex subunit 1	AUG1	1	1	1
	HAUS augmin-like complex subunit 2	AUG2	1	1	1
	HAUS augmin-like complex subunit 3	AUG3	1	1	1
	HAUS augmin-like complex subunit 4	AUG4	1	1	1
	HAUS augmin-like complex subunit 5	AUG5	4	1	1
	HAUS augmin-like complex subunit 6	AUG6	3	1	1
	HAUS augmin-like complex subunit 7	AUG7	1	1	1
	HAUS augmin-like complex subunit 8	AUG8	5	1	9
Polymerization regulator	Targeting protein for Xklp2 family	TPX2	4	1	6
	Targeting protein for Xklp2 family-like 1	TPX2LA	3	2	3
	Targeting protein for Xklp2 family-like 2	TPX2LB	14	1	10
	Cytoskeleton-associated protein 5	CKAP5/XMAP215	2	1	1
Tubulin-folding cofactor	Tubulin-folding cofactor A	TBCA	4	1	1
	Tubulin-folding cofactor B	TBCB	1	1	1
	Tubulin-folding cofactor C	TBCC	1	1	1
	Tubulin-folding cofactor D	TBCD	1	1	1
	Tubulin-folding cofactor E	TBCE	2	1	1
Microtubule severing protein	Katanin p80 subunit B1	KATNB1	2	1	4
	Katanin p60 subunit A1	KATNA1	2	1	1
	Spastin	SPAST	1	1	1
	Fidgetin-like protein 1	FIGNL1	1	1	1

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Category	Protein	Abbreviation	*P. patens*	*M. polymorpha*	*Arabidopsis*
Microtubule binding protein	Microtubule-associated protein, RP/EB family	EB1	4	2	3
	Microtubule-associated protein 65/PRC1	MAP65/PRC1	6	1	9
	Microtubule-associated protein 70	MAP70	6	1	5
	TBCC domain-containing protein 1	TBCCD1	4	1	2
	Microtubule-associated protein SPIRAL1	SPR1	6	1	6
	Microtubule-associated protein SPIRAL2	SPR2	4	1	2
	CLIP-associating protein 1/2	CLASP	4	1	1
	Microtubule-binding protein TCTP	TCTP	2	1	2
	Tubulin-related protein MISATO	MISATO	1	1	1
	Mitotic-spindle organizing protein 1	MZT1	2	1	2
	Mitotic-spindle organizing protein 2	MZT2	1	1	0
	Abnormal spindle-like microcephaly-associated protein	ASP	2	1	1
	Microspherule protein 1	MCRS	1	1	3
	Basic proline-rich protein	BPP	2	1	6
Posttranslational modification factor	Alpha tubulin N-acetyltransferase	ATAT	2	1	0
	Tubulin-tyrosine ligase-like protein 6	TTLL6	1	0	0
	Tubulin-tyrosine ligase-like protein 9	TTLL9	1	1	0
	Tubulin-tyrosine ligase-like protein 12	TTLL12	1	1	1
Centrosomal protein	Centrosomal protein 104	CEP104	2	1	0
	Centrosomal protein 120	CEP120	1	1	0
	Centrosomal protein 131	CEP131	2	1	0
	Centrosomal protein 44	CEP44	1	1	0
	Centrosomal protein 70	CEP70	1	1	0
	Centrosomal protein 78	CEP78	2	1	0
Dynein motor and accessory protein	Cytoplasmic dynein 2 heavy chain	DYNC2H	0	1	0
	Axonemal dynein heavy chain	DNAH	8	9	0
	Dynein intermediate chain	DIC	1	1	0
	Dynein light intermediate chain	DLI	1	1	0
	Dynein light chain LC8-type	DYNLL	5	3	6
	Dynein light chain Roadblock-type	DYNLRB	1	2	0
	Dynein light chain Tctex-type	DYNLT	1	2	0
Kinesin motor and associated protein	Kinesin heavy chain	KHC	78	29	64
	Kinesin light chain	KLC	13	6	6
	Kinesin-associated protein	KAP	0	1	0

MTs. In mammals, tubulin modification can occur in non-axonemal MTs such as endoplasmic and mitotic MTs (Akera et al. 2017; Tas et al. 2017). Therefore, posttranslational modification of tubulin is not exclusive to flagella/cilia. Both flagella-associated and non-flagella-associated modifications may exist in bryophytes.

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TABLE 2 | Gene numbers of flagellum-associated proteins in representative species.

		Chlorophyte	Bryophyte	Brophyte	Lycophyte	Lycophyte	Fern	Gymnosperm		Angiosperm
Category	**Protein**	***Chlamydomonas reinhardtii***	***Physcomitrium patens***	***Marchantia polymorpha***	***Diphasiastrum complanatum***	***Selaginella moellendorffii***	***Ceratopteris richardii***	***Thuja plicata***	***Ginkgo biloba***	***Arabidopsis thaliana***
Tubulin isotype and modification enzyme	ATAT	1	2	1	1	1	1	0	0	0
	δ-tubulin	1	1	1	2	1	1	0	0	0
	ε-tubulin	1	1	1	1	2	1	0	0	0
	TTLL6	3	1	0	2	2	1	0	1	0
	TTLL9	12	1	1	1	1	1	0	0	0
	TTLL12	0	1	1	2	1	1	1	1	1
Kinesin-2	KAP	1	0	1	3[a]	1	1	0	1	0
	Kinesin-2	2	1	1	1	2	1	0	1	0
Cytoplasmic dynein 2	DYNC2H	1	0	1	0	0	0	0	0	0
Centrosomal protein	CEP104	1	2	1	1	0	1	0	0	0
	CEP131	1	2	1	1	1	0	0	2	0
	CEP164	1	1[a]	1	1	1[a]	1	0	0	0
	CEP44	1	1	1	1	2	1	0	0	0
	CEP70	1	1	1	0	0	0	0	0	0
	CEP78	1	2	1	0	0	2	0	2	0

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TABLE 2 | (Continued)

Category	Protein	Chlorophyte	Bryophyte	Brophyte	Lycophyte	Lycophyte	Fern	Gymnosperm		Angiosperm
		Chlamydomonas reinhardtii	*Physcomitrium patens*	*Marchantia polymorpha*	*Diphasiastrum complanatum*	*Selaginella moellendorffii*	*Ceratopteris richardii*	*Thuja plicata*	*Ginkgo biloba*	*Arabidopsis thaliana*
Centriole biogenesis	CEP192/ Spd2	0	0	0	0	0	0	0	0	0
	CEP152/Asl	0	0	0	0	0	0	0	0	0
	CEP63	0	0	0	0	0	0	0	0	0
	CEP57	0	0	0	0	0	0	0	0	0
	PLK4	0	0	0	0	0	0	0	0	0
	STIL/Ana2	1[a]	0	0	0	0	0	0	0	0
	SAS6	1	1	1	1	1	1	0	1	0
	CEP135/ BLD10[b]	1	1	1	0	1	0	0	2	0
	SAS4/CPAP	2	0	1	2	1	1	0	0	0
	CEP120	1	1	1	1	1	1	0	3	0
	SPICE1	0	0	0	0	0	0	0	0	0
	CEP215/Cnn	0	0	0	0	0	0	0	0	0
	CEP295/ Ana1	0	0	0	0	0	0	0	0	0

[a]Core domain is incomplete in at least one gene or exhibits low sequence similarity.
[b]Homologs in *P. patens*, *M. polymorpha*, *S. moellendorffii*, and *G. biloba* are derived from (Koshimizu et al. 2022), which cannot be robustly identified based on sequence similarity.

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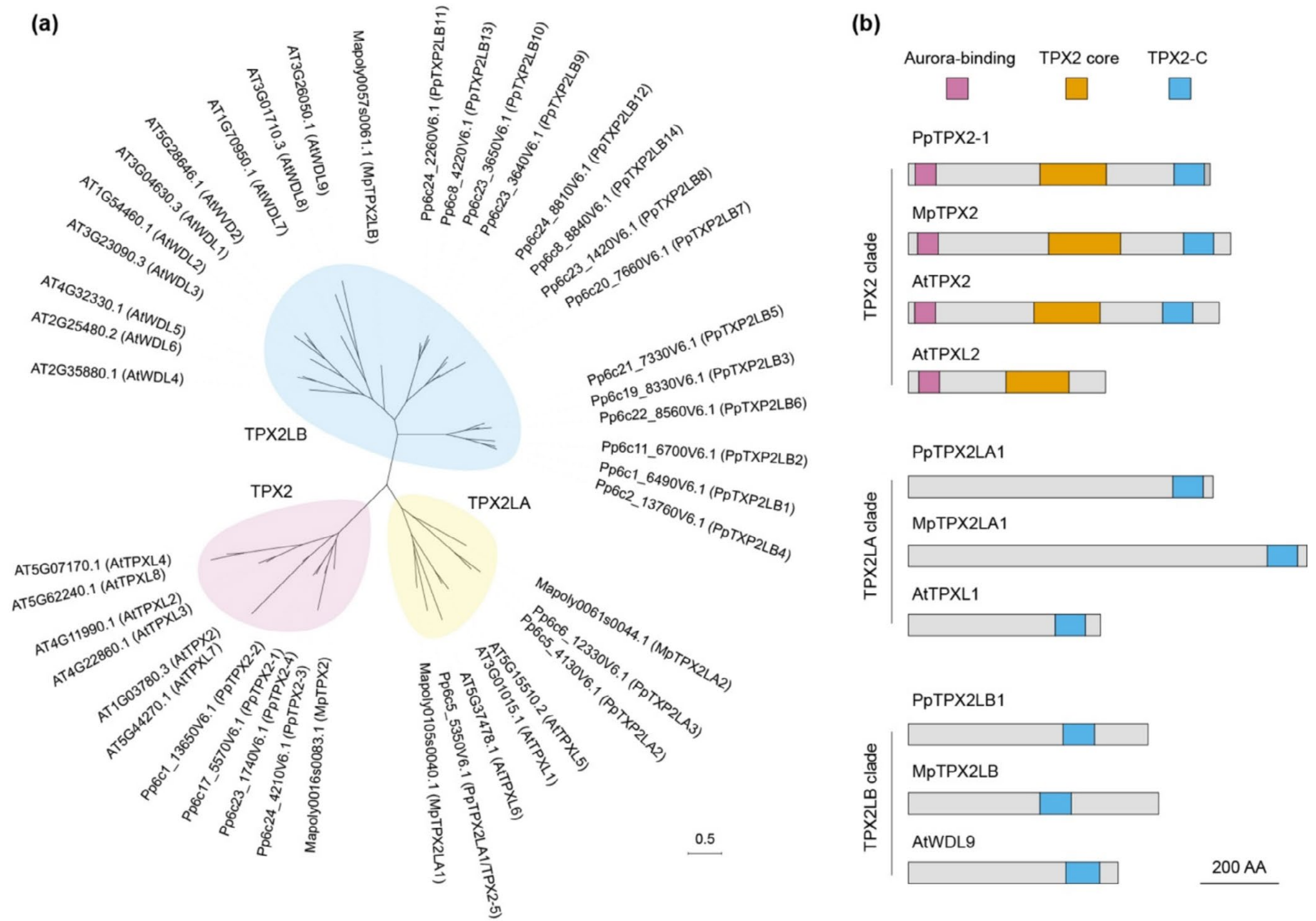

FIGURE 2 | Phylogenetic relationship of TPX2 family and TPX2-like proteins in *P. patens*, *M. polymorpha*, and *Arabidopsis*. (a) Phylogenetic tree of TPX2 family and TPX2-like (TPX2L) proteins comprises three main clades: A TPX2 clade and two TPX2-like clades (TPX2LA and TPX2LB). (b) Domain organization of representative members in each subgroup. Genes in the moss *P. patens* and *Arabidopsis* are named according to (Kozgunova et al. 2022; Smertenko et al. 2021), except that PpTPX2-5 was renamed as PpTPX2LA1.

