

REVIEW

The role of microtubules in secretory protein transport

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ABSTRACT

Microtubules are part of the dynamic cytoskeleton network and composed of tubulin dimers. They are the main tracks used in cells to organize organelle positioning and trafficking of cargos. In this Review, we compile recent findings on the involvement of microtubules in anterograde protein transport. First, we highlight the importance of microtubules in organelle positioning. Second, we discuss the involvement of microtubules within different trafficking steps, in particular between the endoplasmic reticulum and the Golgi complex, traffic through the Golgi complex itself and in post-Golgi processes. A large number of studies have assessed the involvement of microtubules in transport of cargo from the Golgi complex to the cell surface. We focus here on the role of kinesin motor proteins and protein interactions in post-Golgi transport, as well as the impact of tubulin post-translational modifications. Last, in light of recent findings, we highlight the role microtubules have in exocytosis, the final step of secretory protein transport, occurring close to focal adhesions.

KEY WORDS: Golgi complex, Membrane trafficking, Microtubules, Secretory pathway

Introduction

The microtubule cytoskeleton has essential functions in eukaryotes, in particular to ensure cell division and equal partitioning of chromosomes (Vukusic et al., 2019). In mammalian cells, microtubules also have a main role during interphase to regulate intracellular organization (de Forges et al., 2012) and organelle positioning (Bonifacino and Neefjes, 2017).

Microtubules – being organized as one or multiple arrays of polarized tracks that are differently organized depending on the cell type – endow cells with intracellular polarity (Fig. 1). Microtubules comprise α - and β -tubulin heterodimers – α -tubulin being exposed at the microtubule minus end and β -tubulin at the plus end. Minus ends slowly grow and are anchored to microtubule-organizing centers (MTOCs) (Hendershott and Vale, 2014), whereas microtubule plus ends ensure microtubule elongation (Mitchison and Kirschner, 1984). Microtubules are highly dynamic polymers, alternating phases of growth and shrinkage. The dynamic nature of microtubules enables them to ensure exploration of the cytoplasm and fulfil multiple functions. A high number of microtubule-associated proteins (MAPs) exist (Bodakuntla et al., 2019); they structurally regulate the microtubule network itself but also its dynamics and functions in cellular processes. Microtubule diversity is also generated through several post-translational modifications

(PTMs) of tubulin, including acetylation, phosphorylation, polyamination, tyrosination/detyrosination, poly-glutamylolation and poly-glycylation (Janke, 2014), which, recently, became of great interest in diverse cellular processes, including intracellular transport (see below). Tubulin PTMs regulate the microtubule network, as they alter the mechanical properties and stability of microtubules, and influence their interactions with MAPs (Magiera et al., 2018b; Xu et al., 2017).

Molecular motors interact with microtubules and allow the movement of cargos along the microtubule tracks. Motors either directly bind to cargo, or can associate with adaptor or scaffold proteins to ensure the link between the motor and the cargo. Motors use the energy generated by hydrolysis of ATP to move on microtubules with varying directionality and speed. Dynein is a minus-end molecular motor, whereas the large family of kinesin motors can be either plus-end or minus-end directed (Hirokawa et al., 2009; Kardon and Vale, 2009). Although the majority of kinesins are plus-end-directed motors, members of the kinesin-14 family are minus-end-directed motors (Hirokawa et al., 2009; Kardon and Vale, 2009).

Here, we focus on the role of microtubules regarding the organization and regulation of the secretory pathway within mammalian cells. The secretory pathway ensures the transport of proteins from the endoplasmic reticulum (ER) via the Golgi complex to their destination compartment, such as the cell surface, the endosomes or the lysosomes (Boncompain and Weigel, 2018). Microtubules, together with molecular motors, are known to accelerate long-range vesicular trafficking. However, whether they are essential to ensure secretion is still debated (see below). The relationship between microtubules and focal adhesions is intimate, playing a role in the last step of the secretory pathway, i.e. exocytosis. In this Review, we discuss how microtubules contribute to protein secretion from the ER to the Golgi and from the Golgi to the plasma membrane. We focus on kinesin-dependent processes and the role of tubulin modifications in post-Golgi transport. We also discuss the importance of microtubule for cargo exocytosis that occurring near to focal adhesions. Furthermore, we emphasize microtubule-driven secretion in non-polarized cells and discuss their importance in polarized cells, such as neurons.

Microtubules in the homeostasis of secretory pathway organelles

The distribution of intracellular compartments that are necessary for protein secretion is dependent on microtubules (Burkhardt et al., 1997; Vale, 1987), which vary in their cellular organization depending on cell architecture (Fig. 1). The organization of the organelles of the secretory pathway described below corresponds mainly to that of non-polarized cells. The ER is the organelle that constitutes the starting point of the secretory pathway where proteins are translocated through the ER, either post- or co-translationally (Mandon et al., 2013). The ER extends through the entire cytoplasm and its distribution relies on an intact microtubule network, especially to elongate ER tubules (Lee and Chen, 1988). ER tubules interact

