Mechanisms of microtubule organization in differentiated animal cells

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Abstract | Microtubules are polarized cytoskeletal filaments that serve as tracks for intracellular transport and form a scaffold that positions organelles and other cellular components and modulates cell shape and mechanics. In animal cells, the geometry, density and directionality of microtubule networks are major determinants of cellular architecture, polarity and proliferation. In dividing cells, microtubules form bipolar spindles that pull chromosomes apart, whereas in interphase cells, microtubules are organized in a cell type-specific fashion, which strongly correlates with cell physiology. In motile cells, such as fibroblasts and immune cells, microtubules are organized as radial asters, whereas in immotile epithelial and neuronal cells and in muscles, microtubules form parallel or antiparallel arrays and cortical meshworks. Here, we review recent work addressing how the formation of such microtubule networks is driven by the plethora of microtubule regulatory proteins. These include proteins that nucleate or anchor microtubule ends at different cellular structures and those that sever or move microtubules, as well as regulators of microtubule elongation, stability, bundling or modifications. The emerging picture, although still very incomplete, shows a remarkable diversity of cell-specific mechanisms that employ conserved building blocks to adjust microtubule organization in order to facilitate different cellular functions.

Centrosome
A non-membranous organelle which consists of two centrioles surrounded by a proteinaceous matrix that nucleates and anchors microtubules.

Axon
A neuronal compartment that transmits signals.

Dendrites
Neuronal compartments that receive signals.

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Microtubules are long hollow cytoskeletal tubes built from asymmetric subunits, dimers of α-tubulin and β-tubulin. Head-to-tail association of tubulin dimers leads to the formation of polarized microtubule polymers that have two functionally distinct ends — the minus ends, which are often stabilized and anchored at specific cellular sites, and the plus ends, which can grow and shrink rapidly and account for the formation of most microtubule mass. Microtubules can be tens or even hundreds of micrometres long, spanning the length of an average animal cell. Kinesin and dynein motor proteins can walk along microtubules and transport different cargoes. Each motor typically moves on microtubules only in one direction — towards the plus or the minus end. Therefore, the geometry and directionality of microtubules determine the patterns of intracellular transport and guide organelle positioning. Moreover, growing and shrinking microtubules can exert forces that can displace subcellular structures, such as chromosomes during mitosis. Furthermore, bundles of microtubules can withstand compressive forces, and densely packed microtubules contribute to shaping long cell protrusions, such as those in neurons[1], support the disc-like cell shape in platelets and non-mammalian red blood cells[2] or counteract contraction, for example, in heart cells[3]. The shape and organization of microtubule networks thus play a major role in controlling cell morphology, polarity, internal architecture and motility.

Most of our knowledge about microtubule organization and function is derived from studying dividing cells in two-dimensional cultures. These cells form radial, centrosome-anchored microtubule networks during interphase. This led to the 'textbook view' of an animal cell with an aster-like centrosome-driven microtubule organization. However, even in cultured fibroblasts and cancer cells, a significant proportion of microtubules are not attached to the centrosome, and the majority of differentiated animal cells have non-centrosomal microtubule arrays with geometries and densities adapted to the physiology of a particular cell type. For example, in epithelial cells many microtubules run from the apical to basolateral membranes to facilitate polarized transport. Similarly, the axon and dendrites of neurons display different microtubule organizations, with the former having a uniform network of microtubules with the plus ends pointing away from the cell body and the latter featuring many minus end-out microtubules.

The mechanisms underlying the formation and maintenance of such non-centrosomal microtubule

Centrioles
Cylindrical structures with a core of nine microtubule triplets. A centrosome contains two orthogonally arranged centrioles, the mother and the daughter centriole. A mother centriole is assembled one cell cycle earlier than the daughter centriole.

networks are still poorly understood, because microtubule arrays in three-dimensional tissues are much more difficult to visualize and probe. Recent advances in light and electron microscopy and new methods to manipulate protein expression in tissues have made the cytoskeleton in differentiated animal cells accessible to mechanistic studies. In addition, recent cell biological studies have provided key insights into the molecular mechanisms of microtubule nucleation, growth and stabilization. Together, these developments are beginning to reveal how microtubule networks with different geometry, density and stability can be generated by spatial control over microtubule nucleation, minus-end anchoring, plus-end dynamics, severing, sliding, stabilization and bundling by microtubule-associated proteins (MAPs). Here, we review these developments, distil the key principles underlying the formation of specialized microtubule arrays and, then, describe how these principles are used to build microtubule networks that support specific functions in the most abundant cell types in our body — epithelial, neuronal and muscle cells.

Microtubule nucleation and anchoring

The geometry of microtubule networks critically depends on the localization of the sites of microtubule nucleation and anchoring. These sites are traditionally called microtubule-organizing centres (MTOCs), in analogy with centrosomes that organize microtubules within interphase radial microtubule arrays or at the poles of a mitotic spindle[4]. In differentiated cells, however, MTOCs often lack typical centrosome components such as centrioles and may not have an appearance of 'centres' but, rather, occupy large surfaces, for example, the nuclear envelope or areas of the cell cortex. MTOC function depends on microtubule nucleation factors and proteins that stabilize and capture microtubule minus ends.

Microtubule nucleation

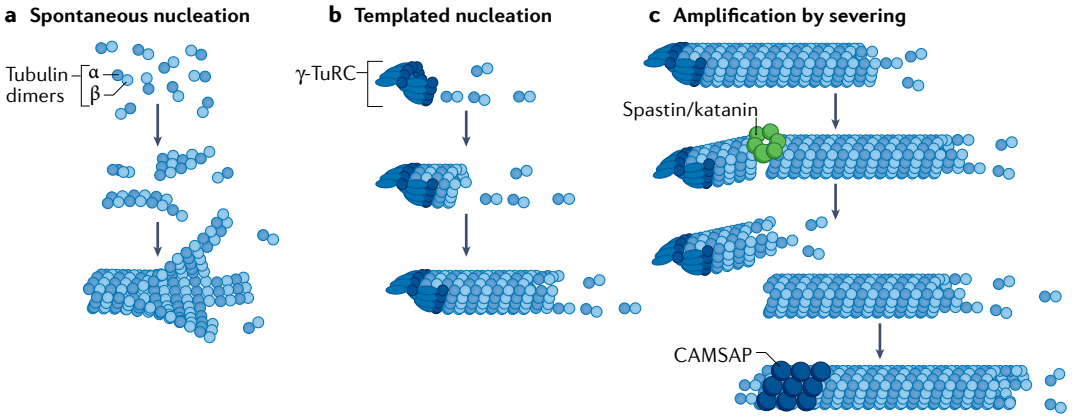
Although microtubules can form from tubulin dimers spontaneously, microtubule formation occurs much faster in the presence of a template that can bring together and position tubulin dimers (BOX 1). A template for de novo formation of microtubules used in most systems

Box 1 | **Microtubule nucleation and amplification**

Microtubules can be nucleated spontaneously, in solutions of purified tubulin in the presence of GTP (reviewed elsewhere[5]). If the concentration of αβ-tubulin dimers is sufficiently high, they can associate into oligomers that can increase in size and, eventually, form a tube (see the figure, part **a**), but the exact nature of intermediate structures is unknown[5]. Factors that can stabilize lateral or longitudinal interactions between tubulin dimers can promote the formation and growth of tubulin oligomers and, thus, stimulate spontaneous microtubule nucleation[5]. Such factors include various microtubule-associated proteins (MAPs), microtubule-stabilizing agents such as Taxol and the GTP analogue GMPCPP, which inhibits GTP hydrolysis by β-tubulin and the associated microtubule-destabilizing conformational changes in tubulin dimers.

An alternative, kinetically more favourable pathway of de novo microtubule formation is outgrowth from an existing template. The major player in this process is the γ-tubulin ring complex (γ-TuRC), which consists of 14 subunits of γ-tubulin that are held in a conical structure by γ-tubulin complex proteins (GCPs) and additional factors. γ-TuRC serves as a microtubule template by positioning tubulin dimers next to each other to initiate microtubule growth (see the figure, part **b**). A flurry of recent structural and biochemical studies revealed the detailed organization of this complex[205–207]. This work showed that γ-TuRC has an asymmetric structure that does not fully match the geometry of the microtubule, which might explain why purified γ-TuRCs are not very efficient microtubule nucleators. Different γ-TuRC-binding proteins and MAPs can increase γ-TuRC nucleation activity and, in this way, control where microtubules are formed in cells (reviewed in REFS[5,6]). The mechanisms of γ-TuRC activation are currently unclear but may involve the adjustment of γ-TuRC conformation so that it would better fit the 13-protofilament microtubule structure.

Finally, pieces of pre-existing microtubules can serve as templates for microtubule outgrowth. Therefore, microtubule severing by the enzymes spastin and katanin, followed by stabilization of the resulting microtubule fragments, provides an efficient way to increase microtubule numbers and, thus, microtubule density. New microtubule ends generated by severing enzymes can be stabilized by the enzymes themselves[208–210], by incorporation of GTP-bound tubulin[113] and by additional MAPs, such as the minus end-stabilizing proteins of the CAMSAP family, which can directly bind to katanin[26] (see the figure, part **c**).