1.2.3 | MT Nucleation and Polymerization Promoting Factors

Regarding MT assembly, key factors associated with nucleation (γ-tubulin ring complex), polymerization (XMAP215/CKAP5 and TPX2), branching (augmin complex), and severing (katanin, spastin, and fidgetin) of MTs, and tubulin folding (tubulin-folding cofactors) are all present in the genomes of *P. patens*, *M. polymorpha*, and *Arabidopsis*. In addition, a unique SPC97-type γ-tubulin complex subunit, which shows a relatively high expression in archegonia (Ortiz-Ramirez et al. 2016), is found in *P. patens* (Table 1). This protein is most similar to GCP2 and has a long C-terminal domain. BLASTing against the genomes of other moss species in Phytozome did not reveal any homologs, suggesting that this gene might be a *P. patens*-specific subunit, although such a function requires further experimental validation. In addition to being stimulated by nucleation factors, MT polymerization is facilitated by CKAP5/XMAP215 and TPX2 proteins (Brouhard et al. 2008; Gard and Kirschner 1987; Wittmann et al. 1998). CKAP5/XMAP215 exists with a low copy number in the genomes of *P. patens*, *M. polymorpha*, and *Arabidopsis*. By contrast, the TPX2 family is greatly expanded (Table 1). Three groups of TPX2 and TPX2-like proteins (TPX2Ls) were characterized based on the phylogenetic relationship (Figure 2), a typical TPX2 group and two TPX2-like groups as previously reported (Boruc et al. 2019; Dvorak Tomastikova et al. 2020; Smertenko et al. 2021). To avoid confusion with the naming of TPX2L proteins as TPXLs in *Arabidopsis*, we refer to the two TPX2-like groups as TPX2LA and TPX2LB (Figure 2). As PpTPX2-5, a protein identified as a TPX2 homolog (Kozgunova et al. 2022), is phylogenetically placed in the TPX2LA group, we renamed this protein PpTPX2LA1. Except for TPX2s, all the TPX2Ls lack key domains found in TPX2. In *Arabidopsis*, five of the TPXLs lack the TPX2-C domain but retain an N-terminal Aurora-binding domain and a central TPX2 core domain (Smertenko et al. 2021). These proteins are phylogenetically related to TPX2 and are absent in *P. patens* and *M. polymorpha*. Other TPX2Ls all lack a canonical TPX2 core domain (Figure 2). Despite the sequence divergence, at least some of the TPX2Ls appear to play a similar role in organizing mitotic MTs as TPX2 does in *Arabidopsis* and *P. patens*, although different subcellular localizations have been observed (Boruc et al. 2019; Kozgunova et al. 2022; Vos et al. 2008). The TPX2LB group is largely expanded in *P. patens* and *Arabidopsis* and contains multiple WDL proteins. Studies in *Arabidopsis* and *M. polymorpha* indicate that WDLs are involved in organizing interphase MTs for cell expansion and development (Champion et al. 2021; Smertenko et al. 2021). The presence of

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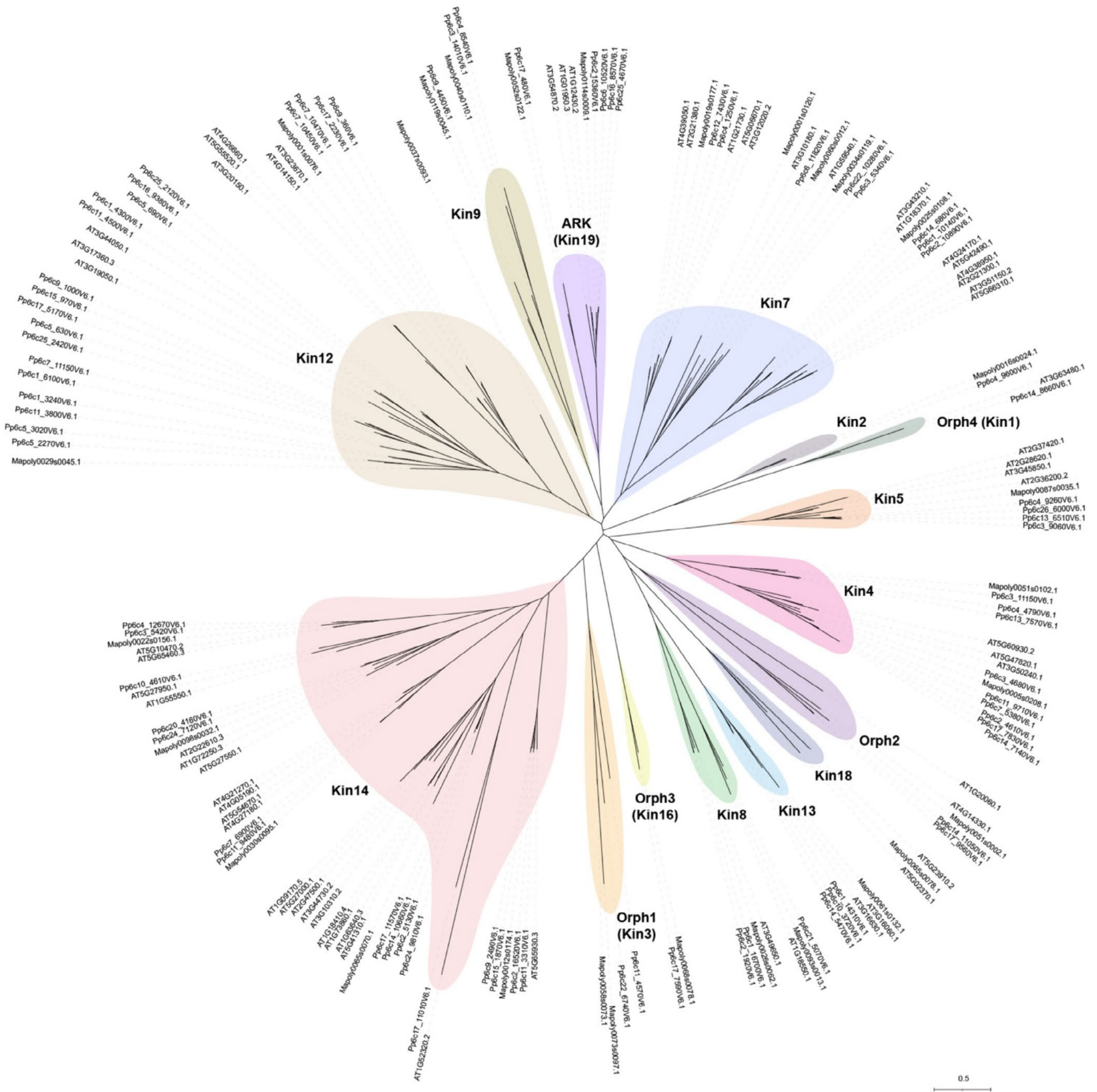

FIGURE 3 | Phylogenetic relationship of kinesins in *P. patens*, *M. polymorpha*, and *Arabidopsis*. A maximum-likelihood phylogenetic tree was constructed using the FASTTREE program, based on full-length sequences aligned with Clustal Omega (Price et al. 2010; Sievers et al. 2011). A nearly identical tree topology was observed when the analysis was repeated using only the motor domain sequences. The placement of orphan kinesins differs slightly from that reported in earlier studies (Miki et al. 2014; Shen et al. 2012). The assignment of orphan kinesins to distinct families is based on our BLAST analysis and previous reports (Lucas and Geisler 2024; Wickstead et al. 2010). The accession numbers and sequences used for tree construction are available in Table S2.

WDL proteins in bryophytes suggests that functional divergence of TPX2Ls has occurred in basal land plants or earlier.

1.2.4 | Kinesins

The MT-dependent motor kinesin superfamily is the largest group of MT-associated factors in all green plants (Lucas and Geisler 2024). Unlike fungi and animals, plants have evolved unique, plant-specific kinesin families, including those containing armadillo repeats (ARK) and unassigned groups (Lucas and Geisler 2024). ARK-containing kinesins are classified within the kinesin-19 family (Lucas and Geisler 2024; Wickstead et al. 2010). Additionally, *P. patens* harbors four unassigned groups, referred to as orphan kinesins (Miki et al. 2014; Shen et al. 2012). Through BLAST analysis, we

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TABLE 3 | Genes encoding intraflagellar transport proteins in the alga *Chlamydomonas reinhardtii*, moss *Physcomitrium patens*, and liverwort *Marchantia polymorpha*.

Protein	IFT complex	*C. reinhardtii*	*P. patens* V6.1	*P. patens* V3.3	*M. polymorpha*
IFT121	IFT-A	Cre11.g475000	Pp6c26_7700	Pp3c26_13860	Mapoly0009s0145
IFT122	IFT-A	Cre01.g065822	Pp6c12_12850	Pp3c12_25770	Mapoly0009s0194, Mapoly0009s0193[a]
IFT139	IFT-A	Cre06.g268800	Pp6c22_930	Pp3c22_1850	Mapoly0181s0006
IFT140	IFT-A	Cre08.g362650	Pp6c12_2590	Pp3c12_5950	Mapoly0052s0084
IFT144	IFT-A	Cre13.g572700	Pp6c20_4740	Pp3c20_6930 / Pp3c20_6940	Mapoly0059s0087
IFT43	IFT-A	Cre06.g251200	None	None	Mapoly0100s0038
IFT172	IFT-B	Cre17.g703900	Pp6c21_4600	Pp3c21_8450	Mapoly0014s0194
IFT20	IFT-B	Cre02.g089950	Pp6c22_2140	Pp3c22_4000	Mapoly0011s0211
IFT22	IFT-B	Cre01.g039200	ND	Pp3c5_3390	ND
IFT25	IFT-B	Cre10.g450350	None	None	None
IFT27	IFT-B	Cre01.g047950	ND	Pp3c24_9710	ND
IFT38	IFT-B	Cre17.g721250	Pp6c9_9850	Pp3c9_19740	Mapoly0094s0074
IFT46	IFT-B	Cre05.g241637	Pp6c7_5900	Pp3c7_9850	Mapoly0014s0167
IFT52	IFT-B	Cre04.g219250	Pp6c12_2090[a]	Pp3c12_4900	Mapoly0059s0029
IFT54	IFT-B	Cre11.g467739	Pp6c15_4970[a], Pp6c15_4980[a]	Pp3c15_9090, Pp3c15_9080	Mapoly0001s0288
IFT56	IFT-B	Cre11.g467616	Pp6c1_18860	Pp3c1_35980	Mapoly0154s0012
IFT57	IFT-B	Cre10.g467000	Pp6c12_7000	Pp3c12_14140	Mapoly0008s0156
IFT70	IFT-B	Cre07.g342200	Pp6c17_1660[a], Pp6c17_1670[a]	Pp3c17_3590, Pp3c17_3590	Mapoly0008s0205
IFT74	IFT-B	Cre01.g027950	Pp6c6_11170[a]	Pp3c6_20640	Mapoly0053s0077[a], Mapoly0053s0076[a]
IFT80	IFT-B	Cre03.g204150	Pp6c12_4380, Pp6c12_4390	Pp3c12_9400, NA	Mapoly0086s0050
IFT81	IFT-B	Cre17.g723600	Pp6c26_5510[a]	Pp3c26_9320	Mapoly0036s0009
IFT88	IFT-B	Cre07.g335750	Pp6c20_1830	Pp3c20_1820	Mapoly0022s0154