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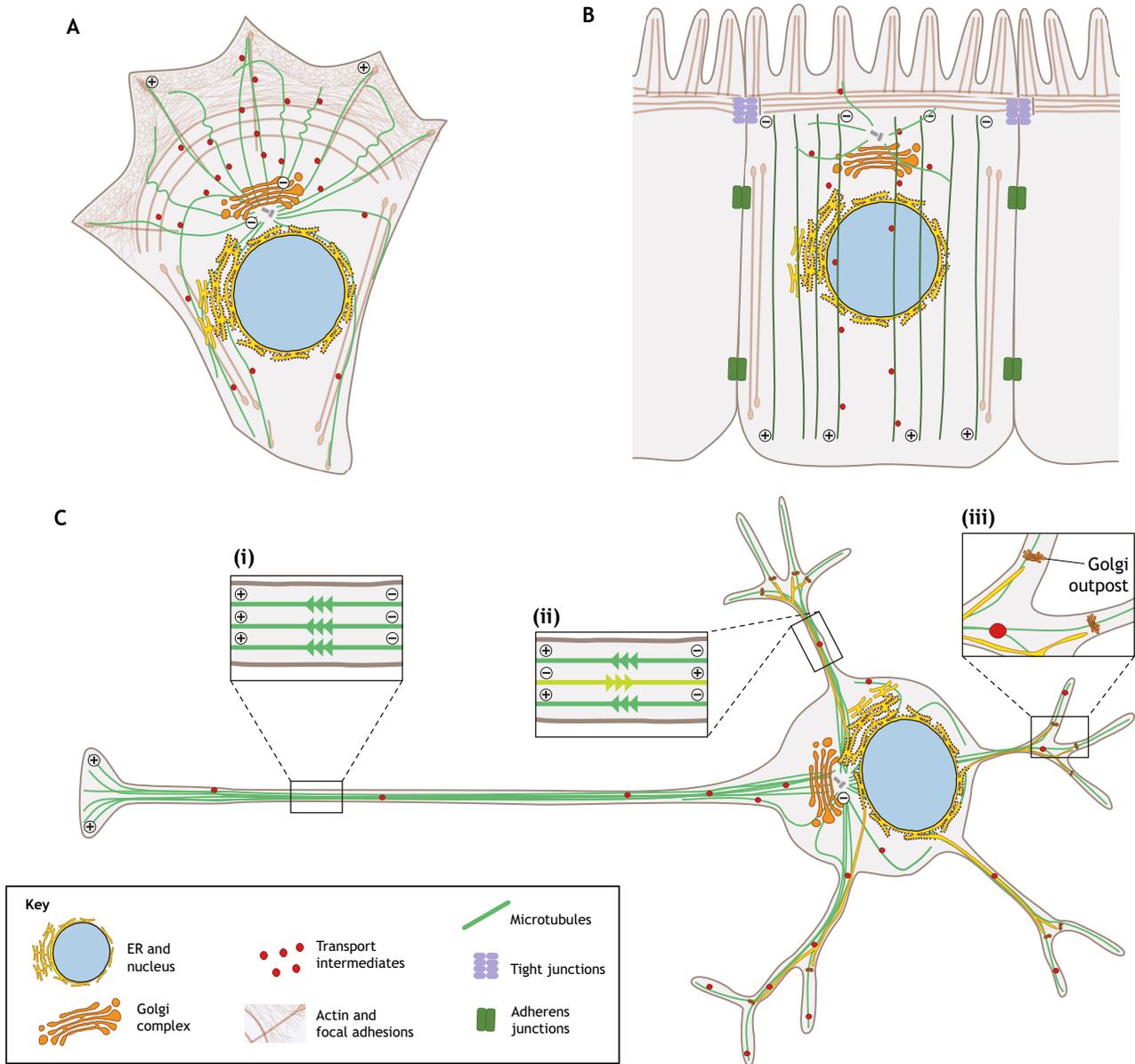


Fig. 1. Microtubule organization and polarity vary in different cell types. (A) In mesenchymal cells or fibroblasts, the microtubule network is mainly astral and emanates from the centrosome and the Golgi complex. The microtubule minus ends are, therefore, in the center and the plus ends mainly at the periphery of the cell. (B) In epithelial cells, centrosomal microtubules are rare. Here, filaments elongate towards the basal pole of the cell, forming a parallel network. (C) In neurons, an astral–central array of microtubules radiates from the centrosome and Golgi at the soma towards their periphery, i.e. the axon and dendrites. (i) Axonal microtubules form bundles of parallel microtubules with the same polarity (minus end at the soma and plus end at the periphery). (ii) In dendrites, by contrast, microtubules form anti-parallel beams of microtubules with mixed polarity and different stability, microtubules oriented from the periphery towards the soma being more stable. (iii) It is possible that Golgi outposts contained in dendrites have a role in the nucleation of microtubules with inverted polarity.

with microtubule plus ends and use the force generated by microtubule polymerization to extend towards the cell periphery (Grigoriev et al., 2008). Removal of microtubules increases the proportion and the frequency of motion of ER sheets, which has also been described as a dense matrix of ER tubules (Nixon-Abell et al., 2016; Terasaki et al., 1986). The ER membrane is connected to microtubules through ER-resident proteins, such as cytoskeleton-associated protein 4 (CKAP4, hereafter referred to as CLIMP-63) and stromal interaction molecule 1 (STIM1) (Grigoriev et al., 2008; Vedrenne et al., 2005) (Fig. 2). CLIMP-63 bears a microtubule-binding domain in its cytosolic part. When overexpressed, CLIMP-63 leads to the re-arrangement of the microtubule network (Klopfenstein et al., 1998). The precise function of CLIMP-63–microtubule

interactions remains unclear but it has been suggested to lie in anchoring the rough ER to the cytoskeleton (Sandoz and van der Goot, 2015). The interaction between STIM1 and growing microtubules, through the plus-end tracker end-binding protein 1 (EB1), has been shown to prevent Ca²⁺ overload (Chang et al., 2018).

