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# **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. Then, we discuss the involvement of microtubules in different trafficking steps, in particular between the endoplasmic reticulum (ER) and the Golgi apparatus, traffic through the Golgi apparatus itself and in post-Golgi processes. A large number of studies have assessed the involvement of microtubules in the transport of cargos from the Golgi apparatus 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. In the last part, in light of recent findings, we emphasize the role of microtubules in exocytosis, which is the final step of secretory protein transport, occurring close to focal adhesions.

## Introduction

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

Microtubules endow cells with intracellular polarity, being organized as one or multiple arrays of polarized tracks differently organized depending on cell types (Fig. 1). Microtubules are composed of heterodimers of alpha and beta-tubulin - alpha-tubulin being exposed at the microtubule minus-end and beta-tubulin at the plus-end. Minus ends slowly grow and are anchored to microtubule-organizing centres (Hendershott and Vale, 2014), whereas microtubule plus-ends ensure their 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 large number of microtubule-associated proteins (MAPs) exist (Bodakuntla et al., 2019), which structurally regulate the microtubule network itself, but also its dynamics and functions in cellular processes. Microtubule diversity is also generated owing to the existence of several post-translational modifications (PTMs) of tubulin, including acetylation, phosphorylation, poly-amylation, tyrosination-detyrosination, poly-glutamylolation and poly-glycylation (Janke, 2014). They recently became of great interest in diverse cellular processes including intracellular transport (see below). Tubulin PTMs regulate the microtubule network by modifying the mechanical properties of microtubules, their stability and by influencing their interactions with microtubule-associated proteins (Magiera et al., 2018b; Xu et al., 2017).

Molecular motors interact with microtubules and allow the movement of cargos along the microtubule tracks. Motors can either directly bind to cargo or, often, be associated 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). Whereas the majority of

kinesins are plus-end directed motors, kinesin-14 family members are, on the contrary, minus-end directed motors (Hirokawa et al., 2009; Kardon and Vale, 2009). Here, we will focus on the role of microtubules in the organization and regulation of the secretory pathway in 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). An intimate relationship between microtubules and focal adhesions do exist, playing a role in the last step of the secretory pathway, the exocytosis. In this review, we will discuss the contribution of microtubules to protein secretion from ER to Golgi and from Golgi to the plasma membrane. We will focus on kinesin-dependent processes and on the role of tubulin modifications in post-Golgi transport. We will also discuss the importance of microtubule for cargo exocytosis occurring in proximity to focal adhesions. Furthermore, we will put emphasis on microtubule-driven secretion in non-polarized cells, but mention 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 the one 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 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 was also 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, also known 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). However, 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  $\text{Ca}^{2+}$  overload (Chang et al., 2018).

The Golgi apparatus is the next compartment in the secretory pathway, receiving cargos from the ER. Functionally, it lies at the centre of the secretory pathway and physically, in many cell types, at the centre of the cell (Boncompain and Perez, 2013). The Golgi complex co-localizes with microtubule minus ends (Martin and Akhmanova, 2018). Microtubules and MAPs, such as calmodulin-regulated spectrin-associated protein 2 (CAMSAP2), myomegalin, EB1, EB3 and dynein, are responsible for the attachment of the Golgi apparatus 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 like ciliobrevin (Firestone et al., 2012). Such perturbation of dynein or microtubule organization induces the formation of Golgi mini-stacks that display a proper *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. Nocodazole-induced Golgi mini-stacks were even shown to facilitate the analysis of the intra-Golgi distribution of Golgi resident proteins or cargos by light microscopy (Dejgaard et al., 2007).