Note: None, no homologs; ND, undetermined; NA, not available.
[a]Fragmented protein.

categorized most of these orphan kinesins into the kinesin-1, 3, and 16 groups, aligning with previous findings (Lucas and Geisler 2024; Wickstead et al. 2010). However, the orphan-2 group remains ambiguous, as its members exhibit sequence similarities with kinesin-1, 2, 5, and 18. Phylogenetic analysis, nevertheless, suggests a closer relationship to kinesin-18. With the recent release of an updated *P. patens* genome (Bi et al. 2024), we also identified revisions to several kinesin genes in this species. As shown in Table S2, three kinesins, kinesin-4IIb (Pp6c3_11150), ARK-like kinesin Pp6c17_480, and orphan-3 Pp6c17_7590, are annotated as fusion products of two genes in the previous genomes (Lang et al. 2018; Rensing et al. 2008). The ARK-like kinesin Pp6c17_480 and a new kinesin-14 member Pp6c17_11010 are novel genes absent in previous genomes. Pp6c17_11010 may not encode a functional kinesin as it lacks a full-length motor domain. The total number of kinesins in *P. patens* remains 78, as previously reported (Miki et al. 2014; Shen et al. 2012). However, after generating a maximum-likelihood phylogenetic tree with FASTTREE (Price et al. 2010), three genes reported by previous studies were reclassified into distinct groups following updates to their sequences (Figure 3) (Miki et al. 2014; Shen et al. 2012). The orphan-4a (Pp6c14_7140/Pp3c14_12630) was placed in the kinesin-4 group. The orphan-4c (Pp6c3_5340/Pp3c3_10820) was placed in the kinesin-7 group. The ARK-like kinesin (Pp6c22_6740/Pp3c22_13180/Pp3c22_13170) was placed in the orphan-1 group. Our analysis also identified a considerable number of putative kinesin light chain proteins (Table 1 and Table S1), yet whether they play a direct role in regulating kinesin remains unclear.

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Kinesin-2 plays a major role in anterograde intraflagellar transport (IFT) (Scholey 2013; Wedaman et al. 1996). Along with the presence of flagella, kinesin-2 is found in bryophytes, lycophytes, ferns, and the flagella-carrying gymnosperm *Ginkgo biloba* (Table 2). There are two subtypes of kinesin-2 members: one exists in a heterotrimeric form comprising two different motor subunits and a kinesin-associated subunit (KAP); the other forms a homodimer (Scholey 2013). Interestingly, only one kinesin-2 motor is present in most land plants (Table 2). KAP-encoding genes were found in *M. polymorpha*, hornworts *Anthoceros agrestis* and *Anthoceros punctatus*, and other flagellar-carrying species analyzed except for *P. patens*. Incomplete KAPs are found in the genomes of the moss *Sphagnum magellanicum* and lycophyte *Diphasiastrum complanatum*. As homodimeric kinesin-2 also regulates intraflagellar transport (Scholey 2013; Snow et al. 2004), albeit in a limited number of organisms and cell types, it appears that either a homodimeric kinesin-2 or a heterotrimeric kinesin-2 with identical motor subunits may have evolved to control the assembly of flagella in bryophytes.

1.2.5 | Dyneins and Intraflagellar Transport Proteins

Axonemal dynein complexes are structural components of the flagella/cilia and comprise two large groups, the outer dynein arms and the inner dynein arms (King 2016). A previous survey reports that outer dynein arms are lost in all land plants, while inner dynein arms are preserved in organisms ranging from bryophytes to gymnosperms (Lucas and Geisler 2022). Consistent with this, we identified axonemal dynein heavy chains in *P. patens* and *M. polymorpha* but not in *Arabidopsis* (Table 1 and Table S1). A complete set of dynein accessory subunits comprising dynein intermediate chains, light intermediate chains, and three types of light chains (LC8, Roadblock, and Tctex) was also characterized. Interestingly, the *Arabidopsis* genome encodes multiple LC8-type light chains (Table 1 and Table S1), which are also present in other flowering plants (Cao et al. 2017). The functions of these light chains remain undetermined. It might be possible that they serve as chaperones to facilitate protein folding independent of being a dynein subunit as their homologs do in animals (Rapali et al. 2011). In addition to axonemal dynein, a cytoplasmic dynein-2 (or IFT dynein) heavy chain was found in *M. polymorpha* but not in *P. patens* or other land plants (Table 1). Cytoplasmic dynein-2 powers retrograde transport in flagella/cilia (Lacey and Pigino 2025). These facts indicate a functional set of motor proteins required for bidirectional IFT, a kinesin-2 for anterograde transport and a cytoplasmic dynein-2 for retrograde transport, is present in liverworts. In animals, the bidirectional IFT requires other macromolecular complexes, namely IFT-A, IFT-B, and BBSome (Lacey and Pigino 2025). Our survey did not reveal BBSome components in *P. patens* and *M. polymorpha*. However, IFT-A and IFT-B subunits were found in both species (Table 3). Some of the IFT-A and IFT-B subunits are clearly fragmented, suggesting that IFT components may undergo evolutionary loss in bryophytes. These facts, along with the absence of cytoplasmic dynein-2 in the majority of land plants and axonemal dyneins in flowering plants, support that flagellar/ciliary components are sequentially lost during plant evolution (Lucas and Geisler 2022).

1.2.6 | Centriole Biogenesis Proteins

In animals, centrioles are generated either through a de novo synthesis pathway or in a templated manner aided by a mother centriole (Breslow and Holland 2019). The assembly of centrioles involves multiple scaffold proteins, initiators, and elongation factors (Banterle and Gonczy 2017; Breslow and Holland 2019). Land plants only build a centriole de novo in spermatogenous cells (Brown and Lemmon 2007). The molecules participating in centriole assembly appear to be highly diverged from those in animals. Many key factors in animals such as PLK4, CEP192, and STIL are absent in land plants and the green alga *Chlamydomonas* (Hodges et al. 2010; Jana 2021). Only three out of the 13 key components (SAS6, CEP120, and SAS4) are found in the majority of centriole-carrying land plants (Table 2). It remains mysterious whether plants employ a distinct pathway for centriole assembly. A recent study identifies a highly diverged CEP135/BLD10 homolog in *P. patens* and *M. polymorpha* (Koshimizu et al. 2022). Other functionally equivalent centriole biogenesis proteins, which may not be identified simply based on sequence similarity, could contribute to centriole assembly. In bryophytes, centrioles still possess the ability to form centrosomes. However, this function is only preserved in the mitosis of spermatogenous cells (Robbins 1984). Centrosomes may be functional in other centriole-carrying species because multiple centrosomal proteins are present in lycophytes and ferns (Table 2). Notably, many centrosomal proteins are still absent (Hodges et al. 2010). If present, the centrosome in these organisms may maintain a limited function as an MTOC. As genes related to centriole biogenesis are typically expressed in spermatogenous cells, it is possible to identify new centriole biogenesis factors through cell type-specific expression analysis (Higo et al. 2016; Koshimizu et al. 2022; Meyberg et al. 2020; Minamino et al. 2022).

1.3 | Major Forms of MT Arrays in Bryophytes

As in other plants, the majority of interphase MTs of bryophytes do not form conspicuous MTOCs due to the absence of a centrosome (Yi and Goshima 2018). Interphase MTs are commonly confined to the cortical cytoplasm in flowering plants, forming a unique arrangement in a 2D plane (Elliott and Shaw 2018). Such well-developed cortical MTs in bryophytes exist mainly in cells of complex tissues such as epidermal cells in moss gametophore stems (Hashida et al. 2020; Spinner et al. 2010) and liverwort thallus (Attrill and Dolan 2024; Furuya et al. 2018), and typical scale cells and photosynthetic filament cells in liverworts (Apostolakos and Galatis 1992, 1993), but are not common in simpler structures including protonemata (Doonan et al. 1985; Nakaoka et al. 2015; Schmiedel et al. 1981), rhizoids (Pressel et al. 2008), gametophore initials (Kosetsu et al. 2017), and food-conducting cells (Ligrone and Duckett 1994). The ordering of cortical MTs requires the MT-severing protein katanin (Attrill and Dolan 2024), MT-associated protein PpTON1 (Spinner et al. 2010), and transcriptional co-repressor MpANGUSTIFOLIA (Furuya et al. 2018), which all regulate cortical MT ordering in flowering plants, suggesting that conserved mechanisms may operate in bryophytes. However, PpANGUSTIFOLIA does not regulate cortical MT organization in *P. patens* (Hashida et al. 2020; Takechi et al. 2021). To what

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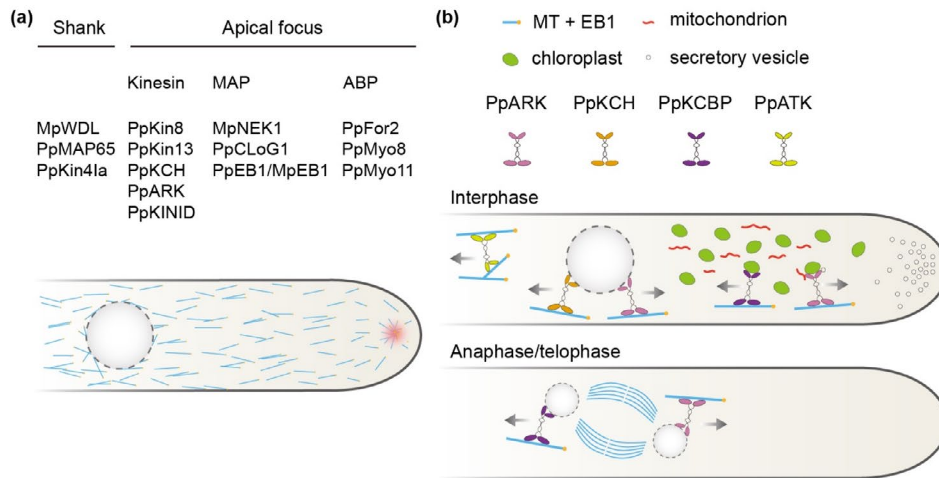

FIGURE 4 | MT organization and kinesin-mediated transport in tip-growing protonemal cells and rhizoids. (a) The MTs in protonemata and rhizoids are preferentially oriented with plus ends toward the tip and form a focus structure in the apical cytoplasm (red). Proteins that localize to MTs in shank region or at the apical MT focus are shown. EB1 specifically associates with MT plus ends. The tip-localized proteins comprise three major groups, kinesin, microtubule-binding protein (MAP), and actin-binding protein (ABP). (b) Multiple kinesins regulate MT-dependent organelle transport. In interphase, the plus-end-directed ARK kinesins play a versatile role in transporting the nucleus, mitochondria, chloroplasts, vesicles toward the tip. The basal transport of nucleus and chloroplasts are driven by KCH and KCBP, respectively. Branched MTs are realigned by the minus-end-direct ATK kinesins toward the base. In anaphase/telophase, the reforming nucleus in apical daughter cells is transported by ARK kinesins anterogradely; retrograde transport of nucleus in the basal daughter cell is mediated by KCBP.

extent the underlying mechanism is preserved in these distant species still remains to be investigated.