The Golgi complex is the next compartment in the secretory pathway, receiving cargos from the ER. Functionally, it lies at the center of the secretory pathway and physically, in many cell types, at the center of the cell (Boncompain and Perez, 2013). The Golgi complex colocalizes with microtubule minus ends (Martin and Akhmanova, 2018). Microtubules and MAPs, such as calmodulin-regulated spectrin-associated protein 2 (CAMSAP2), myomegalin, EB1, EB3 (officially known as MAPRE1 and MAPRE3,

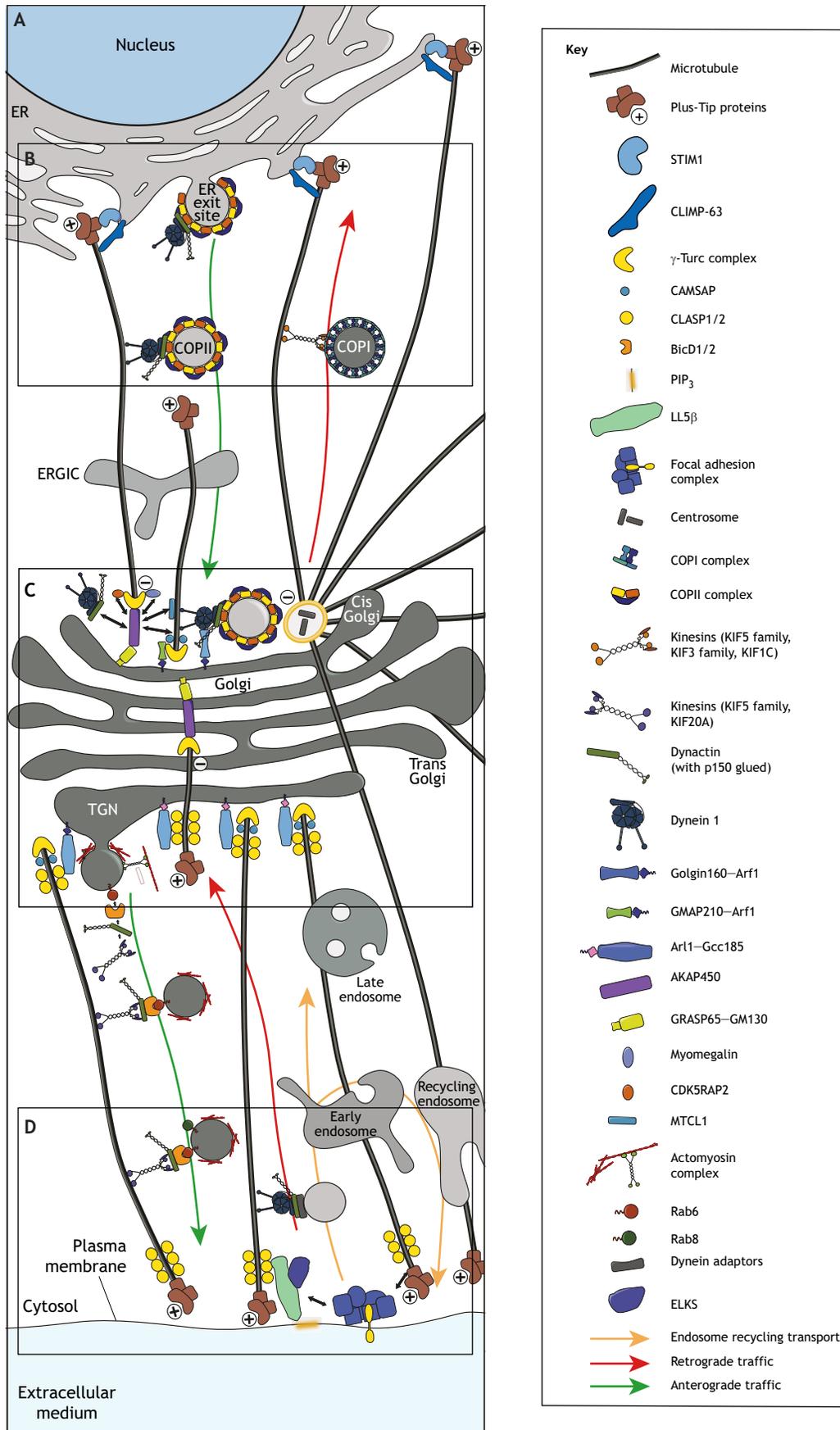


Fig. 2. See next page for legend.