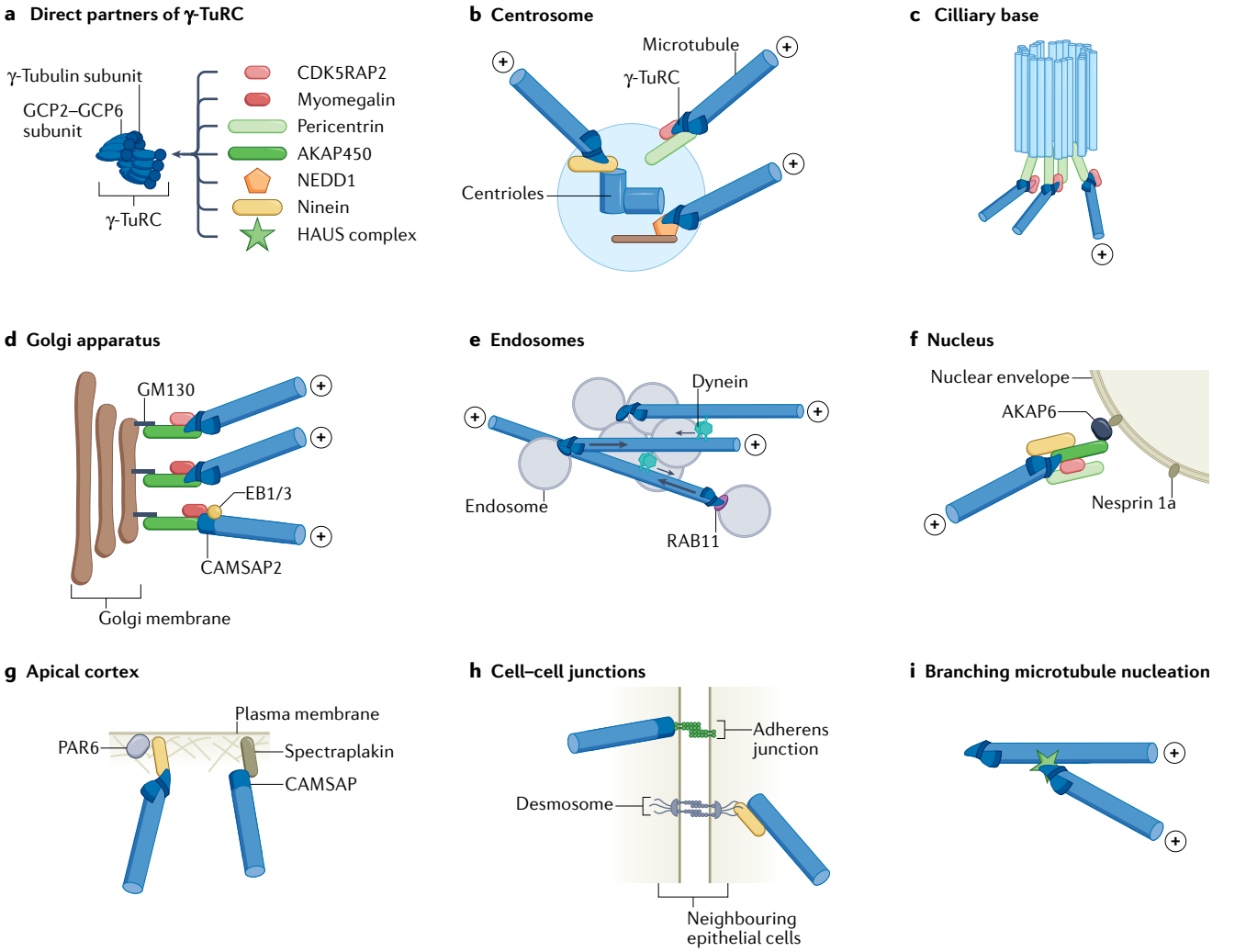


Fig. 1 | **Microtubule-organizing centres found in differentiated animal cells. a** | Key interactors of the γ-tubulin ring complex (γ-TuRC). **b** | Centrosome, the major microtubule-organizing centre (MTOC) in dividing animal cells, with a pair of centrioles surrounded by γ-TuRC-containing pericentriolar material (PCM). Major pathways of γ-TuRC recruitment to the centrosome are through a complex of CDK5RAP2 and pericentrin, through NEDD1 that binds to CEP192 or through ninein. **c** | Ciliary base as an MTOC found at dendrite tips in some neurons in *Caenorhabditis elegans*. Contains worm counterparts of pericentrin and CDK5RAP2. **d** | Golgi apparatus as an MTOC. Depends on the adaptor protein AKAP450, which is recruited by the Golgi matrix protein GM130. AKAP450 binds to γ-TuRC directly, or through CDK5RAP2 or its homologue myomegalin. The complex of AKAP450, myomegalin, EB1/EB3 and CAMSAP2 can also anchor and stabilize microtubule minus ends independently of γ-TuRC at the Golgi. **e** | Endosome cluster as an MTOC found at dendrite tips in some neurons in *C. elegans*. Formed by Rab11-positive endosomes, which tether γ-TuRC and are clustered by cytoplasmic dynein. **f** | Nuclear envelope as an MTOC in muscle cells. γ-TuRC is recruited by a complex of CDK5RAP5 and AKAP450, which through the scaffolding protein AKAP6 interacts with nesprin 1a at the nuclear envelope. Ninein and pericentrin are also involved and also recruited by AKAP450. **g** | Cell cortex as an MTOC. At the cortex of epithelial cells in *C. elegans*, minus ends can be tethered with participation of γ-TuRC and ninein, which is recruited by Par6. An alternative pathway, present in different animal species, includes CAMSAP and spectraplakin, which is bound to the cortical actin–spectrin cytoskeleton. **h** | Microtubule minus-end anchoring at adherens junctions and microtubule attachment to desmosomes occurring via CAMSAP3 and ninein, respectively. **i** | Branching microtubule nucleation. The HAUS complex recruits γ-TuRC to the microtubule surface to nucleate new microtubules, thereby increasing microtubule density. GCP, γ-tubulin complex protein.

is the γ-tubulin ring complex (γ-TuRC) (BOX 1). The ability of γ-TuRC to nucleate microtubules is modulated by γ-TuRC-binding proteins also known as tethering factors, and by MAPs (reviewed elsewhere[5,6]). Binding of γ-TuRC interactors, such as CDK5RAP2 and its homologue myomegalin, pericentrin, AKAP450 and HAUS (also known as augmin) (FIG. 1a; TABLE 1), can stimulate the microtubule nucleation activity of γ-TuRC or position it at specific subcellular sites. γ-TuRC-binding proteins are abundant constituents of the pericentriolar material (PCM) — a proteinaceous matrix surrounding the centrioles in interphase and mitotic centrosomes in dividing cells[6,7]. In differentiated cells, some PCM components are redistributed to other locations, converting them into MTOCs.

When γ-TuRC is depleted, both centrosomal and non-centrosomal microtubules can still form in some cell types[8–11]. Moreover, in some cell types, for example in *Drosophila melanogaster* fat body cells and *Caenorhabditis elegans* embryonic intestinal epithelial cells, microtubules remain robustly organized when γ-TuRC is depleted[12,13]. γ-TuRC-independent nucleation

Table 1 | **Main factors regulating microtubule nucleation and minus-end organization in differentiated animal cells**

Human protein	Function	Paralogues in mammals	Alternative names in mammals	Counterpart in *Caenorhabditis elegans*	Counterpart in *Drosophila melanogaster*
CDK5 (cyclin-dependent kinase 5) regulatory subunit-associated protein 2 (CDK5RAP2)	Activates γ-TuRC and tethers it to PCM and other structures through PCNT and AKAP450	CDK5RAP2 Myomegalin	Centrosomal protein of 215 kDa (CEP215)	Spindle-defective protein 5 (SPD5)	Centrosomin (Cnn)
Myomegalin (MMG)	CM-MMG isoform tethers γ-TuRC, EB-MMG isoform interacts with EB1/EB3 and CAMSAP2 and binds to MT plus and minus ends	CDK5RAP2 Myomegalin	Phosphodiesterase 4D-interacting protein (PDE4DIP), cardiomyopathy-associated protein 2	SPD5	Centrosomin (Cnn)
Pericentrin (PCNT)	Major PCM component, tethers γ-TuRC directly and indirectly		Kendrin	Pericentriolar matrix deficient 1 (PCMD1)	Pericentrin-like protein (Plp)
A-Kinase anchor protein 450 kDa (AKAP450)	Tethers γ-TuRC directly and indirectly, tethers MT minus ends through CAMSAP2, MMG and EB1/EB3		A-kinase anchor protein 9 (AKAP9), AKAP350, CG-NAP, Hyperion, Yotiao	Not identified	Centrosome protein of 309 kDa (CP309)
Neural precursor cell expressed developmentally downregulated protein 1 (NEDD1)	Tethers and regulates γ-TuRC		γ-Tubulin complex protein with WD repeats (GCP-WD), GCP7	Not present	γ-Ring protein with WD repeats (Dgp71WD)
Centrosomal protein of 215 kDa (CEP192)	Major PCM component, indirectly tethers γ-TuRC			Spindle-defective protein 2 (SPD2)	Spd2
Ninein	Tethers and stabilizes MT minus ends, possibly together with γ-TuRC	Ninein Ninein-like protein	Nlp, ninein-like protein (NINL)	Non-centrosomal microtubule array protein 1 (NOCA1)	Blastoderm-specific gene 25D (Bsg25D)
Human augmin complex (HAUS)	Right-subunit complex, binds γ-TuRC and mediates branching MT nucleation			Not present	Augmin
Calmodulin-regulated spectrin-associated protein (CAMSAP)	Stabilizes uncapped MT minus ends	CAMSAP1 CAMSAP2 CAMSAP3	Nezha (CAMSAP3) Marshalin (CAMSAP3)	Patronin (microtubule-binding protein) homologue (PTRN1)	Patronin
Spectraplakin	Cross-links MTs and actin, interacts with proteins that bind to plus and minus ends of microtubules	Microtubule actin cross-linking factor 1 (MACF1) MACF2	Actin cross-linking family protein 7 (ACF7, MACF1) Trabeculin-α (MACF1) Dystonin (DST, MACF2) Bullous pemphigoid antigen 1 (BPAG1, MACF2)	Variable abnormal morphology 10 (VAB10)	Short stop (Shot)
Cytoplasmic linker protein (CLIP)-associated protein (CLASP)	Stabilizes dynamic MTs by preventing catastrophes and promoting rescues, promotes nucleation	CLASP1 CLASP2		Three paralogues of CLASP family of microtubule-binding proteins: CLS1 CLS2 CLS3	Orbit/Mast (Chromosome bows (Chb))
Colonic and hepatic tumour overexpressed gene (chTOG)	Accelerates MT growth at the plus end, promotes MT nucleation		Cytoskeleton-associated protein 5 (CKAP5) Often cited by the name of the *Xenopus* orthologue Microtubule-associated protein of 215 kDa (XMAP215)	Zygote defective 9 (ZYG9)	Mini spindles (Msps)
End binding protein (EB)	Binds to the GTP cap at growing MT ends, recruits numerous other proteins, participates in minus-end regulation	EB1 EB2 EB3	Microtubule-associated protein RP/EB family member 1 (MAPRE1, EB1) RP1, MAPRE2, EB2 EBF3, MAPRE3, EB3	Three paralogues of microtubule EB: EBP1 EBP2 EBP3	Eb1 Three additional related proteins (CG18190, CG15306, CG2955)

GCP, γ-tubulin complex protein; PCM, pericentriolar material; γ-TuRC, γ-tubulin ring complex.