During plant cell division, MTs are organized into well-defined arrays: a PPB in preprophase, a spindle in metaphase and anaphase, and a phragmoplast during telophase and cytokinesis (Liu and Lee 2022; Livanos and Muller 2019). The morphology and assembly of the spindle and phragmoplast in bryophytes exhibit little difference from those in flowering plants (Brown and Lemmon 2011). The PPB mostly occurs in cells of complex tissues and is absent in the majority of protonemal cells and all meiotic cells (Wick 1991). In addition, transitional forms of MT arrays such as polar organizers (POs) in liverworts and nuclear envelope-nucleated MTs in various cells assemble before cell division and contribute to spindle formation (Brown and Lemmon 2007; Yamada and Goshima 2017; Yi and Goshima 2018). Specialized MT arrays, including the plastid-derived axial MT system (AMS) and quadripolar microtubule system (QMS) are involved in the preparation of cell division during mitosis and meiosis, respectively (Brown and Lemmon 1997, 2007, 2013; Shimamura et al. 2003).

Other prominent MT structures include centrioles, flagellar axonemes, and the multilayered structure (MLS), which are present in spermatid mother cells (SMCs) or their daughter cells (spermatids) (Renzaglia and Garbary 2001). Centrioles arise de novo in spermatogenous cells that terminally differentiate into SMCs (Brown and Lemmon 2007; Vaughn and Renzaglia 1998). After mitosis of SMCs, centrioles transform into basal bodies in spermatids and nucleate the assembly of flagella (Moser and Kreitner 1970; Robbins 1984). Beneath the flagellar axoneme is the unique MT structure MLS (Renzaglia and Garbary 2001). The MLS is supposed to serve as a scaffold to maintain spermatid integrity during flagellar beating (Renzaglia and Garbary 2001). In the following sections, we present the current understanding of how the aforementioned MT structures are organized and regulated.

1.3.1 | Endoplasmic MTs in Tip Growth

In bryophytes, interphase MTs are mostly endoplasmic and do not exhibit universal patterns. However, in tip-growing protonemal cells of moss *P. patens* and rhizoids of liverworts, MTs are preferentially aligned along the longitudinal axis with plus ends toward the tip (Figure 4) (Althoff et al. 2022; Attrill et al. 2024; Doonan et al. 1985, 1988; Hiwatashi et al. 2014; Kanda et al. 2023; Lloyd et al. 1985; Schmiedel et al. 1981). At the growing tip, MTs converge and establish an MT focus (Doonan et al. 1985, 1988; Lloyd et al. 1985; Otani et al. 2018; Schmiedel et al. 1981). These MTs are less stable than those in the basal cytoplasm and are not well stained with immunofluorescence microscopy (Doonan et al. 1988; Otani et al. 2018).

The origin of endoplasmic MTs is not well clarified. Evidence indicates that the nucleus and organelles are prominent nucleation sites because MTs frequently associate with these structures (Doonan et al. 1985; Pressel et al. 2008; Schmiedel et al. 1981). Importantly, isolated nuclear membranes and plastids contain γ-tubulin on their membranes and are able to polymerize MTs in vitro (Shimamura et al. 2004). At the molecular level, γ-tubulins are the major factor for MT nucleation, and other polymerization factors such as XMAP215 and augmin play a minor role, as shown in *P. patens* (Nakaoka et al. 2015). Interestingly, a significant portion of MTs in *P. patens* can be nucleated in the absence of γ-tubulin, suggesting the existence of alternative nucleation pathways (Nakaoka et al. 2015). In addition to membrane-associated nucleation, endoplasmic MTs can be generated through an MT-dependent branching mechanism, which is highly conserved in plants (Nakaoka et al. 2015; Yi and Goshima 2018).

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How MTs are organized in parallel in tip-growing cells is unclear. In flowering plants, the ordering of cortical MTs is mainly configured by the generation of branched MTs and the release of nucleated and crossover MTs by katanin-mediated severing (Elliott and Shaw 2018; Yan et al. 2023). The ordering mechanism in *P. patens* may differ from that observed in flowering plants because MT severing in *P. patens* is infrequent, and the angle of MT branching is more variable (Nakaoka et al. 2015). However, branched MTs can be realigned through minus-end-directed transport by the kinesin-14 member PpATKs (Nakaoka et al. 2015; Yamada et al. 2017), which may provide an alternative mechanism (Figure 4b). Other conserved factors could potentially contribute to MT ordering. For example, the MT-binding protein PpMAP65 associates with bundled MTs in the shank region and may contribute to MT alignment; the bundling activity is antagonized by the PpMAP65-binding patterner PpKinesin-4Ia (de Keijzer et al. 2023). PpSPIRAL2 (PpSPR2), a MT minus-end-binding protein which has been shown to stabilize MT minus ends and promote MT severing in *Arabidopsis* (Fan et al. 2018; Nakamura et al. 2018), plays a similar role in stabilizing minus ends in *P. patens* (Leong et al. 2018); a novel MT-binding protein PpCLoG1 specifically tracks the depolymerizing ends and regulates MT dynamicity (Ding et al. 2018).

At the tip of protonemata and rhizoids, endoplasmic MTs are converged to form MT foci (Figure 4a). Numerous cytoskeletal factors, including kinesins, MT-binding proteins, and MT regulators, are enriched there (Champion et al. 2021; Ding et al. 2018; Doonan et al. 1988; Hiwatashi et al. 2014; Kanda et al. 2023; Leong et al. 2020; Otani et al. 2018; Yamada and Goshima 2018). Among these, two kinesins, PpKCH and MpARK, are required for focusing (Kanda et al. 2023; Yamada and Goshima 2018); three other kinesins, PpKinesin-13, PpKinesin-8, and PpKINID1a/b, are involved in the positioning of MT foci (Hiwatashi et al. 2014; Leong et al. 2020). PpEB1, which stabilizes MT plus ends, also localizes to the foci (Hiwatashi et al. 2014). However, its contribution to MT convergence has not been investigated. In *M. polymorpha*, MpNEK1, a NIMA-related protein kinase, negatively regulates MT bundling and focusing, presumably through direct phosphorylation of tubulin (Otani et al. 2018), while MpWDL plays an opposite role in MT bundling, mainly in the shank region of rhizoids (Champion et al. 2021). In *P. patens* protonemal tip cells, kinesin-4Ia colocalizes with bundled MTs in the apical cytoplasm but is not enriched in MT foci. Loss of function of kinesin-4Ia causes hyper-aligned MTs, smaller MT foci, longer MT overlaps, and reduced variability in growth direction, suggesting that it is a negative regulator of focusing, as MpNEK1 is (de Keijzer et al. 2023).

Like MTs, actin filaments as well as various actin-binding proteins (Formin II/PpFor2, Myosin VIII/PpMyo8, and Myosin XI/PpMyo11) also accumulate at the tip of protonemal cells and rhizoids (Figure 4a) (Doonan et al. 1988; Otani et al. 2018; Vidali et al. 2010; Vidali et al. 2009; Wu and Bezanilla 2018). In *M. polymorpha* rhizoids, MT and actin foci are separate, implying that they are differentially organized (Otani et al. 2018). However, in *P. patens* caulonemal tip cells, MTs and actin filaments exhibit colocalization within the foci and are mutually dependent on each other for localization (Wu and Bezanilla 2018; Yamada and Goshima 2018). MTs serve as a transport system that delivers actin nucleation-promoting factors such as PpFor2 (van Gisbergen et al. 2020; Wu and Bezanilla 2018) and PpRopGEFs (Yoshida et al. 2023a) to the tip, while actin may regulate MT organization through actin-binding proteins. For example, PpMyo8, which potentially interacts with MTs, is required for MT convergence (Wu and Bezanilla 2014, 2018).

What is the function of parallel MTs and the MT foci? The MT focus fluctuates along with tip growth, providing an excellent platform for the delivery of secretory vesicles during cell expansion (Orr et al. 2020). The disruption of MTs and actin strongly inhibits vesicle trafficking and tip growth in protonemata and rhizoids (Bibeau et al. 2018; Doonan et al. 1988; Pressel et al. 2008). Although MTs and actin can be recognized as a coherent entity, their functions in tip growth are not identical. At low concentrations, MT inhibitors cause cell swelling and induce ectopic growing tips (Attrill et al. 2024; Champion et al. 2021; Doonan et al. 1988; Wu and Bezanilla 2018). This phenotype is never observed when actin inhibitors are used. Therefore, MTs play a specific role in specifying the growing tip, while actin mainly acts to promote cell expansion. This functional divergence might be common, as similar effects have been recently revealed in liverwort rhizoids (Attrill et al. 2024), although MTs and actin do not show a close association (Otani et al. 2018). The presence of axially aligned MTs and an MT focus is likely a unique feature during tip growth. Such structures are not observed in gametophore bud initials, which undergo diffuse growth (Doonan et al. 1987; Kosetsu et al. 2017).

1.4 | Organization of MTs in Mitosis

1.4.1 | Pre-Mitotic Endoplasmic MTs

In preparation for mitosis, MTs are polymerized around the nuclear membrane and contribute to spindle assembly once the nuclear envelope breaks down (NEB) (Yi and Goshima 2018). This phenomenon was present in a wide range of organisms, including bryophytes and flowering plants. However, the specific patterns of such pre-mitotic MTs vary depending on the species (Figure 5a).

POs are pre-mitotic MTOCs that arise in preprophase/prophase and exist only in liverworts (Figure 5a) (Brown and Lemmon 1990a, 2011). POs can nucleate astral MTs and behave like centrosomes in animals (Brown and Lemmon 2011; Buschmann et al. 2016). However, they are devoid of centrioles (Fowke and Pickett-Heaps 1978). The nucleation of MTs in POs depends on nuclear membrane-associated γ-tubulin (Brown et al. 2004). The focusing of POs involves the MT-severing enzyme katanin (Attrill and Dolan 2024). POs are eliminated in late prophase (Brown and Lemmon 1990a), but seem to play important roles in facilitating division plane selection as other MT structures, such as polar caps in flowering plants, do (Kosetsu et al. 2017; Yamada and Goshima 2017; Yi and Goshima 2018). Despite the lack of strong experimental evidence, there are good correlations that two POs are positioned at opposite sides of the nucleus, with their long axis perpendicular to the future division plane (Brown and Lemmon 1990a, 2011; Buschmann et al. 2016). The lack of such bipolar arrangement leads to a less organized PPB, a ring-like MT array that marks the future division plane (see below) (Buschmann et al. 2016). It is noteworthy