In many cell types, the main microtubule-organizing centre is the centrosome. However, conversely, 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). Whereas a role for the microtubule nucleating factor  $\gamma$ -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 450 kDa (AKAP450) who play the dominant role in nucleation of Golgi microtubules (Wu et al., 2016). The Golgi microtubule-associated protein 210 (GMAP-210) was shown to interact with  $\gamma$ -tubulin (Infante et al., 1999; Rios et al., 2004) and several Golgi proteins physically link Golgi membranes with microtubules such as Golgin-160, which binds to dynein (Yadav et al., 2012). The *trans*-Golgi network (TGN), GRIP- and coiled-coil domain-containing protein 185 (GCC185) was shown to interact with the microtubule-binding proteins CLIP-associating proteins (CLASPs) (Efimov et al., 2007). The *cis*-Golgi protein GM130 binds to AKAP450 allowing microtubule nucleation (Rivero et al., 2009). In addition, the association of both CLASPs and AKAP450 with microtubule cross-linking factor 1 (MTCL1) has been shown to enhance association of microtubule with the Golgi complex (Sato et al., 2014). It was further 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, CDK5RAP2 and myomegalin bind to  $\gamma$ -tubulin ring complexes at the *cis*-face of the Golgi apparatus (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, having 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 apparatus. 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) and (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 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 reach the Golgi apparatus 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 were demonstrated using the fluorescently tagged thermosensitive mutant of viral glycoprotein tsO45 VSVG that could be 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 of Sec23, a component of the COPII coat, and the C-terminal domain of 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 apparatus 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 motors 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 important for membrane recycling (Hirokawa et al., 2009). Importantly, even if 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 apparatus is dispersed and Golgi mini-stacks are formed in apposition of ERES (Cole et al., 1996; Presley et al., 1997). In 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 apparatus have been proposed over the years; to date, the cisternal maturation model and its variations appears to be prevalent one (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 apparatus between stacks of cisternae, which is a difficult endeavour. Although various cargos speeds have been

monitored inside the Golgi apparatus ((Beznoussenko et al., 2014) and our unpublished data), it is complicated to determine if microtubules are important for intra-Golgi trafficking. As described earlier, removal of microtubules strongly perturbs the Golgi apparatus 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 microtubule is to ensure functional maturation of the Golgi complex, but not physical movement of the cargos inside and out from the Golgi apparatus (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 apparatus. 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 the trafficking from the Golgi to the plasma membrane**

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

In 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; Schmoranzer 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, KIFC3 and KIF16B have been shown to be necessary for apical transport in polarized MDCK cells (Astania 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).



### *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. kinesin-1 (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), or 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 apparatus, the small GTPase RAB6 was 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 opposite 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 behaviour 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 may be needed when loading carriers with multiple motors than assembling and disassembling the full complexes each time a carrier should revert 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 the Rab6-BicaudalD1/2-KIF5B pathway (see below).

The role of kinesins may not be restricted to the movement of carriers, but also be important for their immediate release from the TGN: the kinesin 1 KIF5B has been shown to be necessary for the movement of post-Golgi transport carriers from the Golgi apparatus (Grigoriev et al., 2007; Miserey-Lenkei et al., 2010). Membrane

fission at the *trans*-Golgi network indeed occurs through coordinated work by the actin cytoskeleton, the myosin II motor, the GTPase Rab6, Bicaudal D1/2, Dynactin and the kinesin KIF20A (Hoogenraad et al., 2001; Miserey-Lenkei et al., 2017; Miserey-Lenkei et al., 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 motor-cargo interaction.

#### *Secretory transport, motor proteins and tubulin post-translational modifications*

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 kinesin-1 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, the drug-induced acetylation of microtubules has been shown to re-establish transport in neurons in neurodegenerative diseases like 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 epidermal growth factor receptor (EGFR) trafficking kinetics is increased by 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 preferential roads for the intracellular traffic in polarized cells, with a reduction of their level 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., 2018b; Roll-Mecak, 2019). Effects of tubulin PTMs are currently questioned not only *in vitro* but in living cells and in mouse models. For instance, floxed mice for genes coding for cytosolic carboxypeptidases (*CCP1* and *CCP6*) allowed questioning the effects of hyper polyglutamylation on axonal transport (Magiera et al., 2018a). The identification of the molecular players involved in tubulin PTMs and ways to perturb them will for sure shed light on this additional level of regulation of microtubule-based post-Golgi protein transport.

### **Confining exocytosis 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 localized in the leading edge of migrating cells (Schmoranzner et al., 2003). The last step of the secretory pathway occurs close to focal adhesion (FAs) and microtubule-FAs interaction is required. Focal adhesions (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 signalling cascades (mDia, Rho-family complex GTPases with RhoA, Rac and Cdc-42) that will modulate the actin and microtubule networks (Mitra et al., 2005). The balance of activated and inactivated Rac and Rho complexes close to the FAs 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 FAs formation, maturation and dissolution (Stehbens and Wittmann, 2012). Interestingly, microtubules attached to FAs are more stable (Kaverina et al., 1998) and tubulin acetylation of microtubules promotes FAs 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 migration edge. Indeed, depolymerisation of microtubules induces an increase in the size of FAs by stimulating actomyosin- and RhoA-dependent contractility (Rafiq et al., 2019; Ren et al., 1999) and inhibiting detachment of FA proteins (Chang et al., 2008). Two proteins that interact with the microtubule plus end CLASP proteins are ELKS and pleckstrin homology-like domain family B member 2, or LL5 $\beta$  (Lansbergen et al., 2006). LL5 $\beta$  is important for cell migration (Astro et al., 2014) and binds phosphatidylinositol-3,4,5-triphosphate (PIP3) at the plasma membrane and recruits ELKS in close proximity to the FAs. The complex ELKS-LL5 $\beta$  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 $\beta$  supports FAs turnover (Stehbens et al., 2014). Moreover, CLASPs depletion induces a decrease of microtubule density and the loss of microtubules associated with the cortex (Mimori-Kiyosue et al., 2005).