Condensates
Membraneless structures which can form by the physical process of liquid–liquid phase separation, whereby a well-mixed solution of macromolecules such as proteins or nucleic acids spontaneously separates into two phases, a dense phase and a dilute phase.

Axoneme
A microtubule-based cytoskeletal structure that forms the core of a cilium or a flagellum; it contains nine microtubule doublets, and in motile cilia also a central pair of microtubules

may rely on the ability of some MAPs, such as XMAP215 (also known as chTOG) or TPX2, to promote spontaneous microtubule assembly by locally concentrating tubulin, possibly through formation of protein condensates[14,15], or by stabilizing early nucleation intermediates and promoting their growth[16,17]. Good candidates for the latter function are doublecortin, which can stabilize tubulin oligomers[17], and CLASP, a protein that helps convert different incomplete microtubule structures into complete, growing tubes[18]. Altogether, γ-TuRC-independent pathways likely contribute to microtubule nucleation in certain cell types where γ-TuRC expression is low, such as fly fat body cells[13], but γ-TuRC seems to be the kinetically dominant microtubule nucleator in most systems.

Minus-end stabilization and anchoring

In addition to nucleation, another important function of an MTOC is stabilization and anchoring of microtubule minus ends. This function can be carried out by γ-TuRC, which caps microtubule minus ends, together with γ-TuRC activation factors discussed above. An additional protein with a specific role in minus-end anchoring in a broad variety of systems is ninein, which works in conjunction with γ-TuRC through still unclear biochemical mechanisms[11–13,19–23]. An alternative minus-end stabilization pathway is mediated by the members of the CAMSAP/Patronin family (TABLE 1). These proteins bind and stabilize uncapped microtubule ends in a minus end polymerization-dependent manner[24–26]. CAMSAP, similar to other MAPs such as tau[27], can promote de novo microtubule assembly in vitro when its concentration is sufficiently high to form condensates[28]. However, it is unclear whether such concentrations are encountered in cells, and most of the cell biological evidence points to a function of CAMSAPs in stabilizing and tethering the minus ends of microtubules released from the nucleation sites, for example at the Golgi membranes or the cell cortex[24,29–32] (reviewed elsewhere[33]). Importantly, CAMSAPs directly interact and cooperate with the microtubule-severing enzyme katanin[26]. Synergistic action of these proteins can lead to the amplification of acentrosomal microtubules through their detachment from nucleation sites, which can be followed by local capture of stabilized microtubule minus ends[34,35] (BOX 1, see the figure, part **c**).

γ-TuRC-dependent and CAMSAP-dependent mechanisms of microtubule minus-end stabilization and organization can work in parallel, for example, on the Golgi membranes[29] or the apical membranes in epithelial cells[11]. Interestingly, the relative importance of the different pathways is very cell type-dependent — in worms, either the CAMSAP orthologue PTRN1 or the ninein-related protein NOCA1 must be present for proper development of larval epidermis[11], yet in intestinal epidermis, simultaneous loss of PTRN1, NOCA1 and a γ-TuRC component has no strong effect on microtubule organization[12]. It is thus likely that additional pathways of microtubule minus-end organization exist in some tissues.

Cell type-specific MTOCs

The most canonical type of MTOC is the centrosome (FIG. 1b), which can generate microtubules that may be either retained or released, followed by transport and capture elsewhere. During cell differentiation, centrosomes are often inactivated through the loss of PCM components[36,37]. This can occur due to alterations in protein expression or cyclin-dependent kinase signalling[38], or, in the case of ninein in neuronal cells, by switching from a centrosomal to a non-centrosomal splice isoform[39]. Differentiated cells adopt diverse non-centrosomal microtubule configurations, which rely on the formation of a broad variety of MTOCs.

Cells that can move, contract or change their shape typically employ organelle-associated MTOCs, some of which, such as the Golgi membranes or endosomes, can cluster through dynein-mediated minus end-directed transport[40,41]. Such MTOCs can support polarized but dynamic microtubule organization, which can be adjusted during development or cell movement. In static cells, such as epithelia, microtubules are often attached to the cell cortex and generate microtubule networks that are polarized by external cues. At the molecular level, MTOCs display a remarkable diversity, with almost every differentiated cell type studied in some depth demonstrating some original features, as highlighted below, although this view may partly stem from the incomplete understanding of MTOC composition in most systems.

Ciliary base and basal bodies. Whereas the centrosome is often inactivated during cell differentiation, in cells with motile or primary cilia, the mother centriole is repurposed as the ciliary basal body. At the early stages of ciliogenesis, the centrosome retains its MTOC function to facilitate the transport of ciliary components (reviewed elsewhere[42]) or promote formation of the microtubule bundle that repositions the centrosome to the cell surface in order to initiate cilia formation[43]. An interesting case of a cilia-associated MTOC is presented by the sensory neurons of *C. elegans*, which contain a single non-motile cilium at the distal tip of the dendrite: the basal body of this cilium is degraded, but the ciliary base accumulates PCM that extends from the luminal side of the axoneme and nucleates dendritic microtubules that grow towards the nucleus (FIG. 1c). The function of this PCM depends on γ-TuRC as well as the worm counterparts of CDK5RAP2 and pericentrin[44,45].

In multiciliated cells, centrioles are amplified and these newly produced centrioles form basal bodies for ciliary axonemes[46]. Basal bodies, through their appendages called basal feet, organize microtubules at the apical side of the cell[47]. A subcortical microtubule network surrounding and interconnecting the basal bodies ensures their polarized orientation and synchronous beating[47,48]. PCM can participate in centriole formation in such cells[49] and likely also contributes to cortical microtubule nucleation. Both γ-TuRC and the minus end-stabilizing protein CAMSAP3 associate with the basal bodies in a polarized manner, and CAMSAP3 is required for organizing the apical microtubule network[50,51].

Golgi complex and Golgi outposts. In many types of cycling as well as differentiated cells, the Golgi complex serves as a major MTOC (FIG. 1d), which is required for cell polarization, for organization of secretory trafficking

End binding proteins
(EBs). Conserved proteins that specifically bind to growing microtubule ends because they preferentially associate with the microtubule lattice in which β-tubulin is bound to GTP.

Dendritic arborization neurons
Neurons of a larval sensory type in *Drosophila* with specific dendritic morphologies.

Radial glial cells
Progenitor cells responsible for producing neurons of the cerebral cortex.

Oligodendrocytes
Myelinating glia cells of the central nervous system.

Growth cone
An actin-supported extension of a developing or regenerating axon or dendrite.

Fat body
An insect organ distributed throughout the body that has an essential role in energy storage and utilization.

and also for dynein-driven self-assembly of the Golgi ribbon[40,52]. In cultured mammalian cells, the central player in microtubule organization at the Golgi is the scaffolding protein AKAP450, which binds to the *cis*-Golgi matrix component GM130 and can recruit γ-TuRC both directly and indirectly, through CDK5RAP2 and myomegalin[29,53,54]. In a separate pathway, AKAP450 acting together with myomegalin and end binding proteins (EBs) binds CAMSAP2-stabilized microtubule minus ends[29,55]. Another important player in Golgi microtubule organization is CLASP, which interacts with the Golgi membranes through the *trans*-Golgi protein GCC185 (REF.[56]), but may also promote γ-TuRC-mediated nucleation and stabilization of CAMSAP2-bound microtubule minus ends directly[29]. Whether these pathways are conserved is unclear: in *D. melanogaster*, γ-tubulin was shown to be present on Golgi in the soma of neurons, where it participates in generating axonal microtubules, but this process does not depend on the orthologues of pericentrin, AKAP450 or CDK5RAP2 (REF.[57]).

In addition to a centrally located Golgi complex, smaller, distally located membrane compartments bearing Golgi markers, termed Golgi outposts, can serve as MTOCs to organize complex and branched microtubule networks in large cells such as neurons, muscle and glia cells (reviewed elsewhere[58]). In muscles, Golgi outposts supporting orthogonal microtubule grids contain γ-tubulin, AKAP450 and pericentrin[59,60]. In fly dendritic arborization neurons, Golgi outposts were proposed to nucleate dendritic minus end-out microtubules in a manner dependent on γ-tubulin and the fly orthologues of AKAP450 and CDK5RAP2 (REFS[61,62]). However, subsequent work showed that elimination of the two latter proteins does not affect dendritic microtubule polarity[63], and motor-driven relocation of Golgi outposts from dendrites did not remove γ-tubulin from dendrite branch points[64]. These data indicate that alternative pathways of γ-TuRC regulation exist in fly dendrites (see below).

In the extended processes of radial glial cells, outgrowth of microtubules organized in antiparallel arrays occurs from varicosities that are not enriched in γ-TuRC but contain Golgi compartments and CAMSAPs. This suggests that Golgi membranes might capture CAMSAP-stabilized microtubule minus ends that could be generated by severing microtubules nucleated in other cell regions[30]. In oligodendrocytes, Golgi outposts show no γ-tubulin enrichment either, but instead accumulate the protein TPPP that might autonomously promote microtubule nucleation[65] (see BOX 1 for potential mechanisms), illustrating the diversity of Golgi-associated microtubule organization pathways.