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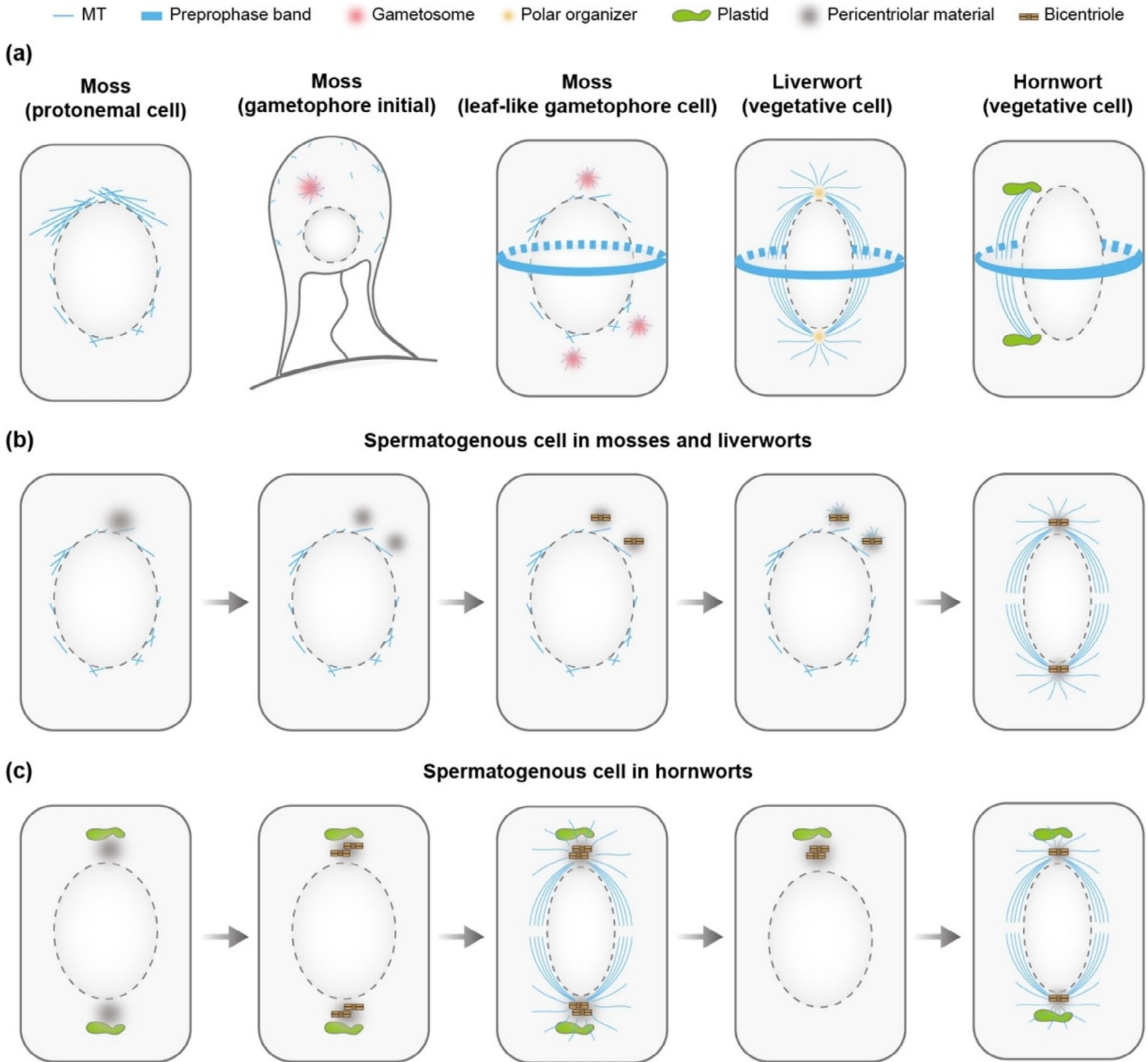

FIGURE 5 | MT organization and origin of bicentrioles in pre-mitotic cells. (a) Distinct forms of pre-mitotic MT arrays in bryophytes. In moss protonemal cells, MTs are mainly nucleated from the nuclear membrane and often shows an asymmetric enrichment at the apical side. A cloudy cytoplasmic microtubule-organizing center (MTOC) called gametosome develops in the apical cytoplasm of gametophore initials in the absence of a preprophase band (PPB). In moss leaf-like gametophore cells, the gametosome and the PPB are both present but not always found in the same cell. In addition, the number of gametosome varies. In liverworts, two centrosome-like structures namely polar organizers (POs) function as the major MTOCs and a PPB develops in preprophase/prophase. In hornworts, all cell divisions are monoplastidic. A single plastid serves as the cytoplasmic MTOC. Once the plastid divides, a pair of plastids migrate to each of the two poles of nuclear surface. An MT band is developed between the plastids and associates with the longitudinally oriented nucleus. This unique array is termed axial MT system (AMS). The PPB is established along with the development of AMS. However, it is asymmetric in size. The portion close to AMS is tight while the other part encircling the nucleus is broad. (b) De novo formation of bicentrioles in moss and liverwort spermatogenous cells. In early prophase, dense pericentriolar materials emerge around the nuclear membrane and split into two halves. Each half synthesizes a bicentriole, of which the proximal central hubs of two centrioles are attached and positioned end-by-end. The bicentrioles mature into two centrosomes and nucleate MTs in late prophase. (c) De novo formation of bicentrioles in hornwort spermatogenous cells. Pericentriolar materials arise from the duplicated plastids. Each pericentriolar region synthesizes a pair of bicentrioles and matures into a functional centrosome. After the first cell division, the daughter cell inherits a single plastid and a pair of bicentrioles. In the second round of cell division, the plastid duplicates. Each bicentriole matures into a centrosome and organizes MTs as an MTOC.

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that the PO axis frequently rotates. Its initial position does not necessarily predict the final division axis. Moreover, suspension cells that have multiple POs can eventually establish a bipolar spindle, indicating that other mechanisms contribute to division plane determination (Buschmann et al. 2016). During the asymmetric division of spores, PO-nucleated MTs are asymmetrically distributed around the nucleus and control basal nuclear migration and subsequent division asymmetry (Attrill et al. 2024). These findings indicate that POs can additionally impact division orientation at an earlier stage. More commonly, POs function as an MT source for spindle assembly because not only MTs but also γ-tubulin derived from POs relocate into the spindle following NEB (Brown and Lemmon 2011; Brown et al. 2004; Yamada and Goshima 2017).

In prophase of moss protonemal cells, abundant MTs emerge and associate with the nuclear membrane (Schmiedel et al. 1981). These MTs are not as focused as those in liverwort POs. Before NEB, the nucleus-derived MTs become asymmetrically enriched at the apical nuclear membrane (Figure 5a) (Doonan et al. 1985; Nakaoka et al. 2012). The development of this asymmetry requires γ-tubulin (Nakaoka et al. 2012), PpTPX2 (Kozgunova et al. 2022), and PpKinesin-13 (Leong et al. 2020), but not augmin (Nakaoka et al. 2012). The positioning of apical nuclear MTs requires the nuclear membrane protein PpSUN2, which also exhibits asymmetric localization (Yoshida et al. 2023b). The nucleus undergoes a rapid short-distance movement immediately before NEB (Leong et al. 2020; Yi and Goshima 2020). The disruption of nucleus-associated MTs and factors impedes nuclear migration (Leong et al. 2020; Yi and Goshima 2020; Yoshida et al. 2023b). The functional significance of this short-distance nuclear migration is unclear. One possible explanation is that asymmetrically distributed MTs finetune the position of the nucleus to ensure the correct localization of the division plane. As observed during side branch formation, the nucleus migrates a long distance from the cell center toward the apical bulge (Schmiedel and Schnepf 1979b). When the nucleus arrives at the base of the bulge, MTs are asymmetrically accumulated at the apical nuclear surface. This asymmetry might be essential for generating forces for the rapid movement of the nucleus into the bulge, which guarantees the correct positioning of the spindle and phragmoplast and orientation of the division plane along the longitudinal axis (Schmiedel and Schnepf 1979a; Yi and Goshima 2020). As in liverworts, nucleus-associated MTs also contribute to spindle assembly (Yamada and Goshima 2017; Yi and Goshima 2018). Upon NEB, the asymmetrically distributed MTs transform into a biased prometaphase spindle, which later develops into a symmetric shape with the supply of newly formed MTs likely through the chromatin and MT-dependent pathways (Nakaoka et al. 2012; Yi and Goshima 2018).

In mosses, the development of gametophore begins with a side branch cell termed gametophore initial (Moody 2019). This cell exhibits little difference in morphology from canonical side branch protonemal cells when produced (Tang et al. 2020). Once obtaining a gametophore initial cell fate either under natural conditions or induced by cytokinin (Brandes and Kende 1968), it becomes bulbous and undergoes several rounds of oblique divisions to initiate gametophore development (Moody 2019). The oblique division of gametophore initials is driven by a distinct MTOC termed gametosome at the apical cytoplasm in prophase (Figure 5a) (Kosetsu et al. 2017). The gametosome does not associate with the nuclear membrane and is not well focused. However, it shows many similarities to other pre-mitotic MTOCs. First, its assembly requires γ-tubulin but not augmin, similar to nucleus-associated MTOCs in protonemal cells (Kosetsu et al. 2017; Nakaoka et al. 2012). Second, the gametosome plays a critical role in spindle orientation as POs in liverworts and polar caps in flowering plants do (Kosetsu et al. 2017; Yi and Goshima 2018). Third, in prometaphase, the gametosome merges into the spindle MTs, thus likely contributing to spindle assembly (Kosetsu et al. 2017). However, reminiscent of POs and nucleus-associated MTOCs (Buschmann et al. 2016; Nakaoka et al. 2012), the gametosome alone is not essential for spindle formation (Kosetsu et al. 2017).

1.4.2 | Preprophase Band

The PPB is a circular array of cortical MTs found in the majority of cells in flowering plants (Gunning 1982; Mineyuki 1999). It transiently forms in preprophase/prophase and disassembles in prometaphase. However, it leaves behind a so-called cortical division zone, enriched with actin and MT-binding proteins, functioning as a guide for division plane orientation (Livanos and Muller 2019). In bryophytes, the PPB is present mostly in complex tissues such as leafy gametophores in mosses (Doonan et al. 1987; Schnepf 1984) and thallus epidermal cells in liverworts (Figure 5a) (Brown and Lemmon 1990a, 2011; Buschmann et al. 2016). Remarkably, it is absent in many cells such as caulonemal tip cells (Doonan et al. 1985; Schmiedel et al. 1981) or side branch cells in protonemata (Schmiedel and Schnepf 1979b), gametophore initials (Kosetsu et al. 2017), and archesporial cells (Brown and Lemmon 1992; Gambardella and Alfano 1990). The morphology of the PPB in bryophytes exhibits great variability among cells and typically does not show a narrow band morphology as seen in flowering plants (Apostolakos and Galatis 1992; Brown and Lemmon 1988b, 2011; Kosetsu et al. 2017). In moss gametophores, only ~50% of leaf cells display PPBs (Kosetsu et al. 2017). When developing a Tmema cell, the protonemata of the moss *Funaria* establish a PPB in the Tmema mother cell (Sawidis et al. 1991). The formation of a PPB in this unique protonemal stage is supposed to trigger an asymmetric division of the Tmema mother cell (Sawidis et al. 1991). In liverworts and hornworts, incomplete or asymmetric PPBs have been observed (Apostolakos and Galatis 1985; Brown and Lemmon 1988b). Despite these divergences, conserved mechanisms appear to regulate PPB assembly and function in bryophytes. For example, katanin and TON1, two well-characterized PPB regulators, have been reported to control PPB assembly in liverworts and mosses, respectively (Attrill and Dolan 2024; Spinner et al. 2010). In addition, as in flowering plants, PPBs frequently interact with cytoplasmic MTs and cooperatively regulate division plane selection (Brown and Lemmon 2011; Buschmann et al. 2016). However, the timing of interactions varies. In liverworts, the PPB arises later than POs; in mosses, it appears earlier than nucleus-associated MTOCs; in hornworts, its assembly is concurrent with the arise of plastid-derived MTs (Brown and Lemmon 1988b, 1990a, 2011; Buschmann et al. 2016). In moss gametophore leaves, both the PPB and the gametosome are formed (Figure 5a) (Kosetsu et al. 2017). The formation of the

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gametosome is independent of the PPB. Some cells can either lack a gametosome or a PPB, implying that the gametosome and PPB are not universally required for cell division (Kosetsu et al. 2017). These observations, together with the absence of the PPB in protonemal cells, support the hypothesis that the PPB has evolved for establishing division polarity in complex tissues (Wick 1991). In addition, the divergence of the PPB in liverworts and hornworts further reflects a transitional development of the PPB in bryophytes.