A large 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). The mechanisms that are responsible for such a localized exocytosis are being uncovered: in particular, the Rho signalling network (comprising guanine nucleotide exchange factor H1 (GEF-H1), the RhoGAP and the Rho effector phospholipase C (PLC)), which regulates the activation of the protein kinase D (PKD) at *trans*-Golgi network (TGN), coordinates the formation of TGN-derived Rab6-positive transport carriers delivering cargo to FAs-apposed regions (Eisler et al.,

2018). Cargos exit the TGN at fission hotspots, the post-Golgi carriers being by 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 events (Grigoriev et al., 2011) (Fig. 2C). The activation of RhoA in the TGN induces a local PKD activation followed by the fission of Rab6 vesicles that will then traffic towards the FAs (Eisler et al., 2018). Moreover, Rab6- and Rab8-positive vesicles have been shown to be transported by KIF5B along microtubules toward regions at the plasma membrane harbouring the protein ELKS and PIP3 that are located adjacent to FAs (Grigoriev et al., 2011; Lansbergen et al., 2006). In addition,  $\alpha$ -testis anion transporter 1 (TAT1)-mediated microtubule acetylation promotes the fusion of Rab6 vesicles with the plasma membrane at FAs (Bance et al., 2019). Stehbens et al. described a preferential exocytosis of membrane-type matrix metalloproteinase-1 (MT1-MMP) close to FAs that occurs using microtubules captured by CLASPs (Stehbens et al., 2014). Additionally, we recently reported that diverse cargos follow the Rab6- and microtubule-dependent pathway 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 does exist and post-Golgi transport is directed to adhesive zones through a subset of microtubules. Some physiological outcomes have been described such as an influence on cell migration and degradation of the extracellular matrix in 2D (Bance et al., 2019). In the near future, it will be interesting to assess if targeted exocytosis to adhesive sites takes place in 3D and to understand the role of microtubules in this process as this would have pathological outcomes for cancer cells such as an influence on their invasive, metastatic capabilities and 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 proper 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 microtubules 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 the difficulty 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 would like to emphasize here that tubulin PTMs constitute another level of regulation and their importance for protein transport is being uncovered. In our opinion, research on the effects of the tubulin PTMs on protein transport and their role in defining subpopulations of microtubules is an interesting 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 cells grown in two dimensions. 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 models, organoids and even in tissues. Deciphering the role of microtubules and identifying molecular regulators of protein secretion in these 3D models will be of particular importance and might highlight novel pathways to be targeted in a pathological context.

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## Figure legends

### **Fig. 1: Microtubule organization and polarity present variability in different cell types.**

**A.** In mesenchymal cells or fibroblasts, the microtubule network is mainly astral and emanates from the centrosome and the Golgi apparatus. The microtubule minus ends are therefore central and the plus ends are mainly peripheral. **B.** In epithelial cells, centrosomal microtubules are rare. In those cells, the filaments elongate towards the basal pole of the cell forming a parallel network. **C.** In the case of neurons, an astro-central array of microtubules irradiates from the centrosome and Golgi at the soma towards the periphery, the axon and the dendrites. **C-1.** Axonal microtubules form bundles of parallel microtubules with the same polarity (minus end at the soma and plus end at the periphery). **C-2.** On the contrary, in the dendrites, microtubules form anti-parallel beams of microtubules with mixed polarity and different stability, the microtubules oriented from the periphery towards the soma being more stable. **C-3.** It is possible that Golgi outposts contained in dendrites could play a role in the nucleation of microtubules with inverted polarity.