Endosomes. Although the role of Golgi outposts in organizing minus end-out microtubules in neuronal dendrites in *D. melanogaster* is contested, recent evidence points to the involvement of the endosomal compartments. In dendrites of fly neurons, γ-TuRC was shown to localize to early, Rab5-positive endosomes with participation of the components of the Wnt signalling pathway[66]. Furthermore, during development of certain sensory neurons of *C. elegans*, Rab11-positive endosomes form a γ-TuRC-containing MTOC in the dendritic growth cone[41] (FIG. 1e). This MTOC is formed by dynein-mediated clustering and moved to the dendritic tip by kinesin 1 (REF.[41]). The ability of recycling, Rab11-positive endosomes to associate with γ-TuRC is conserved in vertebrate cells, where these endosomes contribute to spindle formation and pericentrin accumulation at the centrosome[67,68].

Mitochondria. An interesting example of an organelle-based MTOC is provided by *D. melanogaster* spermatids. Here, γ-TuRC is bound to giant mitochondria through a testes-specific isoform of the CDK5RAP2 orthologue centrosomin (TABLE 1), organizing microtubules that support elongation of sperm cells[69]. Whether mitochondria contribute to microtubule nucleation in other contexts is currently unknown.

Nuclear envelope. Nuclei can serve as robust MTOCs to generate microtubules that control nuclear positioning as well as overall cell architecture. In mammalian heart and skeletal muscle cells, and in osteoclasts, this function relies on a specific isoform of the nuclear envelope protein Nesprin 1, which acts together with the spectrin repeat-containing adaptor AKAP6 to recruit AKAP450 and pericentrin that, in turn, bind to γ-TuRC to nucleate microtubules[60,70] (FIG. 1f). The AKAP6–AKAP450 interaction also leads to a tight association between the nucleus and Golgi membranes, which act together in microtubule organization with the participation of ninein[70], whereas no function for CAMSAPs has been reported in muscle cells or osteoclasts. A different type of nuclear MTOC is assembled in *D. melanogaster* fat body cells: it contains an orthologue of nesprin, a spectraplakin (a microtubule and actin cross-linking protein with multiple spectrin repeats) and the fly counterparts of CAMSAP, ninein and XMAP215, but not γ-TuRC[13]. In this system, microtubule nucleation seems to occur in a γ-TuRC-independent manner.

Cell cortex. In polarized epithelial cells, microtubules are typically organized vertically, with the plus ends directed towards the basal side, and the minus ends linked to the apical cortex or to cell–cell junctions. Apical microtubule organization depends on the major polarity regulators, PAR3, PAR6 and aPKC[71–73]. Recent work showed that in *C. elegans* epidermis cells, Par6 directly binds the ninein orthologue NOCA1 (REF.[74]) (FIG. 1g). NOCA1 participates in the formation of apical MTOCs together with γ-TuRC, but their exact contribution depends on the cell type and the NOCA1 isoform[11,12,74]. In worm epidermis, Par6 also regulates the CAMSAP orthologue PTRN1 (REF.[74]), which acts in a γ-TuRC-independent pathway of apical minus-end organization[11]. Similarly, in mammalian epithelia, apical MTOCs contain the CAMSAP family member CAMSAP3, ninein and γ-TuRC[20,31,75]; whether PAR proteins directly associate with these factors in mammals is unclear. Binding of CAMSAP3-decorated microtubule minus ends to the apical cortex depends on its interaction with spectraplakin[31]. This complex is conserved in flies, where it was shown to involve spectrin, a large actin-binding binding cortical scaffold[32,76].

In fly salivary glands, the orthologues of CAMSAP, spectraplakin and the severing enzyme katanin act together to release microtubules from the centrosome and promote their capture at the apical cortex[35]. The orthologue of spectraplakin as well as a conserved protein WDR62, which regulates katanin during mammalian cell division[77,78], also participate in the formation of apical MTOCs in worm intestine[73]. Furthermore, mammalian CAMSAP3 can also directly link microtubule minus ends to adherens junctions[24], whereas ninein can connect microtubules to desmosomes in skin cells by binding to desmoplakin[22] (FIG. 1h). Altogether, CAMSAP and ninein define two key pathways of cortical minus-end attachment in epithelial tissues throughout the animal kingdom.

Microtubule branches. Microtubules can serve as platforms for nucleating new microtubules: the γ-TuRC-binding protein complex HAUS, acting together with TPX2, leads to formation of microtubule 'branches' that emerge at very shallow angles, resulting in amplification of parallel microtubule arrays[79] (FIG. 1i). Branching nucleation is important for the formation of both axonal and dendritic microtubule arrays in mammalian and fly neurons[61,80,81] and contributes to neuronal activity-dependent microtubule nucleation in presynaptic boutons[82]. Whether augmin participates in microtubule nucleation in differentiated cells other than neurons is unclear.

Cytokinetic bridges and mid-body remnants. During cytokinesis, the central spindle is transformed into a mid-body with two antiparallel overlapping bundles of densely packed microtubules, which, together with associated vesicles, guide abscission of daughter cells to complete cell division. However, during early stages of mouse embryonic development, the cytokinetic bridge is retained and, through the minus end-stabilizing activity of CAMSAP3, is converted into an acentriolar MTOC. This MTOC is required for polarized transport of adhesion molecules and establishment of polarity in the daughter cells[83]. The mid-body remnant, which is asymmetrically inherited by one daughter cell, can also participate in primary cilia formation by promoting concentration and trafficking of ciliary components in polarized epithelial cells (reviewed elsewhere[84]).

Microtubule dynamics and stabilization

Whereas the distribution and activity of MTOCs define the overall geometry of microtubule networks, processes that control microtubule dynamics, stabilization, bundling and sliding further determine microtubule length, density and organization.

Microtubule growth and shrinkage

Microtubules can grow from their plus and minus ends by adding tubulin dimers. Both α-tubulin and β-tubulin bind GTP, and whereas the GTP molecule bound to α-tubulin is not hydrolysed, the GTP bound to β-tubulin is hydrolysed after the dimer is incorporated into the microtubule lattice. As a result, a cap of subunits containing GTP-bound β-tubulin (GTP-tubulin) is present at the growing microtubule ends, whereas the rest of the microtubule shaft contains GDP-tubulin[85–87] (FIG. 2a). GTP hydrolysis and subsequent phosphate release, which is thought to occur with some delay, trigger conformational changes in tubulin that lead to complex, still incompletely understood, structural transitions that destabilize the microtubule structure[88–90]. Therefore, if the GTP cap at the microtubule tip is lost, a microtubule switches to shrinkage[85–87]. Growth of microtubule plus ends (the ends where β-tubulin is exposed) is intrinsically faster than growth of the minus ends, and is therefore responsible for generating most of the microtubule mass. Microtubule plus-end growth is specifically accelerated by the microtubule polymerase XMAP215 (REF.[91]). Microtubule minus-end polymerization can also be physiologically important, because CAMSAP-mediated stabilization of minus ends depends on their growth[26]. Interestingly, robust minus-end elongation was observed in fly and zebrafish neurons, where the fly orthologue of CAMSAP was shown to be required for populating neuronal dendrites with microtubules that point with their minus ends away from the cell body[92].

Growth and shrinkage of microtubule plus ends are regulated by a plethora of factors that specifically bind to microtubule tips and either promote their growth and prevent depolymerization (such as XMAP215 or CLASP) or trigger microtubule shrinkage and pausing (such as members of the kinesin 4, 8 and 13 families) (reviewed elsewhere[87,93]). Robust growth increases microtubule density and allows microtubules to support cell protrusions[94] or remodel intracellular membranes such as the endoplasmic reticulum[95]. Inhibition of microtubule growth can be used to ensure that microtubules terminate at the cell margin[96], and depolymerase-controlled microtubule disassembly is needed to suppress or remove cell protrusions, for example, to inhibit axonal branching and promote axonal pruning during neuronal development[97,98].

Some isoforms of microtubule plus-end regulators are specifically expressed in differentiated cells. For example, among the three mammalian members of the EB family, EB1 predominates in dividing cells whereas EB3, the member with the highest affinity for microtubule tips, is upregulated in differentiated cells[99,100]. Another example is the kinesin 4 KIF21B, which is specifically expressed in neurons and immune cells, where it restricts microtubule growth[101,102]. In immune cells, KIF21B keeps microtubules short, so that the whole microtubule network can be quickly relocated during the formation of immunological synapse (see also section Microtubule network diversity in cells)[101]. However, the majority of factors controlling microtubule dynamics and local microtubule density are present in both dividing and differentiated cells, and the spatial and temporal control of their activities is often achieved through phosphorylation (for interesting examples from neuronal development, see REF.[103]).

Microtubule stabilization and bundling

Because microtubules are often highly dynamic and undergo frequent transitions between growth and shrinkage (catastrophes), as well as reverse transitions

Adherens junctions
Protein assemblies at cell–cell junctions in epithelial and endothelial cells; they contain transmembrane proteins called cadherins and are linked to the actin cytoskeleton.

Desmosomes
Adhesive protein complexes localized to intercellular junctions, responsible for maintaining the mechanical integrity of tissues.

Presynaptic boutons
Neurotransmitter-producing knoblike enlargements at the end of an axon involved in forming a synapse with another neuron.

Immunological synapse
The interface between an antigen-presenting cell or a target cell and a lymphocyte, such as a B cell or T cell, or a natural killer cell.

Catastrophes
Abrupt transitions from microtubule growth to shortening associated with loss of a GTP cap; they can occur spontaneously or be triggered by obstacles to microtubule growth or different cellular factors.