Fig. 2. Molecular machinery linking microtubules to main intracellular trafficking pathways. (A) Overview of organization of microtubules and their interplay with main secretory organelles, i.e. ER, Golgi complex and plasma membrane. (B) Microtubules can bind to the ER directly through CLIMP63 or indirectly through interactions between plus-end proteins and ER proteins, such as STIM1. COPII vesicles containing secretory cargos are formed at ER exit sites (ERES). They interact with microtubules through binding of p150glued with the COPII coat component Sec23. The presence of dynein 1 enables movement of COPII vesicles toward the Golgi complex. (C) At the center of these anterograde and retrograde pathways, the Golgi complex has a main role as trafficking hub but also as MTOC, owing to its proximity to the centrosome and its intrinsic microtubule nucleating (through γ -TuRC and Golgi protein-containing complexes, such as AKAP450, GM130 and GRASP65) and capture capabilities (through interactions between Golgi proteins and microtubule minus-end stabilizing proteins, such as γ -TuRC complexes, and CAMSAP and CLASP proteins). At the *cis*-face of the Golgi complex, several proteins mediate the interaction between microtubules and Golgi membranes, such as the golgins GMAP210 and golgin160 that link to Golgi membranes through Arf1. At the *trans*-face of the Golgi complex, the microtubule-interacting proteins CLASP1/2 bind to the Golgi protein GCC185, which is bound to Arl1. Post-Golgi carriers are formed at the TGN. The actomyosin complex is necessary to ensure fission of the post-Golgi carriers. Rab6 decorates the cargo-containing vesicles and interacts with BicD1/2 and molecular motors. Among these motors, KIF5B and KIF20A mediate transport along microtubule towards the cell periphery. (D) On the other end of the trafficking pathway, microtubule plus-end complexes interact with focal adhesion complexes and PIP₃-interacting proteins, such as LL5 β .

respectively) and dynein, are responsible for the attachment of the Golgi complex to the minus ends, and for its central localization (Corthesy-Theulaz et al., 1992; Sandoval et al., 1984; Wang et al., 2014; Wu et al., 2016; Yang et al., 2017). Dynein inhibition or microtubule depolymerization induces the reversible fragmentation and the dispersion of the Golgi complex to the extent that we employ Golgi morphology as a read-out for dynein activity or the efficiency of dynein-inhibiting drugs, such as ciliobrevin (Firestone et al., 2012). Such perturbation of dynein or microtubule organization induces the formation of Golgi mini-stacks that display a correct *cis*-to-*trans* organization (Cole et al., 1996; Trucco et al., 2004), indicating that microtubules are not required for the organization of the Golgi stacks themselves. By using light microscopy, Nocodazole-induced Golgi mini-stacks were even shown to facilitate the analysis of the intra-Golgi distribution of Golgi-resident proteins or cargos (Dejgaard et al., 2007).

In many cell types, the main MTOC is the centrosome, even though microtubules can also be nucleated from Golgi membranes (Chabin-Brion et al., 2001; Efimov et al., 2007). These non-centrosomal microtubules are important for the perinuclear localization of the Golgi complex (Hoppeler-Lebel et al., 2007), for polarized cell migration (Wu et al., 2016) and for directed post-Golgi transport (Efimov et al., 2007; Miller et al., 2009) (Fig. 2A,D). Although a role for the microtubule-nucleating factor γ -tubulin in Golgi-near microtubule nucleation has been reported (Chabin-Brion et al., 2001), it is Golgi-associated microtubule-binding proteins, such as CAMSAP2 and A-kinase anchor protein 9 (AKAP9, hereafter referred to as AKAP450), who play the dominant role in the nucleation of Golgi microtubules (Wu et al., 2016). The Golgi-associated microtubule-binding protein 210 (TRIP11, hereafter referred to as GMAP210), was shown to interact with γ -tubulin (Infante et al., 1999; Rios et al., 2004) and several Golgi proteins physically link Golgi membranes with microtubules, such as golgin subfamily A member 3 (GOLGA3, hereafter referred to as golgin160), which binds to dynein (Yadav et al., 2012). The *trans*-Golgi network (TGN) GRIP- and coiled-coil domain-containing protein 185 (GCC2, also known as GCC185) was

shown to interact with the microtubule-binding proteins CLIP-associating proteins (CLASPs) (Efimov et al., 2007). The *cis*-Golgi protein GOLGA2 (hereafter referred to as GM130) binds to AKAP450, allowing microtubule nucleation (Rivero et al., 2009). In addition, the association of both CLASPs and AKAP450 with microtubule crosslinking factor 1 (MTCL1) has been shown to enhance association of microtubules with the Golgi complex (Sato et al., 2014). It has further been demonstrated that, through GM130, AKAP450 and myomegalin, Golgi membranes are connected to the microtubule-binding proteins CAMSAP2 (Wu et al., 2016), and EB1 and EB3 (Yang et al., 2017). The proteins AKAP450, CDK5 regulatory subunit-associated protein 2 (CDK5RAP2) and myomegalin bind to γ -tubulin ring complexes at the *cis*-face of the Golgi complex (Gavilan et al., 2018; Wang et al., 2010). It should be noted that several isoforms of myomegalin exist; two of them, CM-MMG and EB-MMG, have different functions, promoting microtubule nucleation and restricting microtubule growth, respectively (Roubin et al., 2013).

In conclusion, microtubules are important for intracellular distribution and shaping of organelles, including those involved in secretory protein transport. Recent work has shed light on the importance of minus-end regulation and growth at the Golgi, affecting microtubule nucleation and organization of the Golgi complex. Microtubules are also involved in the distribution of other cellular organelles, such as endosomes, lysosomes and mitochondria (see Bonifacino and Neefjes, 2017; Pu et al., 2016; Lin and Sheng, 2015 for recent reviews).