### **Fig. 2: Molecular machineries linking microtubules to main intracellular trafficking roads.**

**A.** Overview of microtubule organization and of their interplay with main secretory organelles (ER, Golgi apparatus and plasma membrane). Panels **B**, **C** and **D** represent close ups, zooming in on the main microtubule-organelle interfaces. **B.** Microtubules can bind to the ER directly through CLIMP63 or indirectly through interactions between plus-tip proteins and ER proteins like STIM1. **C.** On the other extremity of the trafficking road, microtubule plus-ends complexes interact with focal adhesion complexes and PIP3-domain-interacting proteins such as LL5 $\beta$ . **D.** At the center of these anterograde and retrograde roads, the Golgi apparatus plays a major role as a trafficking hub but also as a microtubule-organizing center owing to its proximity to the centrosome and its intrinsic microtubule nucleating (through  $\gamma$ -TuRC and Golgi protein-containing complexes) and capture capabilities (through the

interaction between Golgi proteins and microtubule minus-ends stabilizing proteins such as  $\gamma$ -TuRC complexes and CAMSAP and CLASP proteins).

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**Box 1: The role of microtubules in polarized protein trafficking in neurons**

Neurons are polarized cells composed of a cell body, a long axon and numerous branched dendrites (Fig. 1C). Their arborisation allows them to receive, process and transmit information. However, neuronal organisation challenges the secretory protein transport with the need to carry cargos over long distances, up to a meter 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, with plus ends extending outward in the axon when mixed anti-parallel orientation - polarized bundles with opposite orientation (Tas et al., 2017) - is observed in dendrites in mammalian neurons (Yau et al., 2016), which also harbour 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 have been shown to selectively mediate cargo transport in the axon such as kinesin-1, which does not enter dendrites, whereas kinesin-3 mediates transport in both axon and dendrites (Tas et al., 2017). This motor selectivity might depend on tubulin PTMs, such as acetylation and detyrosination for axonal selectivity of kinesin-1 (Cai et al., 2009; Konishi and Setou, 2009). Kinesin-3, which is a non-selective motor, has been proposed to bind to tyrosinated microtubules (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 detyrosinated microtubules are abundant in both axon and dendrites (Hammond et al., 2010). In dendrites, acetylated microtubules are oriented with their minus ends out, accounting for the plus end-directed kinesin-1 inefficiency to mediate transport in axons (Tas et al., 2017). In addition, kinesin-3 is negatively regulated by dynamic microtubules for the transport of synaptic vesicles (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).