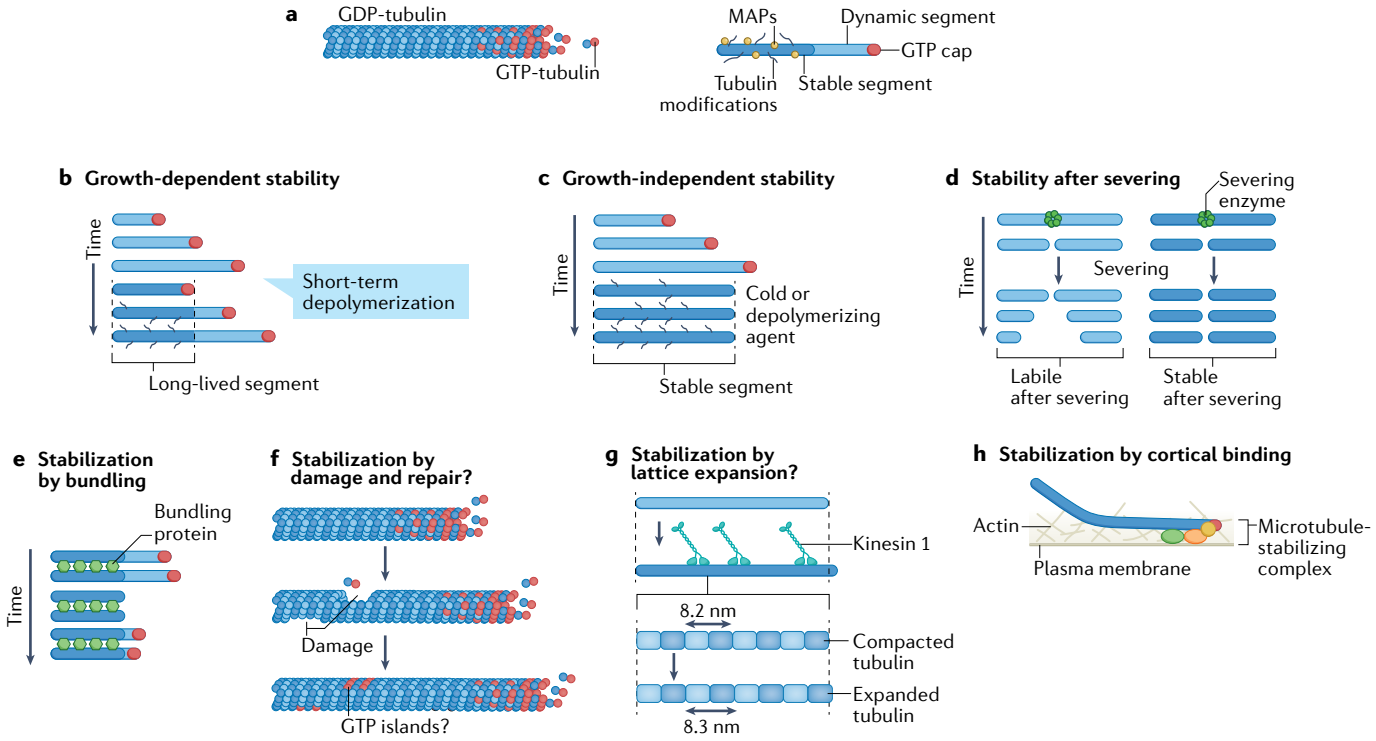


Fig. 2 | **Regulation of microtubule growth and stabilization. a** | Two representations of a growing microtubule with a cap of GTP-bound tubulin. Older parts of a microtubule can accumulate post-translational modifications, which can contribute to regulation of microtubule stability. Stability can also be achieved by binding of microtubule-associated proteins (MAPs). **b**–**d** | Definitions of microtubule stability: a microtubule segment can become long-lived even if its end is very dynamic if the depolymerization episodes are short and followed by regrowth, resulting in net extension of the microtubule (part **b**); some microtubules are stable and do not depolymerize even though they lack the stabilizing GTP cap (this typically occurs due to the action of specific MAPs) (part **c**); microtubules with stable microtubule lattices do not depolymerize after breakage or severing (part **d**). **e** | Some MAPs specifically bind to two or more microtubules to form bundles, which prevents shrinkage of microtubules. **f** | Loss of GDP-tubulin dimers from microtubule lattice ('damage') can lead to incorporation of new GTP-bound β-tubulin (GTP-tubulin) ('repair') and, perhaps, lattice stabilization. **g** | Kinesin 1 walking on a microtubule could have a stabilizing effect by converting the tubulin dimers in the lattice to an axially extended conformation. **h** | Cortical complexes can capture and stabilize microtubule plus ends.

(rescues), most microtubule mass turns over rapidly. As indicated above, growing microtubules are stabilized by their GTP caps, which are lost when microtubules switch to shrinkage[85]. Some microtubules can live longer than others, and these are often called stable microtubules. Importantly, there are multiple ways to define microtubule stability[104]. A microtubule that does not undergo catastrophes maintains its GTP cap and could be considered a stable microtubule, because its lattice has a long lifetime. However, microtubules that grow and shrink, and thus acquire and lose GTP caps, can also acquire long-lived segments if the average length gained during growth is greater than the length lost during shrinkage (FIG. 2b). In both cases, the stable, long-lived nature of the microtubule lattice depends on the behaviour of the microtubule plus end.

In a more stringent and widely used definition of stability, stable microtubules should persist also when the GTP cap is lost, for example, during depolymerization induced by cold or by small molecules, such as nocodazole, or after severing (FIG. 2c,d). In many cases, such GTP cap-independent microtubule stability depends on the presence of lattice-associated post-translational modifications (see the next section) or MAPs. In particular, MAP6 (or STOP, stable tubule only) is well known for protecting microtubules from cold or drug-induced depolymerization. Recent work revealed that MAP6 accumulates in a periodic pattern in the lumen of microtubules, which could contribute to stabilization, and induces them to coil[105]. Besides MAP6, many other MAPs can contribute to microtubule stability (for a recent review, see REF.[106]). Some of these MAPs also cause microtubule bundling, which explains why microtubule bundles are often more stable than individual microtubules[106]. An interesting example is TRIM46, a bundler of parallel microtubules that has a low affinity for individual microtubules (FIG. 2e). When this protein was added to a reconstitution experiment with dynamic microtubules, no shrinkage was detected in microtubules cross-linked by TRIM46 (REF.[107]). Recent work in *Drosophila* suggested that the fly orthologue of TRIM46, Trim9, has a similar role in neuronal dendrites in the fly, although in this case microtubule stabilization additionally requires cooperation with EB1 and the kinesin 5 KLP61F (REF.[108]).

Next to the ability to resist drug, protein or temperature-induced depolymerization, microtubule stability could be reflected in the response to mechanical stresses, such as compression or bending. Whereas forces exerted on microtubules can induce their depolymerization[109] or even breakage[110], the presence of MAPs or modifications can make microtubules more resilient and, thereby,

Rescues
Abrupt transitions from microtubule shortening to growth associated with regaining a GTP cap; these events are thought to be induced by cellular factors.

Axon initial segment
A plasma membrane-associated compartment at the base of an axon, which generates and shapes the action potential.

Focal adhesions
Integrin-containing multi-protein assemblies that establish mechanical links between intracellular actin bundles and the extracellular matrix.

Z-discs
Fine dense lines forming sarcomere boundaries, which stabilize actin filaments and allow force transmission between sarcomeres.

Sarcomeres
Basic contractile units of a muscle fibre; they contain actin and myosin filaments.

Gap junction
A channel that physically connects adjacent cells, mediating rapid exchange of small molecules.

increase their lifetime[110]. Furthermore, when microtubules do break upon bending or get severed by severing enzymes, newly generated ends could either quickly depolymerize or remain stable over time (FIG. 2d), and this stability likely correlates with the ability to resist other challenges. Nonetheless, it is important to discriminate the different aspects of microtubule stability discussed above, because this might help explain how a subset of microtubules becomes stabilized.

A recently proposed mechanism for microtubule stabilization is the incorporation of GTP-tubulin in existing lattices after the removal of GDP-tubulin — a process referred to as microtubule repair (reviewed elsewhere[111]) (FIG. 2f). In vitro experiments have shown that free tubulin can become incorporated into microtubules damaged by bending, severing enzymes or motor proteins[112–115]. The GTP-tubulin patches that result from this repair process could contribute to lattice stabilization, for example by providing rescue sites that halt depolymerization and promote growth[116,117]. For long-lasting effects this would require that the GTP does not get readily hydrolysed in those repair patches. Another potential mechanism for lattice stabilization is the conversion of GDP-tubulin from a compacted state to a slightly more expanded GTP-like state. Recent work has shown that kinesin 1 can induce this transition[118,119] (FIG. 2g). Remarkably, sub-stoichiometric decoration with these motors already results in microtubule elongation that would require conformational changes to the expanded state in the majority of tubulin dimers. This suggests that the kinesin-induced axial extension of individual tubulin dimers can be propagated to neighbouring subunits to induce more global changes in the lattice configuration. This, in turn, could promote the recruitment of additional motors and MAPs that preferentially bind microtubules with an expanded-type lattice and lead to further stabilization and modification.

Microtubule–cell cortex interactions

Both microtubule tips and shafts can be specifically tethered to the cell cortex through a large variety of molecular links that direct microtubule-based transport towards specific cortical sites or that control the mobility and positioning of microtubule arrays by promoting or counteracting motor-based sliding (see section Microtubule sliding and reorientation). In neurons, interactions of microtubules with the cortex can restrict microtubule mobility and promote polarized microtubule organization[120]. By providing a stable protein delivery route, these stabilized microtubule networks can generate positive feedback that promotes the formation and maintenance of membrane domains, such as the axon initial segment[107].

Similar to the mechanisms of cortical minus-end attachment described above, cortical tethering of microtubule shafts or plus ends can be achieved by direct coupling to the actin–spectrin cytoskeleton, for example, by microtubule–actin cross-linking proteins such as spectraplakin (reviewed elsewhere[121]). Furthermore, a cortical microtubule stabilization complex, which contains CLASP and several scaffolding proteins and is present in mesenchymal and epithelial cells, attaches microtubule plus ends to the vicinity of focal adhesions and coordinates adhesion with secretory trafficking to promote epithelial polarity or cell motility (reviewed elsewhere[122]) (FIG. 2h). This CLASP-containing complex is also present on the muscle side of neuromuscular junctions, where it controls delivery and organization of acetylcholine receptors[123]. Another large protein assembly present in muscle cells, the dystrophin-associated protein complex (DAPC), connects the Z-discs of the contractile units, sarcomeres, to the extracellular matrix as well as to subcortical microtubules and intermediate filaments; it serves as a hub for mechanotransduction and protects muscle cells from exercise-induced injury[124]. Dystrophin can bind to microtubules directly but also controls microtubule organization indirectly[125], and some additional components, such as liprin-α1, are shared between DAPC and the cortical microtubule stabilization complex[126]. Membrane localization of DAPC requires ankyrins, scaffolding proteins interacting with β-spectrin[127]. Ankyrins can connect to microtubules in other ways — for example, through EBs[128] or through the microtubule-binding protein CRMP[120].