Microtubules in ER-to-Golgi and intra-Golgi trafficking

After their translocation in the ER, cargos are concentrated in specialized export domains, named ER exit sites (ERES), from where COPII-coated vesicles are formed (Bannykh et al., 1996). Microtubules are not required to generate force to ensure budding of COPII vesicles, since they can still form spontaneously in their absence (Presley et al., 1997). COPII vesicles then fuse to create the ER-Golgi intermediate compartment (ERGIC) (Brandizzi and Barlowe, 2013; Xu and Hay, 2004). These pre-Golgi carriers have to be transported to the Golgi complex owing to the separation of ER and Golgi complex in mammalian cells. Microtubule-based movement of ER-to-Golgi transport carriers and the role of dynein therein have been demonstrated by using the fluorescently tagged thermosensitive mutant of viral glycoprotein tsO45 VSVG when tracked in pre-Golgi structures moving towards the Golgi (Presley et al., 1997; Scales et al., 1997). The interaction between ERES and microtubules is ensured by binding Sec23, a component of the COPII coat, and the C-terminal domain of dynactin subunit 1 (DCTN1, hereafter referred to as p150glued), a component of the dynein activator complex (Watson et al., 2005) (Fig. 2B). Dynein and its accessory subunits mediate minus-end-directed transport, allowing ER-to-Golgi vesicles to reach the centrally located Golgi complex from the dispersed ERES (Burkhardt et al., 1997; Palmer et al., 2009) (Fig. 2A,B). However, kinesins are also present on pre-Golgi vesicles (Lippincott-Schwartz et al., 1990) and, as a consequence, the presence of both kinds of motor generates opposing forces, which allow bi-directional movement of vesicles on microtubules (Brown et al., 2014). More specifically, kinesins that are present on pre-Golgi vesicles ensure Golgi-to-ER retrograde motility, which is crucial for membrane recycling (Hirokawa et al., 2009).

Importantly, even though the involvement of microtubules in ER-to-Golgi transport has been clearly demonstrated over the last decades, this transport still occurs in the absence of microtubules

(Cole et al., 1996). As indicated above, without microtubules the Golgi complex is dispersed and Golgi mini-stacks are formed in apposition of ERES (Cole et al., 1996; Presley et al., 1997). Under these conditions, transport of newly synthesized cargos occurs efficiently since COPII vesicles are still formed, and ERES and Golgi mini-stacks are in close proximity.

Several models that describe how cargos are transported inside the Golgi complex have been proposed over the years; to date, the cisternal maturation model and its variations appears to be the prevalent one (Grasse, 1957; for reviews also see Boncompain and Perez, 2013; Boncompain and Weigel, 2018; Glick and Luini, 2011). According to this model, new Golgi cisternae form by coalescence of ER-derived membranes and secretory proteins are conveyed inside the cisternae. The identity of Golgi compartments is ensured by constant retrograde transport of Golgi-resident proteins. To understand the role of microtubules in intra-Golgi transport, it is important to monitor and quantify the movement of cargos or Golgi enzymes inside the Golgi complex between stacks of cisternae – a difficult endeavor. Although various cargo speeds have been monitored inside the Golgi complex (Beznoussenko et al., 2014; our unpublished data), it is complicated to determine whether microtubules are important for intra-Golgi trafficking. As described earlier, removal of microtubules strongly perturbs the Golgi complex structure and induces the dispersion of Golgi mini-stacks throughout the cytoplasm (Cole et al., 1996; Trucco et al., 2004). It is, thus, essential to establish approaches where microtubules can be fully disassembled while preventing cargo transport and, subsequently, monitor transport. We developed the retention using selective hooks (RUSH) system to synchronize the trafficking of different cargos (Boncompain et al., 2012) and monitor their transport through the cisternae in absence of microtubules (Fourriere et al., 2016). Our results indicate that microtubules are dispensable for intra-Golgi transport, and that the role of the microtubule is to ensure functional maturation of the Golgi complex but not physical movement of the cargos inside and out from the Golgi complex (Fourriere et al., 2016).

Therefore, microtubules appear not to be essential for ER-to-Golgi and intra-Golgi protein transport. However, microtubules, when present, are used to facilitate the movement of transport carriers from the ER to the Golgi complex. Nonetheless, the challenges to assess intra-Golgi transport, especially in the absence of microtubules, limit our current understanding of the role of microtubules in intra-Golgi transport.

Role of microtubules in trafficking from the Golgi to the plasma membrane

As mentioned above, in the absence of microtubules protein secretion still occurs in rather small cells through vesicle diffusion (Cole et al., 1996; Fourriere et al., 2016; McCaughey et al., 2019).

Under physiological conditions, cargos leave the TGN inside vesicular, granular or tubular structures that use microtubules to achieve a fast and directed transport (Cole and Lippincott-Schwartz, 1995; Schmoranzler and Simon, 2003; Toomre et al., 1999). Cargos trafficking from the TGN to downstream compartments use various kinesins as molecular motors (Hirokawa et al., 2009). Reported in most mammalian cell types, this kinesin-dependent transport of cargos is particularly important in highly polarized cells, and KIF5B, KIF5C, KIF3 and KIF16B have been shown to be necessary for apical transport in polarized MDCK cells (Astanina and Jacob, 2010; Jaulin et al., 2007; Noda et al., 2001; Perez Bay et al., 2013). Microtubules are also of particular importance in trafficking pathways in neurons (see Box 1, Fig. 1).