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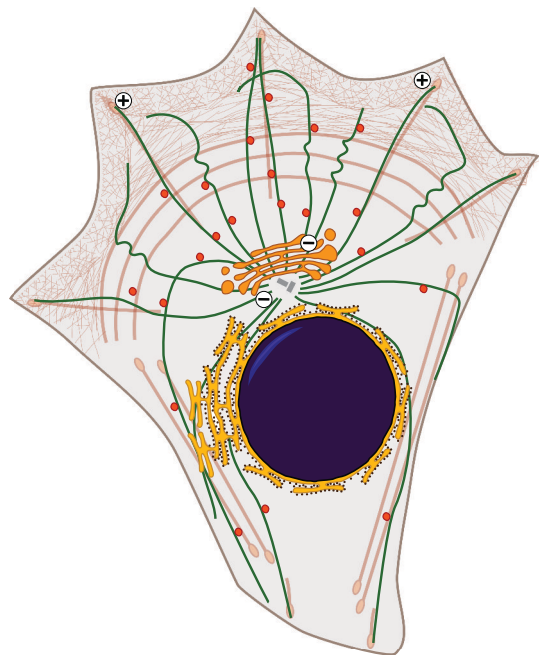
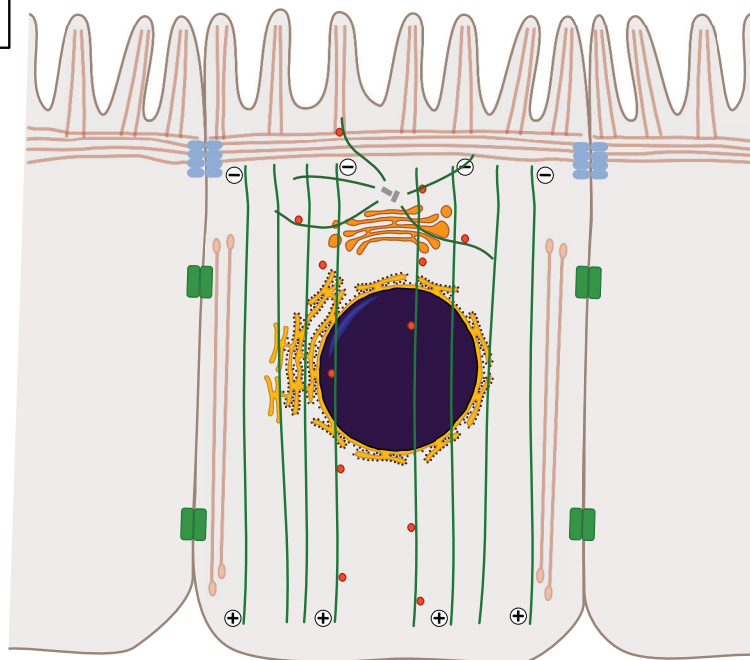