Another major player in microtubule attachment to the cortex is the minus end-directed motor cytoplasmic dynein together with its accessory complex dynactin. For example, in cardiomyocytes, microtubule plus-end tethering through the complex of dynactin with EB1 was reported to guide transport of gap junction components to N-cadherin-positive adherens junctions[129]. However, the most important function of the cortical dynein–dynactin complex is to slide microtubules along the cortex or pull on their outermost plus ends and, in this way, centre or relocate microtubule networks (see section Microtubule sliding and reorientation).

Post-translational modifications

After polymerization, tubulin dimers within the microtubule lattice can undergo various post-translational modifications, including acetylation, detyrosination and polyglutamylation (for recent reviews, see REFS[130,131]). This creates a new dimension of biochemical microtubule heterogeneity and allows cells to diversify their network by creating different microtubule subsets that serve different functions. Post-translational modifications are strongly enriched in specialized compartments, such as cilia or axons. Naively, this suggests that the activity of the modifying enzymes is restricted to certain compartments and will result in a homogeneous modification of microtubules within that compartment. Nonetheless, high-resolution microscopy has revealed that different subsets of microtubules often coexist in the same compartment[132], which raises many questions about how these different microtubule subsets are established and maintained.

Because many tubulin modifications accumulate slowly and are not generally reversed within the microtubule lattice, they accumulate on older microtubules (FIG. 2b,c), and stable microtubules are therefore often highly modified[131]. Furthermore, some modifications also promote microtubule stability. Acetylation makes microtubules more resistant against mechanical wear, and thereby promotes microtubule longevity[110].

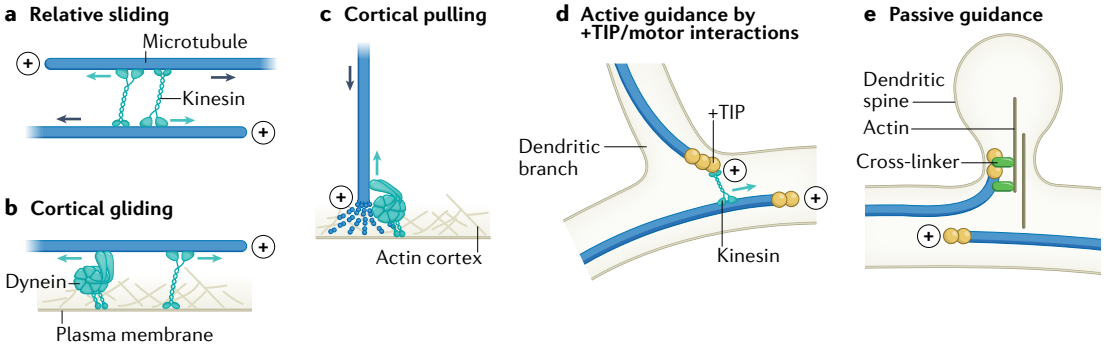


Fig. 3 | **Microtubule sliding and reorientation. a** | Motor proteins can organize microtubules by sliding them against each other. **b** | Motor proteins attached to cell cortex can organize microtubules by gliding. **c** | Dynein attached to cell cortex can pull on microtubule plus ends while they depolymerize and, in this way, affect positioning of entire microtubule networks, such as a mitotic spindle. **d** | Kinesin can guide growing microtubules by associating with plus end-tracking proteins (+TIPs) at their ends and walking along other microtubule tracks. **e** | Passive cross-linkers between microtubules and actin can redirect microtubule growth along actin filaments by associating with +TIPs.

Detyrosination reduces microtubule disassembly by kinesin 13 family members[133]. Increased levels of polyglutamylation promote binding of the severing enzymes spastin and katanin but reduce their activity[134]. As a result, severing is initially promoted upon increasing polyglutamylation, but inhibited for heavily polyglutamylated microtubules, such as those in cilia and centrioles. As such, highly polyglutamylated microtubules could also be protective for neighbouring microtubules, because they serve as a sink for severing enzymes.

The existence of different microtubule subsets also provides opportunities to create specialized transport routes for different cargoes. For example, kinesin 1 preferentially interacts with microtubules that are acetylated and detyrosinated[135,136], whereas kinesin 3 prefers dynamic, tyrosinated microtubules[136,137]. However, given the limited effects of microtubule modifications in experiments with purified components[138], these strong preferences are most likely not directly caused by these modifications but mediated by MAPs or microtubule lattice configurations[118,139].

The most direct evidence for important functional roles of microtubule modifications comes from studying genetic diseases caused by mutations in modifying enzymes or from mouse models in which these enzymes have been removed (see REF.[140] for a recent review). For example, hyperglutamylation of α-tubulin caused by depletion of the deglutamylase CCP1 results in reduced motility of many different microtubule-based cargoes in mouse model and infantile-onset neurodegeneration in humans[141–144]. Furthermore, knockout of the two enzymes that initiate tubulin glycylation (TTLL3 and TTLL8) causes male infertility in mouse because the lack of glycylation in the sperm tail axonemes perturbs sperm swimming, most likely through impaired dynein activity[145].

Microtubule sliding and reorientation

Microtubules can be redistributed or redirected in order to promote a specific organization of the cytoskeleton and allow its adaptation to dynamic cell processes and behaviours. In many cases, this involves the use of motor proteins that move entire microtubules, focus microtubule minus ends or redirect growing plus ends. In all these cases, the specific directionality of motors helps form a microtubule network with a well-defined organization. Motor-driven sliding of entire microtubules along each other contributes to the formation of bipolar mitotic spindles by pushing apart spindle poles (FIG. 3a), whereas dynein-dependent focusing of minus ends organizes the spindle poles. Moreover, dynein-mediated sliding of microtubules along the cortex (FIG. 3b) or pulling on their plus ends (FIG. 3c) is crucial for mitotic spindle positioning, which determines the cell division plane and thus directs the choice between proliferation and differentiation during tissue development (reviewed elsewhere[146]). Dynein-driven pulling at the plus ends also participates in centring[147] or relocation of interphase centrosomes. The latter process is important for the formation of immunological synapse in immune cells[148]. The molecular details of cortical dynein machinery in mitosis have been studied in great detail[146], and the corresponding interphase complexes are also starting to be revealed. For example, in fly axons, dynein-dependent sliding moves minus end out-oriented microtubules back to the cell body and thereby promotes a uniform plus end out-oriented microtubule array[149,150]. In this case, dynein is cortically anchored through the dynein adaptor Spindly[151].

Apart from driving cargo transport, the major plus end-directed motor, kinesin 1, can also drive motility of the microtubules themselves, either by cross-linking microtubules (sliding, reviewed elsewhere[152]) or, perhaps, by attaching to static structures (gliding) (FIG. 3a,b). This process causes cytoplasmic streaming in fly oocytes and promotes neurite initiation in fly neurons[153,154]. At branch points of fly dendrites, the uniform minus end-out microtubule orientation of dendrites is maintained by another member of the kinesin family, kinesin 2, which associates with growing plus ends to bias their growth towards the plus ends of pre-existing microtubules so that they would all point in the same direction[155] (FIG. 3d). In the cell body, kinesin 2 motors furthermore ensure that microtubule plus ends are biased towards the axon[57].

Cytoplasmic streaming
Intracellular movement of the fluid substance (cytoplasm), transporting nutrients, macromolecules and organelles.

In addition to motor-driven guidance, microtubule growth can be directed along existing microtubules or other cytoskeletal elements through passive cross-linkers. For example, the penetration of microtubules into dendritic spines, which is important for controlling neuronal activity from the post-synaptic side, requires bending of polymerizing microtubules along actin fibres emerging from the spines[156,157] (FIG. 3e). In a similar fashion, actin bundles can guide microtubules in the axonal growth cone during axonal pathfinding[158–160].

Microtubule network diversity in cells

The combinations of molecular mechanisms described above can generate microtubule networks with widely different geometries and densities. Below, we describe the microtubule organization in the best-studied cell types of our body — fibroblasts, immune, epithelial and muscle cells and neurons. These different organizations are uniquely related to specific cell functions as highlighted below. For a more general discussion on how different microtubule network geometries control transport patterns, see REF.[161].

(Semi-)Radial networks in motile cells

Motile differentiated cells often contain radial, centrosome-anchored microtubule networks, likely because they allow quick alterations in cell polarity. For example, immune cells can reorient their centrosome within a few minutes and dock it at the site of immune synapse to create a focal point for trafficking and secretion (FIG. 4a), allowing target killing or immune cell activation[162]. This rapid reorientation requires microtubules to be short and sparse[101]. As the microtubule density in immune cells is typically low, microtubules do not provide direct mechanical support but can still promote cell integrity. They can prevent large, branched cells, such as dendritic cells, from breaking into pieces during migration, because local microtubule depolymerization in cell protrusions remote from the MTOC triggers actomyosin contractility and retraction of these remote protrusions[163]. Microtubules in immune cells are predominantly centrosomal, and during an immune response, microtubule nucleation at the centrosome can be increased by enhanced recruitment of PCM components[164].

Motile epithelial and endothelial cells as well as fibroblasts have a semi-radial microtubule system centred around the centrosome and the associated Golgi complex[40,52] (FIG. 4b). The activity of the Golgi as the secondary MTOC can play an important role in increasing microtubule density and promoting microtubule-based transport to the leading cell edge (reviewed elsewhere[40]). The synergy between the Golgi MTOC in the cell centre and microtubule plus end-stabilizing complexes at the cell cortex allows generation of dense and stable microtubule arrays facing the direction of cell migration[40,165]. Such microtubule architecture is needed for directional vesicle trafficking, control of cell adhesion and, possibly, also for providing mechanical support to large cell protrusions, which are particularly important in soft three-dimensional environments where adhesion is insufficient to support cell shape[94,166]. Because dynamic microtubule rearrangements are important for cell migration, both stabilizing and destabilizing microtubule-targeting agents can modulate cell movements, for example, during angiogenesis[167].