Box 1. The role of microtubules in polarized protein trafficking within neurons

Neurons are polarized cells comprising a cell body, a long axon and numerous branched dendrites (Fig. 1C). This arborization allows them to receive, process and transmit information. However, neuronal organization challenges the secretory protein transport with the need to carry cargos over long distances, i.e. up to 1 m in motor neurons. Microtubules are key players in the organization of neurons and in polarized transport (Kelliher et al., 2019). Microtubules differ in their polarity and stability between axon and dendrites. Plus ends extend outward in the axon, whereas mixed anti-parallel orientation (Tas et al., 2017) is observed in dendrites within mammalian neurons (Yau et al., 2016), which also harbor microtubule-dependent Golgi outposts (Gardioli et al., 1999; Horton and Ehlers, 2003); (Fig. 1C). Dynein and kinesins are necessary to ensure anterograde and retrograde movements (Bentley and Banker, 2016). In the axon, dynein drives retrograde transport and kinesins drive anterograde transport, whereas dynein drives bidirectional transport in dendrites (Kapitein et al., 2010). Certain motors, such as KIF5A, KIF5B and KIF5C, have been shown to selectively mediate cargo transport in the axon, which does not enter dendrites. KIF1A, however, mediates transport in both axon and dendrites (Tas et al., 2017). This selectivity of a motor protein might depend on PTMs of tubulin, such as acetylation and deetyrosination in the case of axonal selectivity of KIF5A, KIF5B and KIF5C (Cai et al., 2009; Konishi and Setou, 2009). KIF1A and KIF1C are non-selective motor that has been proposed to bind microtubules comprising tyrosine residues at their C-terminal end (Guardia et al., 2016; Lipka et al., 2016). However, the preferential binding of these motors to PTMs of tubulin does not fully explain axonal motor selectivity because acetylated and deetyrosinated microtubules are abundant in both axon and dendrites (Hammond et al., 2010). In dendrites, orientation of acetylated microtubules is such that their minus ends point outwards, accounting for the plus-end-directed inefficiency of kinesin-1 proteins to mediate axonal transport (Tas et al., 2017). In addition, transport of synaptic vesicles mediated by members of the kinesin-3 family (e.g. KIF1A and KIF1C) is negatively regulated by dynamic microtubules (Guedes-Dias et al., 2019). Defects in microtubule-dependent trafficking have pathological implications, and contribute to neurodevelopmental disorders (Lasser et al., 2018) and neurodegenerative diseases (Brunden et al., 2017).

Motors and adaptor proteins in post-Golgi transport

Recruitment of kinesins to carriers is reversible and determines the final destination of the cargo (Akhmanova and Hammer, 2010). As diverse kinesins are involved in the transport of chosen carriers, a specific recruitment of the motors must occur. Carriers can bind kinesin directly, e.g. members of the kinesin-1 family heavy chain proteins (KIF5A, KIF5B and KIF5C) (Kamal et al., 2000); however, most of the time, adaptors and intermediates are needed. Such a function was shown for the receptor-recycling retromer complex (Hunt et al., 2013; Wassmer et al., 2009) and for adaptor protein 1 (AP-1) (Campagne et al., 2018; Delevoye et al., 2009), which enable the recruitment of kinesins on endosomal membranes. At the level of the Golgi complex, the small GTPase Rab6 has been shown to recruit KIF20A to Golgi membranes to promote the fission of post-Golgi carriers (Miserey-Lenkei et al., 2017) (Fig. 2D).

Intriguingly, different molecular motors, sometimes with opposing directionality, can be found on the same carrier, leading to a bi-directional movement of the carrier (Hirokawa et al., 2010; Vale, 2003) in a tug of war. Each class of motor can be specifically activated and alternates between having the role of a motor or of a passenger (Derr et al., 2012; Kunwar et al., 2011; Muller et al., 2008; Welte, 2004). Importantly, it has been shown that several motors are activated upon binding to their receptor on the carriers (Derr et al., 2012; Kunwar et al., 2011); cycles of binding and release from the carriers could also be the basis of bi-directional

movement. This behavior may be surprising as it appears costly for the cell and reduces the mean transport speed, but it has been proposed that these cycles avoid road blocks along busy microtubule tracks (Telley et al., 2009) and help in the proofreading of wrongly oriented carriers (Ally et al., 2009; Jolly and Gelfand, 2011). Also, less energy might be needed when loading carriers with multiple motors as compared with assembling and disassembling the full complexes each time a carrier reverts its direction (Welte, 2004). In the context of post-Golgi transport, loading of multiple motors on carriers might require adaptors, for which little is known except when regarding the pathway comprising Rab6, protein bicaudal D homologs 1 and 2 (BICD1 and 2) and KIF5B (see below).

The role of kinesins might not be restricted to the movement of carriers but also important for their immediate release from the TGN: KIF5B has been shown to be necessary for the movement of post-Golgi transport carriers from the Golgi complex (Grigoriev et al., 2007; Miserey-Lenkei et al., 2010). Membrane fission at the *trans*-Golgi network, indeed, occurs through coordinated work of the actin cytoskeleton, the myosin II motor, the GTPase Rab6, BICD1/2, dynactin and the kinesin-like protein KIF20A (Hoogenraad et al., 2001; Miserey-Lenkei et al., 2017, 2010) (Fig. 2D). Rab6 has also been shown to directly interact with KIF1C, which inhibits interaction of the motor with microtubules and slows down the motility of post-Golgi carriers (Lee et al., 2015a). However, as noted above, our observation that Golgi-to-plasma membrane traffic can occur efficiently in the absence of microtubules (Fourriere et al., 2016) suggests a supportive but not essential role for kinesins in post-Golgi cargo movement.