1.4.3 | Plastid-Derived MTOC

Pre-mitotic MTOCs in monoplastidic cell division of bryophytes mostly originate from a single plastid, a phenomenon that occurs in all types of mitotic and meiotic divisions in hornworts and is found in the meiotic sporocytes of mosses and a few types of cells in liverworts (Brown and Lemmon 1997).

In hornworts, the mitotic division of vegetative cells begins with the elongation of the plastid and its positioning in parallel with the fusiform nuclear surface in preprophase (Brown and Lemmon 1988b). This cellular arrangement occurs before any obvious changes in MT organization, thus being the earliest mark of division polarity. The plastid then functions as a MTOC to nucleate MTs aided by γ-tubulin (Brown and Lemmon 2011). As the plastid-derived MTs run over the long axis of the plastid and nucleus, this MT array is termed the axial microtubule system (AMS) (Figure 5a). When the AMS is established, an asymmetric PPB, comprising a small tight portion over the plastid isthmus and a broad region encircling the nucleus, simultaneously develops perpendicular to the AMS (Brown and Lemmon 1988b, 2011). The plastid-derived MTs frequently interact with the PPB (Brown and Lemmon 1985, 1988b, 2011). In late prophase or later, the plastid divides, and the daughter plastids are positioned at each of the nuclear poles. Following NEB, the PPB is disassembled; MTs of the AMS transform into the prometaphase spindle (Brown and Lemmon 1988b). As the AMS is placed on one side of the nucleus, the prometaphase spindle is obviously asymmetric with more MTs on the AMS side. In mosses, the final mitotic division of SMCs is also monoplastidic and similarly depends on an AMS (Gambardella and Alfano 1990). However, the PPB is not formed in this process. Notably, not all monoplastidic divisions require an AMS. In the archesporial cells of the liverwort *Monoclea gottschei*, POs function as the major pre-mitotic MTOCs; neither AMS nor PPB develops (Brown and Lemmon 1992).

1.4.4 | Spindle and Phragmoplast

All land plants form two distinct MT arrays—the spindle and the phragmoplast—to facilitate cell division. The spindle ensures the equal segregation of chromosomes into daughter cells, while the phragmoplast directs the transport of secretory vesicles to mediate cell plate assembly and expansion (Liu and Lee 2022; Smertenko et al. 2018). The morphology of the spindle and phragmoplast in bryophytes is similar to that in flowering plants (Brown and Lemmon 2011). In general, the spindle is in a barrel shape with broad poles, although mild variations have been reported. For example, in moss protonemata, the metaphase spindle poles narrow in anaphase (Doonan et al. 1985); during side branch formation, the spindle and phragmoplast are asymmetric, with the apical pole more focused (Doonan et al. 1986). As in flowering plants (Lee and Liu 2019; Liu and Lee 2022; Muller 2019; Yamada and Goshima 2017), the assembly of the spindle and phragmoplast involves conserved MT nucleators and regulators. These include γ-tubulin (Brown and Lemmon 2011; Brown et al. 2004), augmin (Nakaoka et al. 2012), katanin (Attrill and Dolan 2024), kinesin-5 (Miki et al. 2014), and TPX2 (Kozgunova et al. 2022). During the transition from spindle to phragmoplasts, multiple MT bundling factors and kinesins, including PpMAP65 (Kosetsu et al. 2013), PpKINID (Hiwatashi et al. 2008), PpKinesin-4Ia (de Keijzer et al. 2017), and PpNACK/kinesin-7 (Naito and Goshima 2015) are involved in the establishment of MT interdigitation and the maintenance of phragmoplast bipolarity, thus ensuring the accurate delivery of vesicles to the cell center for cell plate assembly. Recently, the kinesin-12 family has been identified as the long-sought motor protein responsible for transporting vesicles during cell plate assembly. Functional defects in these kinesins result in delayed deposition of cell plate materials, as well as impaired phragmoplast expansion and disassembly (Yamada et al. 2025). How MT regulators are orchestrated to mediate phragmoplast assembly is still under investigation. A recent study identifies new drugs that regulate MT organization and phragmoplast formation, likely through affecting the phosphorylation status of MT regulators, implying the involvement of kinases as upstream regulators (Kimata et al. 2023).

The positioning and orientation of the spindle and phragmoplast are critical for the determination of a division plane (Livanos and Muller 2019). In moss caulonemal tip cells, the phragmoplast rotates to establish an oblique division plane; this process depends on MTs (Doonan et al. 1985; Schmiedel et al. 1981). PpKinesin-12I and its putative interacting protein PpREN are likely involved in division plane orientation as their homologs do in *Arabidopsis* (Lipka et al. 2014; Muller et al. 2006; Stockle et al. 2016), yet the underlying mechanism remains to be investigated (Miki et al. 2014; Yi and Goshima 2020, 2022). Additionally, the actin-dependent motor Myo8 interacts with periphery MTs in phragmoplasts and regulates division orientation (Wu and Bezanilla 2014; Wu et al. 2011). The involvement of an MT-associated myosin in division plane orientation has only been reported in mosses. However, recent studies from maize and *Arabidopsis* identify Myosin XI as an integral component of a cortical division zone complex (Huang et al. 2024; Nan et al. 2023), which comprises multiple MT-binding proteins (Livanos and Muller 2019). Myosin XI is recruited to the division site likely through direct binding with MT-binding proteins such as the kinesin-12 member POKs and facilitates the consolidation of the multiprotein complex (Huang et al. 2024; Nan et al. 2023). These findings suggest that an MT/actin cytoskeleton-associated motor assembly is widely involved in the guidance of spindle and/or phragmoplast orientation.

In moss gametophore initials, the division plane is more obliquely oriented than those in protonemal cells, a feature marking the transition from 2D development to 3D development (Harrison et al. 2009; Moody 2019). The orientation of the spindle is guided by an apical cytoplasmic MTOC, the gametosome, as mentioned earlier (Kosetsu et al. 2017). Recently, the MT polymerization

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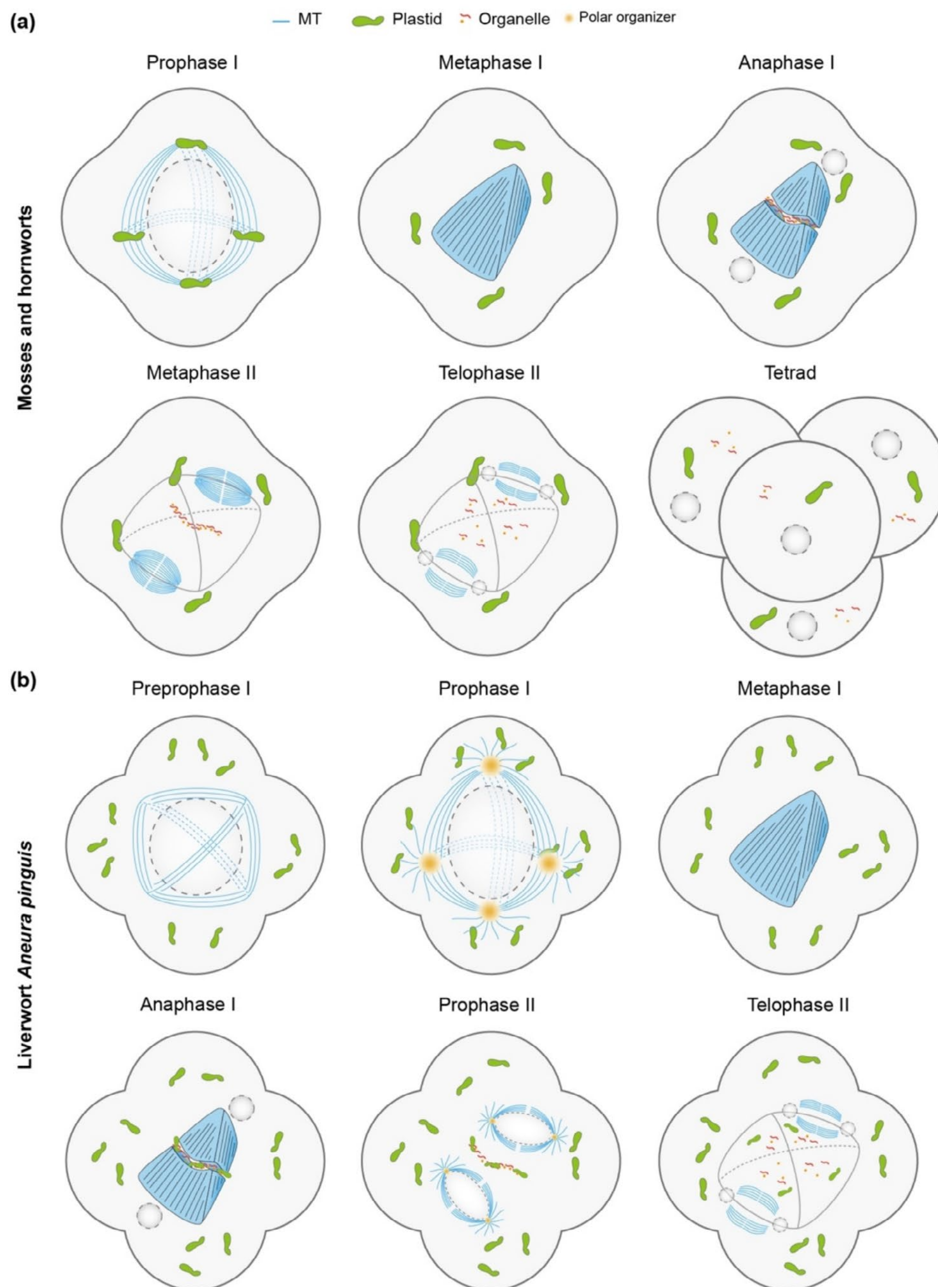

FIGURE 6 | MT organization in meiotic cells. (a) Meiotic division of sporocytes in mosses and hornworts. In early prophase I, a single plastid divides twice, producing four daughter plastids. In late prophase I, the plastids are positioned at poles in a tetrahedral arrangement. Each plastid functions as an MTOC for MT nucleation. Six MT bands connect each pair of plastids and establish the quadripolar system (QMS). In metaphase I, the spindle is organized in a tetrahedral shape with two broad poles positioned at a right angle. Cytokinesis is incomplete in meiosis I. When the chromosomes are separated, an organelle band, which consists of mitochondria, endoplasmic reticulum, and lipid droplets, forms in the presumptive division plane. Meiosis II of daughter nuclei occurs simultaneously. The metaphase spindle is assembled by MTs derived from the pole region of meiosis I. During cytokinesis, the organelles become dispersed and are distributed into each spore domain together with a single plastid. (b) Meiotic division of sporocytes in the liverwort *Aneura pinguis* (*Jungermanniopsida*). Meiotic divisions in liverworts are highly variable. Except for some species of *Jungermanniopsida* and *Marchantiopsida* (e.g., *Blasia*), the majority of meiotic division in liverworts is polyplastidic. Pre-meiotic MTs could be organized by plastids, nuclear membrane, or polar organizers (POs). In deeply lobed sporocytes, quadripolarity is established in preprophase by girdling MT bands before the QMS is developed. These MTs likely originate from the nuclear membrane. In liverwort *Aneura pinguis*, POs function as the major MTOCs for QMS development in meiosis I and for spindle formation in meiosis I and II. The division process is similar to that in mosses and hornworts, except that plastids are present in the organelle band.

promoting factor PpTPX2 has been reported to play a role in spindle positioning. Loss of function of *tpx2* results in a basally located spindle (Kozgunova et al. 2022). Interestingly, this phenotype can be rescued with the actin inhibitor Latrunculin A, suggesting that MT-actin crosstalk is involved in spindle positioning. In protonemal tip cells, the disruption of kinesin-13 also causes abnormal spindle migration (Leong et al. 2020). These data highlight a potential role of spindle anchoring in division

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plane selection, albeit the underlying molecular mechanism remains to be explored.