Microtubule networks in epithelia

Columnar epithelial cells form sheets that serve as barriers and display a different intracellular organization towards their apical versus their basolateral sides. To establish and maintain this polarized organization, these cells build a polarized microtubule network that facilitates direct transport routes to both sides[168] (FIG. 4c). As discussed above (in the section Microtubule nucleation and anchoring), the formation of this network is driven by the enrichment of microtubule-nucleating and anchoring complexes at the apical cortex or, in the case of multiciliated cells, the basal bodies of the cilia (FIG. 4d). This results in a uniform microtubule array that grows towards the basolateral surface. Beyond transport, this longitudinal microtubule array may also serve other functions, such as shape control and morphogenesis, as reducing its density results in decreased actomyosin-driven apical constriction during epithelial tube formation[35,169].

Many epithelial cells, furthermore, feature a dense microtubule network underneath the apical surface. These microtubules are believed to contribute to planar cell polarity, which is the uniform polarization within the plane of a sheet of cells (for a review, see REF.[170]). In the fly wing, key planar cell polarity proteins, such as Frizzled and Dishevelled, have been shown to move over apical microtubules to achieve their correct localization (that is, proximal or distal)[171,172]. In the multiciliated ependymal cells that line the ventricles of the mouse brain, CAMSAP3 mediates the formation of an apical microtubule network that is important for broadening their apical domain and proper ventricle formation, possibly through the positioning of lysosomes that mediate mTORC1 activation, which, in turn, regulates cell morphology[173]. Apical microtubules, furthermore, contribute to the proper positioning and coordinated beating of cilia in the apical plane of multiciliated cells[47,48,174].

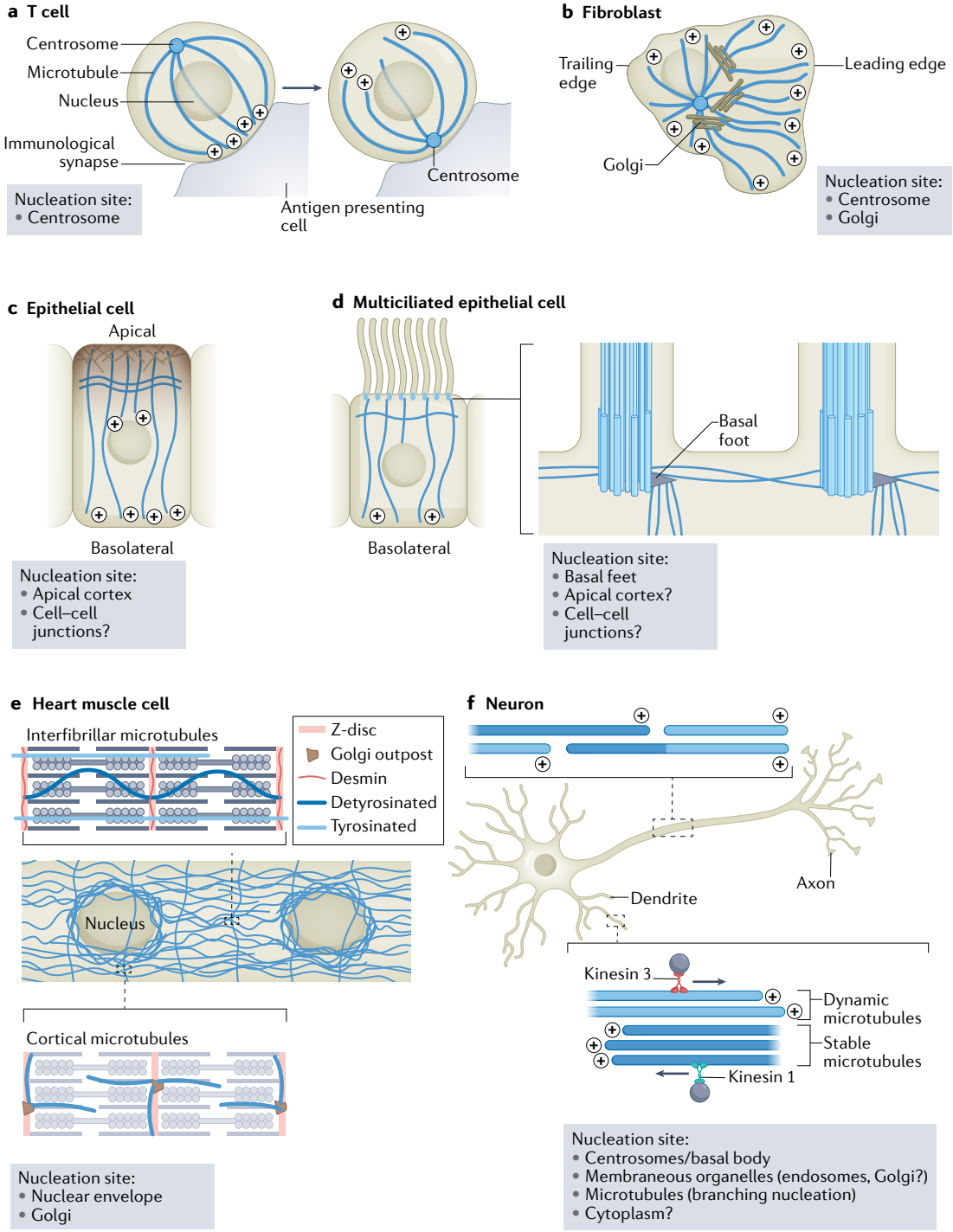
Microtubule networks in muscle cells

Differentiated muscle cells contain both longitudinally arranged microtubules that run parallel to myofibrils (interfibrillar microtubules) and orthogonal cortical microtubule grids[3,59,175] (FIG. 4e). Increased microtubule density is found around the nuclei and associated Golgi membranes, which serve as major MTOCs and are located centrally in cardiomyocytes, but extruded to the cell periphery in skeletal muscles[60,70]. Cortical microtubule grids are organized by Golgi outposts (FIG. 4e), and the organization of microtubules running parallel to Z-discs depends on the DAPC[59,125,175]. Similar to other large cells, microtubules in muscle cells are important for organelle positioning (for example, distribution of nuclei, sarcoplasmic reticulum and mitochondria) and for trafficking of transmembrane proteins (reviewed elsewhere[3]). By serving as transport highways, microtubules participate in organizing specialized membrane

Dendritic spines
Membranous protrusions from a neuronal dendrite that receive input from an axon at the synapse.

Dendritic cells
Antigen-presenting cells that form an important role in the adaptive immune system.

mTORC1
(Mammalian (or mechanistic) target of rapamycin complex 1). A protein complex that functions as a nutrient, energy and redox sensor and controls protein synthesis.



domains, such as neuromuscular junctions in skeletal muscles[123] and the cardiomyocyte intercalated discs, intercellular contact sites containing gap junctions and desmosomes[3].

Recent work showed that desmin intermediate filaments located at the Z-discs of sarcomeres in cardiomyocytes specifically interact with stable, detyrosinated microtubules[176] (FIG. 4e) and also serve as sites of microtubule stabilization and rescue[177]. Whereas the molecular basis of these interactions still needs to be uncovered, their physiological importance has been convincingly demonstrated: they increase cardiomyocyte stiffness and inhibit relaxation[3,176]. Increased microtubule density, MAP-driven stabilization and detyrosination impair cardiomyocyte contractility and are associated with heart failure of different aetiology[178–180]. Control of microtubule dynamics and post-translational modifications thus emerges as a promising therapeutic route for treating heart disease[178–180].

Microtubule organization in neurons

Because of their extreme dimensions, neurons heavily depend on long-range transport along microtubules. Not only do microtubule-based motors help deliver proteins,

◀ Fig. 4 | **Microtubule organization in major mammalian cell types. a** | Immune cells have a sparse and dynamic centrosomal microtubule network that can be rapidly reoriented during formation of immunological synapse to facilitate polarized secretion of cytokines towards the antigen-presenting cell or lytic molecules towards a cell targeted for cytotoxic killing. **b** | In fibroblasts and other mesenchymal cells, a radial microtubule network formed by the centrosome with the closely associated Golgi apparatus can be reoriented depending on the direction of cell migration to control polarized transport of vesicles and macromolecules, such as focal adhesion components, to and from the leading and trailing cell edges. **c** | In some epithelial cells, microtubule minus ends are tethered at the apical cortex whereas the plus ends extend to the basal side to create a polarized array. These microtubules control polarized transport and organization of the epithelial cell. In addition, a microtubule meshwork present at the apical side can help shape the apical cell. **d** | In multiciliated epithelial cells, basal feet (appendages of the basal bodies) organize the apico-basal microtubule network, required for polarized cell organization and transport, whereas an apical cortical meshwork participates in regulating planar cell polarity (coordinated alignment of cells across the tissue plane) and ensures uniform orientation of cilia. **e** | In cardiomyocytes, microtubule minus ends are tethered to the nuclear envelope with closely apposed Golgi membranes; Golgi outposts form additional microtubule-organizing centres (MTOCs). Cortical microtubules form an orthogonal grid, with transversal microtubules positioned along Z-discs. Interfibrillar microtubules run parallel to actomyosin fibres and perpendicular to Z-discs. Cardiomyocytes feature a population of stable, detyrosinated microtubules; these interact with desmin at Z-discs and buckle during cell contraction. Similar to other cell types, microtubules in muscle cells guide membrane transport and control organelle positioning. In addition, microtubules play mechanical roles in these cells, whereby buckling microtubules resist the load of contraction and stiffen the cell. **f** | In mammalian neurons, axons have a microtubule array with uniform plus end-out polarity, whereas dendrites feature microtubules of both orientations. These microtubules organize into polarized bundles that differ in stability and composition (that is, minus-end microtubules are more stable than plus end-out microtubules) and serve as rails for long-range transport and as a scaffold for organelle positioning and mechanical support of neurites. In addition, signalling complexes at microtubule plus ends have local regulatory roles.

mRNA and organelles across the enormous length of the axon, they also contribute to the sorting of the different cargoes to either axons or dendrites[161,181,182]. The organization of the neuronal microtubule cytoskeleton facilitates both of these processes. Microtubules in neurons are more abundant and stable than in most other cell types, likely due to the presence of numerous neuron-specific MAPs[1,104]. Interestingly, to organize and stabilize their microtubules, neurons also use some MAPs that in dividing cells act specifically in mitotic spindle assembly, such as kinetochore proteins, which provide an additional set of tools to shape microtubule arrays[183–186].