Molecular motors, especially the plus-end-directed kinesins, have been demonstrated to accelerate cargo transport along microtubules but might not be essential for post-Golgi transport. The recruitment of motors to membranes might require adaptor proteins, which bring specificity to the interaction between motor and cargo.

Secretory transport, motor proteins and PTMs of tubulin

As introduced above, tubulin is subjected to PTMs (Janke, 2014). PTMs lead to the formation of sub-populations of microtubules (Tas et al., 2017) and some kinesins are able to distinguish between these microtubule populations (Cai et al., 2009). For example, KIF5B preferentially moves on the most-stable microtubules *in vivo* (Kaul et al., 2014). A higher stability of microtubules is associated with tubulin acetylation, which increases the affinity and speed of the kinesin-1 KIF5C along microtubules (Cai et al., 2009; Hammond et al., 2010; Reed et al., 2006). However, acetylation might not be essential for trafficking, and the mobility of kinesin-1 on acetylated or de-acetylated microtubules appears to be similar *in vitro* (Walter et al., 2012). Given the recent discovery of the molecular players involved in tubulin acetylation, its biological consequences are only emerging (Janke and Montagnac, 2017).

Interestingly, PTMs of tubulin have also been associated with defects in transport: abnormally high tubulin poly-glutamylation causes defects in neuronal transport (Magiera et al., 2018a). Furthermore, drug-induced acetylation of microtubules was shown to re-establish transport in neurons of patients diagnosed with neurodegenerative diseases, such as Charcot-Marie-Tooth and Huntington's (d'Ydewalle et al., 2011; Dompierre et al., 2007), as well as for mutations in leucine-rich repeat serine/threonine-protein kinase 2 (LRRK2) by inhibiting aberrant interactions of mutated LRRK2 with de-acetylated microtubules (Godena et al., 2014). Interestingly, it has been shown that the trafficking kinetics of

epidermal growth factor receptor (EGFR) is increased in response to inhibition of histone deacetylase 6 (HDAC6) and microtubule acetylation (Deribe et al., 2009; Lee et al., 2015b). Detyrosinated microtubules are preferentially chosen by KIF5C (Dunn et al., 2008; Hammond et al., 2010) and they seem to be preferred roads for the intracellular traffic in polarized cells, with a reduction of their levels leading to strong decrease in apical protein secretion (Zink et al., 2012).

Tubulin PTMs and the resulting microtubule diversity are of recent focus. At the same time, several studies question the effects of tubulin PTMs on the function of motors and on intracellular transport, and their link to diseases (Magiera et al., 2018a,b; Roll-Mecak, 2019). The identification of molecular players involved in tubulin PTMs and of ways to perturb them will, for sure, shed light on this additional level of regulation of microtubule-based post-Golgi protein transport.

Confining exocytosis by using microtubules and a localized fusion machinery

The final step of the secretory pathway is the arrival of cargos at the plasma membrane for subsequent exocytosis. Microtubules deliver vesicles to particular plasma membrane domains that are located in the leading edge of migrating cells (Schmoranzler et al., 2003). The last step of the secretory pathway occurs close to focal adhesions (FAs) and interaction between microtubule and FA is required. FAs constitute an adhesive platform at the plasma membrane composed of more than 180 proteins, which link actin and integrin networks to the extracellular matrix (Haase et al., 2014). FAs are very dynamic and are able to adjust their shape, position and composition according to the needs of the cell and to the rigidity and composition of the cell substrate (Riveline et al., 2001). Through their permanent contact between the cytoskeleton and the extracellular matrix, FAs play a role in the formation, maintenance and transmission of forces between the cell and the environment (Chen et al., 2015). On a molecular level, focal adhesion kinase (FAK) receives signals from growth factors and integrins and, in turn, recruits new FA proteins or adaptors to the adhesive region and activates signaling cascades (mDia, Rho-family complex GTPases with RhoA, Rac and Cdc-42) that will modulate the actin and microtubule networks (Mittra et al., 2005). The balance between activated and inactivated Rac and Rho complexes close to the FA region is important for the targeting of FAs by microtubules and for cell polarity (Small and Kaverina, 2003).