1.5 | Organization of MTs in Meiosis

As in flowering plants, all meiotic divisions in bryophytes do not involve a PPB (Wick 1991). However, pre-meiotic MTs are well organized in preparation for two consecutive divisions that produce four spores from a single spore mother cell (sporocyte) (Brown and Lemmon 2013). In hornworts and mosses, a single plastid functions as the major pre-meiotic MTOC, while in liverworts, three forms of MTOCs, that is, plastids, nuclear membranes, or POs, all exist, and the division can be either monoplastidic or polyplastidic (Brown et al. 2010).

In mosses, the single plastid divides twice and produces four daughter plastids in prophase. Each plastid is positioned at one of the tetrahedral poles and functions as an MTOC (Brown and Lemmon 1982b, 1987b). MTs between the poles interact with each other and establish six MT bands, namely a quadripolar MT system (QMS) that encircles the nucleus (Figure 6a) (Brown and Lemmon 1982b, 1987b; Busby and Gunning 1988a, 1988b). In metaphase I, the four poles of QMS transform into two broad poles whose long axes are oriented at a right angle that places the pole edges in tetrahedral positions (Brown and Lemmon 1987b; Busby and Gunning 1988a, 1988b). Cytokinesis does not occur until the end of meiosis II. Instead, an organelle band enriched with mitochondria, endoplasmic reticulum, and lipid droplets forms at the presumptive division plane (Brown and Lemmon 1982a; Brown and Lemmon 1987a; Busby and Gunning 1988a, 1988b). The phragmoplast is assembled by interzonal MTs perpendicular to the organelle band (Brown and Lemmon 1982a; Brown and Lemmon 1987a). The second division in daughter nuclei occurs simultaneously. Each of the metaphase II spindles is organized by a pair of plastids with its poles positioned toward the future spore domains (Brown and Lemmon 1982a; Brown and Lemmon 1987a; Busby and Gunning 1988a, 1988b). Phragmoplasts are assembled between pairs of daughter nuclei seemingly without any contribution of MTs from the organelle band. The final cytokinesis perfectly partitions the cytoplasm and nuclei into each spore domain.

QMS-assisted meiosis is highly conserved in hornworts and involves plastid-derived AMS-like arrays (Brown and Lemmon 1990b, 2013). Notably, each of the plastids in meiosis is able to nucleate a parallel AMS along its long axis, as in mitosis. However, the first AMS does not directly contribute to spindle assembly. Only after plastid division do AMSs derived from the elongated daughter plastids, which become located at the opposite poles of the nucleus, transform into a QMS. Meanwhile, the second division occurs, and the four daughter plastids are positioned at the tetrahedral poles (Brown and Lemmon 1990b; Brown and Lemmon 1997).

Sporocytes of liverworts commonly undergo polyplastidic division, but monoplastidic division is also found in some species such as *M. polymorpha*, *Blasia pusilla*, and *Dumortiera hirsuta* (Brown et al. 2010; Shimamura et al. 2003). In monoplastidic sporocytes, the development of the QMS resembles that observed in mosses and hornworts, with plastids serving as the primary MTOCs (Brown et al. 2007; Shimamura et al. 2004). In polyplastidic cells, POs, which form de novo in prophase, or the nuclear envelope, are responsible for MT nucleation during QMS development (Figure 6b) (Brown and Lemmon 2004, 2008, 2009). Therefore, three types of MTOCs derived from plastid, nuclear envelope, and PO are functional in liverworts (Brown and Lemmon 2013). Despite this divergence, γ-tubulin is a universal nucleation factor for all types of MT organization (Brown and Lemmon 2004, 2008, 2009; Brown et al. 2007; Shimamura et al. 2004), as it is found in mosses (Shimamura et al. 2004).

In addition to assembling the spindle and phragmoplast, MTs also play a role in setting up the quadripolarity of sporocytes. The cytoplasm of sporocytes in preparation for meiosis commonly undergoes ingrowth in early prophase, which leads to the formation of quadrilobes in most bryophytes (Brown and Lemmon 2013). The extent of quadrilobing varies in species (Brown and Lemmon 2013). In mosses, hornworts, and some liverworts, the lobing occurs at differential stages in prophase and is associated with the tetrahedral positioning of the plastids (Brown and Lemmon 1982b, 1987b; Brown and Lemmon 1990b; Brown et al. 2010). In *Jungermanniopsida* of liverworts, of which the sporocyte division is polyplastidic, the lobing of the sporocyte correlates with unique interlocking MT bands that delimit the future spore domains early before the QMS is developed (Figure 6b) (Brown and Lemmon 2006, 2009). The origin of these MTs is not well defined but appears to involve the nuclear membrane. In any case, γ-tubulin is clearly localized in these bands (Brown and Lemmon 2006, 2009). When the QMS either originated from POs or the nuclear envelope is developing, the MT bands are completely disassembled (Brown and Lemmon 2006, 2009). These behaviors highly resemble the PPB in mitosis and represent the earliest mark of quadripolarity. The development of interlocking MT bands is also observed in monoplastidic *Blasia* of liverworts. These MT arrays are transformed from a single MT band in close proximity to the nuclear membrane (Brown et al. 2010). The plastid is not involved in nucleating MTs at this stage, although it is essential for QMS development (Brown et al. 2010). The way of quadripolarity establishment in liverworts is highly variable. The involvement of interlocking MT bands has been only reported in species of *Jungermanniopsida* and *Marchantiopsida* (e.g., *Blasia*) with deeply lobed sporocytes. In addition, unlike other species, *Blasia* uses both interlocking MT bands and plastid-derived QMS for quadripolarity initiation (Brown et al. 2010). In certain species, a quadrilobing process (e.g., in *Reboulia hemisphaerica*) or even a quadripolarity during the entire meiosis (e.g., in *Conocephalum conicum*) is lacking (Brown and Lemmon 1988a; Brown et al. 2010). This diversity indicates the importance of MTs in polarity establishment during reproductive development in early land plants.

1.6 | Centriole, Centrosome, and Flagellum

In bryophytes, the haploid SMC in antheridia produces two spermatids through mitotic division, a process that involves genuine centrosomes (Moser and Kreitner 1970). Centrosomes are formed de novo in spermatogenous cells and are initiated

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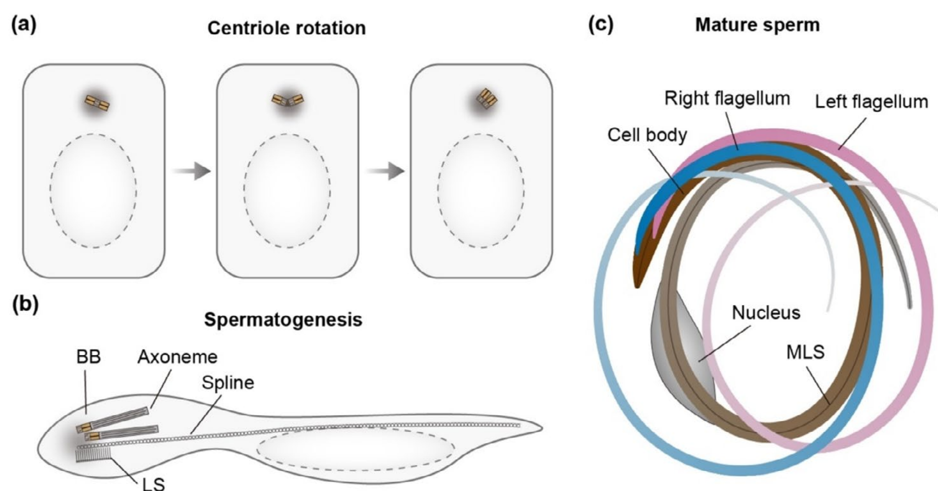

FIGURE 7 | The development of flagella. (a) Centriole preparation for basal body organization. The spermatid inherits a bicentriole from spermatid mother cells as shown in Figure 5. The centrioles subsequently rotate and are eventually positioned side-by-side. (b) The development of flagella and the multilayered structure (MLS). At a later stage, the cell body of spermatids undergoes dramatic reorganization. The two centrioles mature into basal bodies (BBs) and nucleate axoneme assembly. In mosses and liverworts, the BBs are placed in staggered positions, while in hornworts they are arranged in parallel. During BB transition, the MLS is developed underneath the BB. The mature MLS typically comprises four layers. The uppermost layer is a long plate of MTs called spline, which runs along the cell body and is associated with the nucleus. The other layers constitute the lamellar strip (LS), a short proteinaceous band below the anterior portion of the spline. The LS is derived from pericentriolar materials and functions as the MTOC for spline assembly. (c) A model of mature sperm in mosses. Adapted from (Renzaglia and Garbary 2001).

from a dark staining structure around the nuclear membrane (Figure 5b,c) (Robbins 1984). This structure has no cartwheels and radial spokes as seen in a typical centriole, and even MTs, but appears to already have MT-nucleating activity and may contain γ-tubulins (Robbins 1984; Shimamura et al. 2004). At later stages, it separates into two lobes. Each lobe migrates to the future spindle poles and matures into a bicentriole-based centrosome, which clearly nucleates MTs for spindle assembly (Robbins 1984). In contrast to perpendicular orientation in animals, the two centrioles in each centrosome are oriented end-to-end and joined by their extended cartwheels at the proximal ends (Moser and Kreitner 1970). In telophase, the two centrioles are separated and reoriented in parallel with their proximal ends side by side (Figure 7a) (Moser and Kreitner 1970). The de novo formation of centrioles depends on conserved centriole biogenesis factors SAS6, CEP135/BLD10, and POC1. Among these, SAS6 is required for cartwheel assembly, while CEP135/BLD10 and POC1 regulate cartwheel elongation and centriolar wall assembly (Gomes Pereira et al. 2021; Koshimizu et al. 2022; Meyberg et al. 2020). Notably, the flagellar axoneme in bryophytes is acetylated and polyglutamated (Gomes Pereira et al. 2021; Koshimizu et al. 2022; Meyberg et al. 2020; Minamino et al. 2022). These modifications are presumably generated by the ATAT and TTLL enzymes (Table 1). In addition, posttranslational modification can be regulated by centriole biogenesis factors such as CEP135, yet the underlying mechanism remains to be investigated (Koshimizu et al. 2022; Meyberg et al. 2020).