Axons display a uniform microtubule array where all plus ends are oriented distally, which facilitates straightforward division of labour between plus and minus end-directed motors in driving anterograde and retrograde transport, respectively (FIG. 4f). Importantly, the axonal microtubule network is a mosaic of many different microtubules, because axonal microtubules are typically much shorter than the axon itself[187]. Careful analysis of axonal transport and microtubule organization in *C. elegans* revealed that the distance travelled by cargoes before pausing is determined by microtubule length and that cargoes often pause at microtubule ends[188]. Axonal microtubules are often heavily modified and stabilized (reviewed in REFS[104,181]), which could ensure long-term stability of axonal transport. Nonetheless, dynamic microtubules are still present throughout the axon. Recent work has shown that growing microtubule ends are enriched near en passant synapses (synapses that are not located at axon terminals). The growing microtubule ends contribute to proper delivery of synaptic vesicle precursors by promoting the detachment of the plus end-directed kinesin KIF1A, which has a reduced affinity for the GTP-tubulin at the plus end[189]. Conversely, proteins enriched at dynamic plus ends, such as CLIP-170, promote the loading of dynein motors onto microtubules to initiate retrograde transport of different cargoes[190,191].

The dendrites of flies and worms also display uniform microtubule orientations, but here all minus ends are pointing outward[192,193]. This difference in orientation facilitates straightforward sorting of cargoes between axons and dendrites by activating either plus or minus end-directed motors, respectively[194]. By contrast, dendrites of mice and rat neurons feature both minus and plus end out-oriented microtubules[195,196] (FIG. 4f). As a result, various motors can drive bidirectional transport over this microtubule network[197,198]. Nonetheless, microtubules with the same polarity often cluster together (FIG. 4f), so that motors that switch to neighbouring microtubules are likely to continue moving in the same direction[137]. Furthermore, in rat hippocampal neurons, oppositely oriented microtubules have different properties[137]. Nocodazole-resistant stable microtubules are predominantly oriented minus end out, whereas the majority of dynamic microtubules are oriented plus end out. This organization is believed to exclude kinesin 1 (which prefers stable microtubules) from dendrites, while ensuring that kinesin 3 (which prefers dynamic microtubules) can drive long-range anterograde transport within dendrites. Thus, microtubule organization within dendrites supports motor selection to regulate transport between dendrites and the soma.

As discussed in earlier sections, parallel bundling and stabilization of microtubules by TRIM46 and other MAPs, directed nucleation by the HAUS complex and motor-based sorting all contribute to the uniform orientation of axonal microtubules[80,107,149,150,199–201]. The mechanisms that underlie the selective stabilization of minus end-oriented microtubules in mammalian dendrites with mixed orientations are less clear. Important players, such as CAMSAP2 and the kinesin 14 KIFC1, have been identified in recent years[202,203], but an integrated model for microtubule organization in mammalian dendrites is still missing.

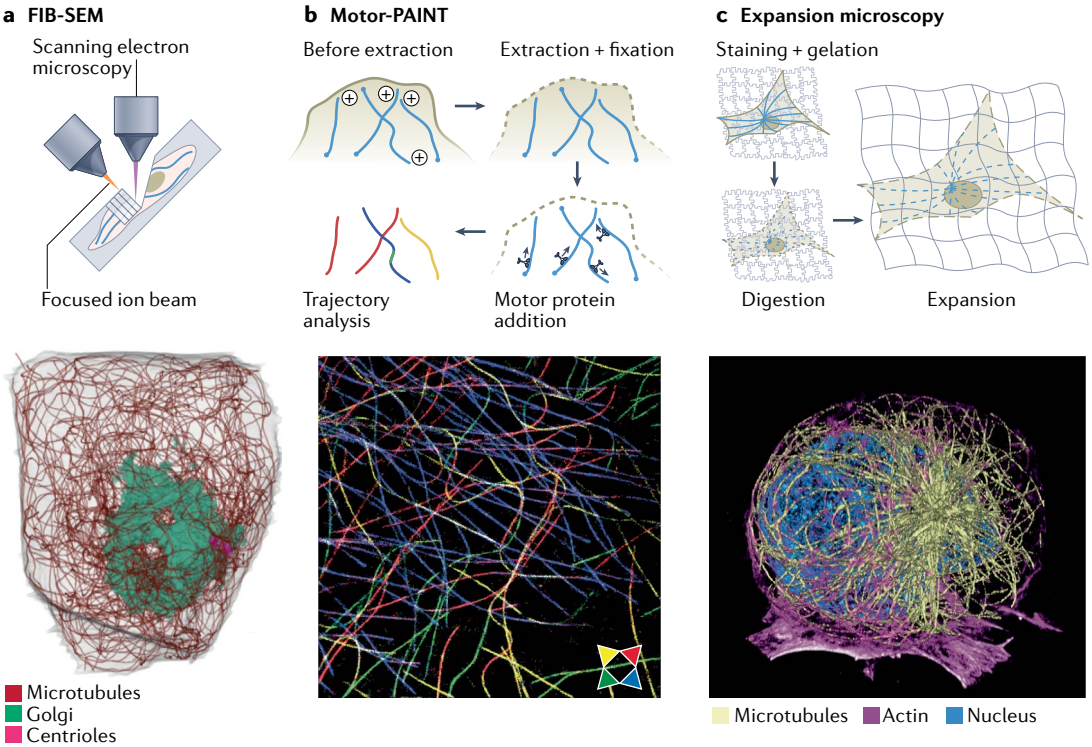
Outlook

Microtubule organization is well understood in cycling cells cultured on flat surfaces, but imaging microtubules in three-dimensional differentiated cells, particularly within intact tissues, has proven to be much more challenging due to the small cell sizes, high microtubule density and poor accessibility of such samples to conventional microscopy. Recently, major progress has been made in manipulating and imaging the cytoskeleton in tissues, and further progress is expected due to advances in high-resolution three-dimensional microscopy, such as expansion microscopy and cryo electron tomography (BOX 2). Combined with increasingly effective ways to knockout genes and tag endogenous proteins in specific

Box 2 | **Technologies for mapping dense microtubule networks**

Individual microtubules are easy to visualize by fluorescence microscopy in both live and fixed cells. However, achieving a complete quantitative description of dense, three-dimensional microtubule networks present in most differentiated cells represents a major challenge. Advances in electron tomography and focused ion beam milling combined with scanning electron microscopy (FIB-SEM) (see the figure, part **a**, top), in principle, allow very thorough analysis of microtubule networks[211,212]. For example, the entire microtubule network of insulin-producing β cells was reconstructed at high resolution (see the figure, part **a**, bottom; reprinted from REF.[212], CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/)), revealing microtubule geometry and interactions with different membrane organelles[212]. Although the resolution of electron microscopy is very high, it is also extremely laborious, making the throughput low. Furthermore, whereas electron microscopy-based analyses allow exploring microtubule interactions with surrounding organelles, they are more difficult to combine with detection of specific molecules or post-translational modifications.

These problems can be addressed by super-resolution fluorescence microscopy. Earlier super-resolution techniques, such as single-molecule localization microscopy or stimulated emission depletion microscopy, yield a tenfold increase in the lateral resolution as compared with conventional light microscopy, and current developments focus mostly on optimizing the fluorescent probes to increase labelling density and brightness or reduce probe size to limit linkage errors[213]. A variant of single-molecule localization microscopy, Motor-PAINT, allows one to determine both the position and orientation of single microtubules. This is achieved by detergent-based cell extraction followed by fixation to preserve the cytoskeleton, addition of fluorescent kinesins, single-molecule imaging and subsequent trajectory reconstruction[137] (see the figure, part **b**, top; adapted from REF.[137], CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/); and bottom; reprinted from REF.[137], CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/)). However, these techniques are currently insufficient for resolving dense three-dimensional microtubule networks in tissues. Among the available techniques, the most promising for addressing this challenge is expansion microscopy, where samples stained with fluorescent probes are embedded in a gel that is isotropically swollen before imaging[214] (see the figure, part **c**; bottom image, derived from the data in REF.[215], is a courtesy of Hugo Damstra). Recent advances in expansion microscopy allow tenfold expansion, and combination of antibody staining for specific cellular components with more general stains for proteins and membranes that provide cellular context[216]. As all techniques have their advantages and drawbacks, one can expect that, in the future, correlative approaches combining different imaging modalities will be needed to obtain full descriptions of microtubule networks, including microtubule localization, number, length, orientation, modifications and the composition of the associated microtubule-associated proteins (MAPs).



tissues and organs, these approaches bear great promise to reveal complete three-dimensional maps of microtubules and the functions of their regulators, for example in small transparent animals, such as worms or zebrafish. Detailed understanding of cytoskeletal architecture and function in cells within mammalian tissues also becomes increasingly feasible, particularly through improvements of intravital imaging and the broad use of cultured genetically tractable tissue models such as organoids. Such understanding is essential, because it will provide a much clearer view on how microtubule-based processes contribute to organism development and organ physiology. Moreover, imaging of microtubules and their regulators in their native tissue environment

is important from a medical perspective — it will open up the possibilities to optimize the activity and reduce the toxicity of microtubule-targeting agents, such as Taxol or colchicine. These compounds have been used for decades to treat cancer and inflammation, but we still lack the information on the dynamic changes they exert on the cytoskeleton within tissues. Recent interest in targeting microtubules to treat cardiovascular disease[3] and neurodegeneration[204] makes such studies increasingly relevant.

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