Microtubules are tethered at adhesion sites and influence formation, maturation and dissolution of FAs (Stehbens and Wittmann, 2012). Interestingly, microtubules attached to FAs are more stable (Kaverina et al., 1998), and tubulin acetylation of microtubules promotes FA turnover and cell migration (Bance et al., 2019; Dubois et al., 2017). Microtubules determine cellular polarity by modulating the distribution of FAs, which are continuously formed and disassembled at the leading edge. Indeed, depolymerization of microtubules induces a size increase of FAs by stimulating actomyosin- and RhoA-dependent contractility (Rafiq et al., 2019; Ren et al., 1999), and by inhibiting detachment of FA proteins (Chang et al., 2008). Two proteins that interact with the microtubule plus-end CLASP proteins are the ELKS/Rab6-interacting/CAST family member 1 (ERC1, hereafter referred to as ELKS) and the pleckstrin homology (PH)-like domain family B member 2 (PHLDB2, hereafter referred to as LL5 β) (Lansbergen et al., 2006). LL5 β is important for cell migration (Astro et al., 2014), binds phosphatidylinositol (3,4,5)-trisphosphate (PIP₃) at the plasma membrane and recruits ELKS in close proximity to FAs (Paranavitane et al., 2003). The complex

between ELKS and LL5 β acts like a platform to recruit CLASPs, who will then attach and stabilize microtubules (Fig. 2C) (Lansbergen et al., 2006). It has been shown that the direct recruitment of CLASPs by LL5 β supports FA turnover (Stehbens et al., 2014). Moreover, depletion of CLASP proteins induces decrease of microtubule density and the loss of microtubules associated with the cortex (Mimori-Kiyosue et al., 2005).

A high number of factors (e.g. microtubule plus-end tracking proteins, scaffolding factors, actin-binding proteins, docking machinery components) coordinate microtubule attachment at the cell membrane and localized exocytosis (Noordstra and Akhmanova, 2017 preprint). The mechanisms responsible for such localized exocytosis are being uncovered. In particular, the Rho signaling network – comprising guanine nucleotide exchange factor H1 (GEF-H1), the RhoGAP and the Rho effector phospholipase C (PLC) – which regulates activation of protein kinase D (PKD) at the *trans*-Golgi network (TGN), coordinates the formation of TGN-derived Rab6-positive transport carriers delivering cargo to FA-apposed regions (Eisler et al., 2018). Cargos exit the TGN at fission hotspots, the post-Golgi carriers being Rab6 (Miserey-Lenkei et al., 2017) (Fig. 2D). Indeed, both Rab6 and Rab8 play a key role in the attachment and fusion of vesicles to the plasma membrane, and are required for exocytosis (Grigoriev et al., 2011) (Fig. 2C). Activation of RhoA in the TGN induces localized activation of PKD followed by fission of Rab6 vesicles that then traffic towards FAs (Eisler et al., 2018). Moreover, Rab6- and Rab8-positive vesicles were shown to be transported by KIF5B along microtubules, towards regions at the plasma membrane that harbor ELKS and PIP₃, and are located adjacent to FAs (Grigoriev et al., 2011; Lansbergen et al., 2006). In addition, α -testis anion transporter 1 (TAT1)-mediated microtubule acetylation promotes fusion of Rab6 vesicles with the plasma membrane at FAs (Bance et al., 2019). Stehbens et al. have described a preferential exocytosis of membrane-type matrix metalloproteinase-1 (MT1-MMP) close to FAs, which occurs when using microtubules captured by CLASPs (Stehbens et al., 2014). Additionally, we have recently reported that diverse cargos follow the Rab6- and microtubule-dependent pathway, thereby sustaining exocytosis close to FAs, and that the carriers used a sub-population of microtubules toward FAs (Fourriere et al., 2019).

Thus, an intimate relationship between microtubules and FAs exist, and post-Golgi transport is directed to adhesive zones through a subset of microtubules. Some physiological outcomes have been described, such as influence on cell migration and degradation of the extracellular matrix of cells cultured in 2D (Bance et al., 2019). In the near future, it will be interesting to assess whether targeted exocytosis to adhesive sites takes place in 3D culture, and to understand the role of microtubules in this process as this would have pathological outcomes for cancer cells, such as an influence on invasive, metastatic capabilities and their interaction with the cellular microenvironment.

Conclusions and perspectives

Microtubules are polarized tracks that stage-manage cell shape, mitosis and intracellular trafficking. They are essential to ensure correct organelle positioning and dynamics. The role of microtubules in the early steps of anterograde protein transport has been established over the past two decades, demonstrating that they are dispensable for ER-to-Golgi transport of carriers but facilitate this step when present. Our understanding of the role of microtubules in intra-Golgi transport is very limited, mainly because of difficulties to assess this transport step. At the Golgi

level, microtubules do have an important role by nucleating from Golgi membranes and organizing the Golgi through their minus-end dynamics. Microtubules might not be essential for post-Golgi transport but clearly accelerate the transport of carriers, especially in polarized cells, such as neurons (see Box 1, Fig. 1C), where transport over long distances is required.

We like to emphasize here that PTMs of tubulin constitute another level of regulation, and their importance for protein transport is being uncovered. In our opinion, to investigate the effects tubulin PTMs have on protein transport and the role tubulin PTMs in defining subpopulations of microtubules is an interesting research track to follow for the next years.

Recently, microtubules were assigned a key role in the delivery of exocytic cargos close to adhesion sites in 2D cell culture models. Microtubules are connected to FAs through protein interactions and, together, they sustain efficient targeted delivery of post-Golgi transport carriers. The role of microtubules in this transport step has clear physiological and pathological implications, as polarity cues and proteins involved in cell migration or cancer cell invasion are delivered by protein transport. An open question in the field remains the existence of targeted delivery of exocytic cargos in cells grown in three dimensions, such as invading cancer cells, in 3D-polarized cell culture models, organoids and even in tissues. To decipher the role of microtubules and to identify molecular regulators of protein secretion in these 3D models will be of particular importance, and might highlight novel pathways that can be targeted in a pathological context.

Competing interests

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