During spermatogenesis, the two centrioles mature into basal bodies which initiate axoneme elongation at a later stage (Figure 7a,b) (Renzaglia and Garbary 2001). In hornworts, the basal bodies lie parallel and are structurally identical (Carothers et al. 1977). In mosses and liverworts, the two basal bodies eventually separate and become staggered (Kreitner and Carothers 1976; Paolillo Jr. et al. 1968). Along with the maturation of the basal body, the MLS, another component of the locomotory apparatus of sperm, develops (Figure 7b,c) (Paolillo Jr. et al. 1968; Renzaglia and Garbary 2001). The mature MLS typically comprises four proteinaceous layers, of which the uppermost layer, namely the spline, is a long band of acetylated MTs (Gomes Pereira et al. 2021; Renzaglia and Garbary 2001). The spline spans the entire cell body, associates with the upper surface of the nucleus, and is overlaid by basal bodies at its anterior terminal region (Renzaglia and Garbary 2001). The lower layers constitute a plate-like structure called the lamellar strip, which is placed underneath the spline and basal body and above the mitochondrion (Renzaglia and Garbary 2001). The lamellar strip is derived from pericentriolar materials of the previous mitotic centrosome in SMCs and functions as an MTOC for spline MT assembly (Bernhard and Renzaglia 1995; Miller et al. 1983; Vaughn and Renzaglia 1998). The conserved centrosomal protein Centrin is an integral component of the lamellar strip (Vaughn et al. 1993). As the lamellar strip resembles the striated fibers of the basal body in other organisms, it has been proposed to play additional roles in regulating centriole maturation and flagella anchorage (Gomes Pereira et al. 2021).

1.7 | MT-Dependent Intracellular Transport

Early transmission electron microscopy studies have revealed a close association between MTs and organelles, suggesting the involvement of MTs in organelle positioning in bryophytes (Pressel et al. 2008). In recent years, the kinesin motors responsible for organelle transport have been unraveled. The best-studied cargos are the nucleus and chloroplasts.

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During caulonemal cell growth in mosses, the nucleus undergoes constant migration along with the growing tip and thus is always located around the cell center (Pressel et al. 2008). The positioning of the nucleus is not a passive process but requires MTs (Schmiedel and Schnepf 1980). Recently, two oppositely directed kinesin families, PpKCH and PpARK, have been shown to generate a tug-of-war force on the nucleus (Figure 4b) (Miki et al. 2015; Yamada and Goshima 2018; Yoshida et al. 2023a). In the absence of PpKCH, the localization of interphase nuclei shifts towards the apical part of the cell, while when PpARK is deleted, the nuclei are basally located. The directional transport of the nucleus by PpKCH and PpARK is consistent with the orientation of endoplasmic MTs with the plus ends toward the cell tip (Hiwatashi et al. 2014). In liverwort rhizoids, nuclear positioning also depends on ARK-mediated anterograde transport (Kanda et al. 2023). How motor proteins directly transport the nucleus is still under investigation. The available evidence in *P. patens* suggests the involvement of nuclear membrane proteins as a linker to the motors (Yoshida et al. 2023b). In telophase, the reforming nucleus rapidly moves to the cell center. PpARK kinesin is required for anterograde nuclear transport in the apical daughter cell (Miki et al. 2015). However, another minus-end-directed motor, PpKCBP, rather than PpKCH, regulates the retrograde movement of the nucleus in the basal daughter cell (Figure 4b) (Yamada et al. 2017). Interestingly, PpKCBP also directly associates with chromosomes and appears to directly transport them away from the cell equator in telophase (Yoshida et al. 2019). In addition to mediating transport, MTs may play a role in nuclear anchoring as MT inhibitors reduce the force needed for centrifugation-induced displacement of the nucleus (Schmiedel and Schnepf 1979a, 1980). During side branch formation, the long-distance nuclear migration in the subapical cells apparently involves MT-dependent transport (Schmiedel and Schnepf 1979a; Yi and Goshima 2020). The actin-based cell polarization and bulge formation can strongly impact nuclear migration by altering MT reorganization (Yi and Goshima 2020). Because MT orientation is biased toward the apical side as in tip cells and nuclear migration is highly directional and asymmetric, a plus-end-directed motor is supposed to transport the nucleus (Yi and Goshima 2020). Although not demonstrated yet, ARK kinesin is a good candidate (Miki et al. 2015; Yoshida et al. 2023a).

The transport of organelles is similarly balanced by oppositely directed kinesins (Figure 4b). In *P. patens*, the disruption of PpKCBP results in apically localized chloroplasts (Yamada et al. 2017; Yoshida et al. 2019). When PpARK is depleted, the chloroplasts become basally located (Yoshida et al. 2023a). ARK kinesin is a versatile motor akin to kinesin-1 in animals. It not only transports the nucleus and chloroplasts but also regulates the positioning of multiple organelles, including mitochondria and secretory vesicles (Miki et al. 2015; Yoshida et al. 2023a). This versatility of ARK kinesin for organelle transport is conserved during the apical growth of rhizoids in *M. polymorph* (Kanda et al. 2023). It is tempting to speculate that the organization of other organelles, such as vacuoles, whose morphology and position are in close association with MTs during cell growth and division (Oda et al. 2009; Pressel et al. 2008), might also be governed by ARK kinesins.

In subapical cells of *P. patens* and surface cells of *M. polymorpha*, chloroplasts are uniformly distributed. The maintenance of this localization pattern requires the kinesin-14 member KAC proteins (Shen et al. 2015; Yamamoto- Negi et al. 2024). Chloroplasts avoid high-energy light illumination but move to the light-illuminated region when the intensity is low (Wada and Kong 2018). The loss of function of KACs also causes strong defects in light responses in *P. patens*, *M. polymorpha*, and *Arabidopsis* (Shen et al. 2015; Suetsugu et al. 2012; Suetsugu et al. 2010; Yamamoto- Negi et al. 2024). Interestingly, KACs do not regulate MT dynamics, polymerization, and depolymerization, but are required for chloroplast-associated actin bundling (Shen et al. 2015; Suetsugu et al. 2010). Consistent with this function, KAC proteins lack an MT-binding capacity but are able to bind actin filaments in vitro (Suetsugu et al. 2010). The regulation of chloroplast localization under distinct light conditions is complex and involves both actin and MTs (Sato et al. 2001; Wada and Kong 2018). More factors related to MTs might be identified in the future. For instance, in addition to KACs, PpKinesin-4II has been shown to regulate blue light-induced chloroplast avoidance in mosses (MacVeigh-Fierro et al. 2017).

1.8 | Tropic Growth and Stress Response

The protonemal cells in bryophytes exhibit high plasticity in adaptation to environmental changes. Typical responses include the change of growth direction upon unidirectional light illumination and gravity vector change. Early studies have shown that MTs in apical cells are oriented along the long axis of filaments and in close proximity to organelles under unidirectional light (Burgess and Linstead 1981). Treatment with the MT inhibitor colchicine destroys the phototropic growth of protoplast-regenerated protonemata (Burgess and Linstead 1981), indicating critical roles of MTs in specifying a new growing apex. A similar function of MTs has been shown to regulate gravitropic growth of tip cells in dark-grown protonemata, during which the protonemal cells grow in the opposite direction of the gravity vector (Schwuchow et al. 1990). Gravitropic response requires the MT-associated protein PpTON1 (Spinner et al. 2010), PpKCH kinesin (Li et al. 2021), and PpKinesin-4Ia (de Keijzer et al. 2023). Interestingly, there are four highly similar KCH members in *P. patens*; only PpKCHb is essential for gravitropic growth (Li et al. 2021). Functional studies with truncated and chimeric proteins indicate that the specificity of KCHb lies in its N-terminal portion, which comprises a calponin homology (CH) domain, two coiled-coil motifs, and a motor domain, and does not require the putative cargo-binding tail (Li et al. 2021). PpKCHb is enriched at the apical MT/actin focus and can potentially bind both MTs and actin filaments (Li et al. 2021; Yamada and Goshima 2018). However, its motor activity, but not the actin-binding CH domain, is involved in gravitropic growth. It is proposed that unknown cargos are transported by PpKCHb toward the basal cytoplasm, thus indirectly impacting the localization of the MT/actin foci and growth direction (Li et al. 2021). Unlike PpKCHb, PpKinesin-4Ia is not present in the MT/actin foci (de Keijzer et al. 2023). Gravitropic response is delayed but not completely blocked in the absence of kinesin-4Ia. Kinesin-4Ia appears to indirectly regulate gravitropic response by attenuating MT dynamicity (de Keijzer et al. 2023). It is noteworthy that the lack of the aforementioned factors does not impair protonemal growth in general but specifically influences gravity responses. Therefore, gravitropic growth involves the

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positioning rather than the assembly of the machinery required for tip growth.

2 | Concluding Remark

Being the extant lineages closely related to the land plant ancestor, bryophytes represent indispensable models for understanding evolutionarily adaptations of plants (Donoghue et al. 2021; Harrison 2017). Over the last twenty years, advancements in genetics, cell biology, and molecular biotechnology in bryophytes have led to an unprecedented era for studying bryophyte development and physiology at the molecular and subcellular levels (Bi et al. 2024; Bowman et al. 2017; Frangedakis et al. 2021; Ishizaki et al. 2016; Kohchi et al. 2021; Li et al. 2020; Naramoto et al. 2022; Rensing et al. 2020; Rensing et al. 2008). Notably, MTs play an essential role in intracellular organization, cell division, and development throughout the life cycle (Naramoto et al. 2022; Wu et al. 2018; Yamada and Goshima 2017; Yi and Goshima 2018). Future endeavors will be made toward identifying MT regulators and generating a molecular network that organizes MTs to accomplish distinct processes. In addition, an increasing number of studies suggest crucial roles of MTs in bryophyte adaptation to land environments. For example, desiccation causes a dramatic decrease in MT abundance surrounding the chloroplasts in moss gametophores (Wang et al. 2009) and food-conducting cells (Pressel et al. 2006). The disruption of MTs prevents rehydration and likely plant survival on dry lands (Pressel et al. 2006). Although our understanding of MTs in environmental adaptation is still fragmented, future studies could benefit from the development of new tools for MT imaging and the use of various bryophyte models for MT study. For example, recently modified imaging systems enable optimal analysis of MT organization under distinct conditions (Bascom Jr. et al. 2016; Hiwatashi and Murata 2023; Kozgunova and Goshima 2019; Yoshida and Kozgunova 2023). Some of the bryophyte species such as the moss *Syntrichia caninervis* can tolerate severe water loss and survive under extreme conditions (Li et al. 2024); the amphibious liverwort *Riccia fluitans* can live in either a water-submerged form or a land form (Althoff et al. 2022). Research on these distinct species can provide valuable insight into MT-mediated environmental adaptation during land colonization.

Author Contributions

Conceptualization: P.Y. Drafting: Z.Y., Y.G., Y.C., E.K., and P.Y. Figure preparation: P.Y.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All data used in this study is available within the manuscript.

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