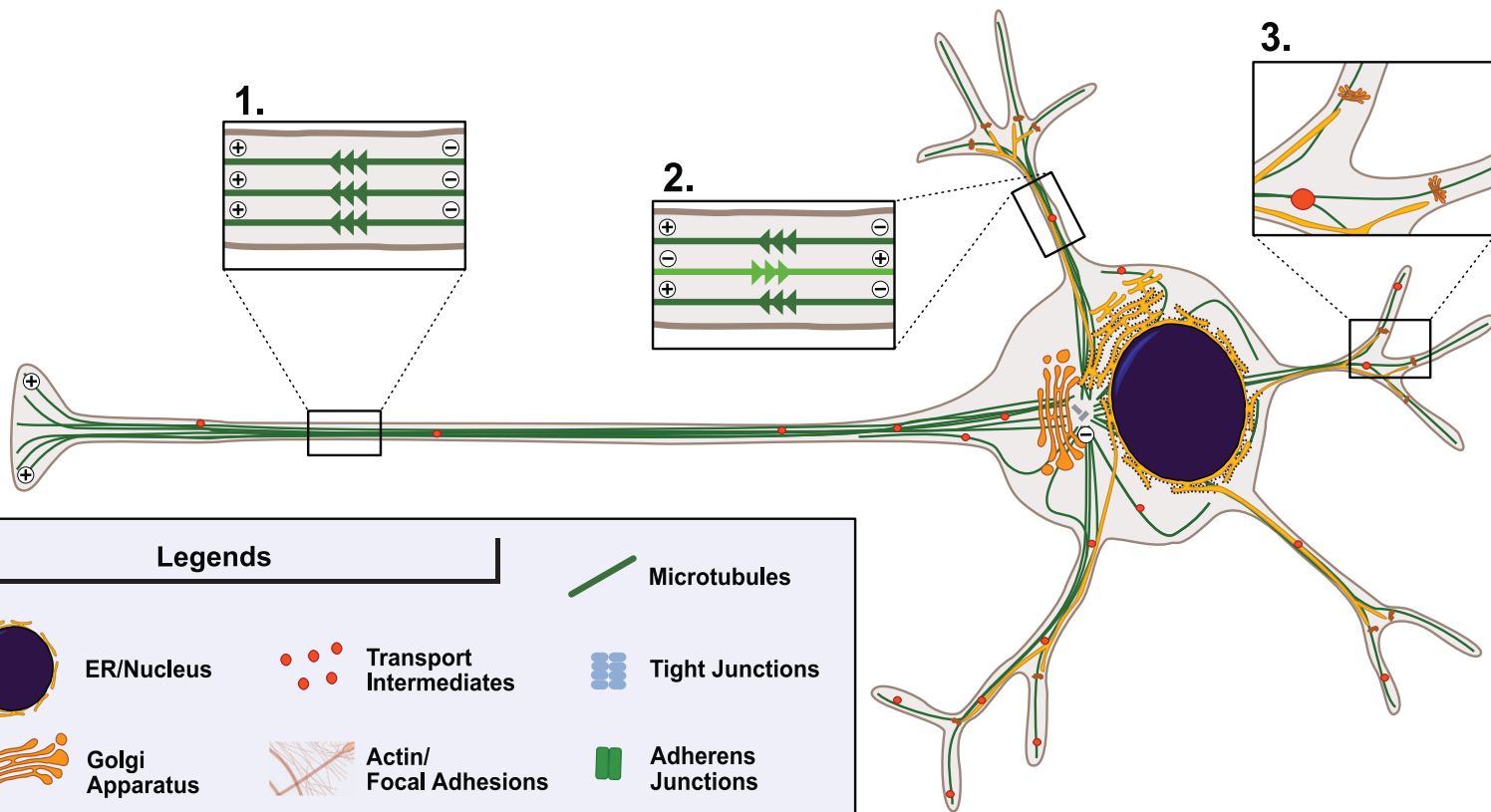
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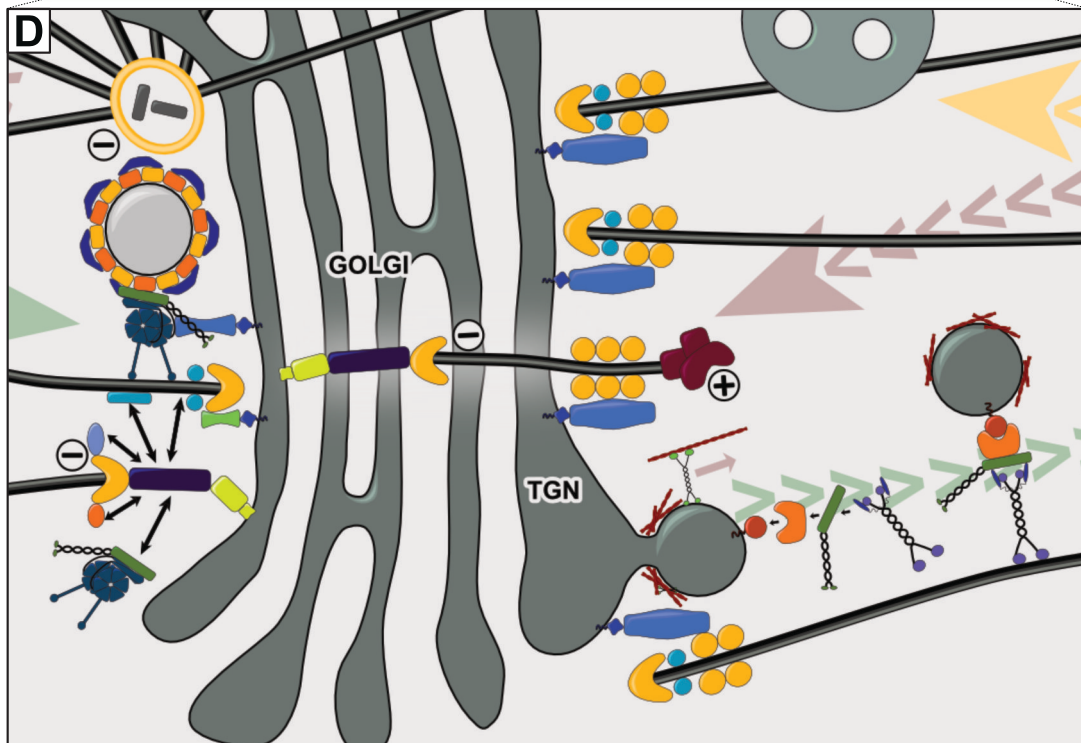
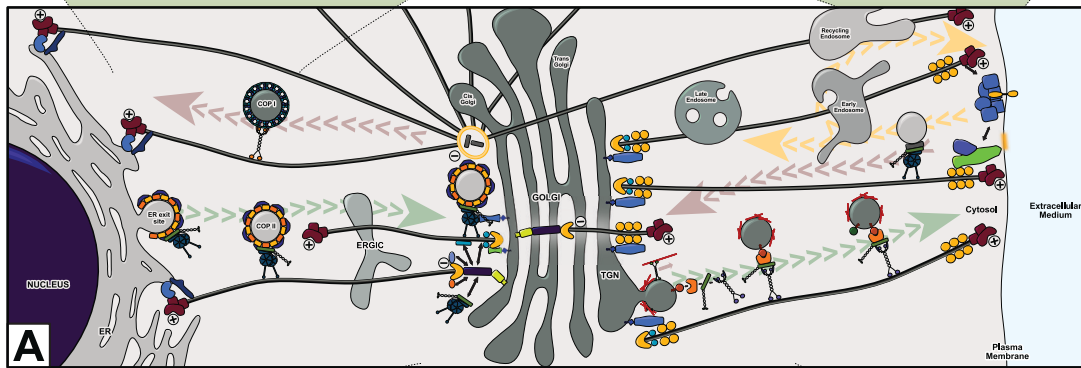
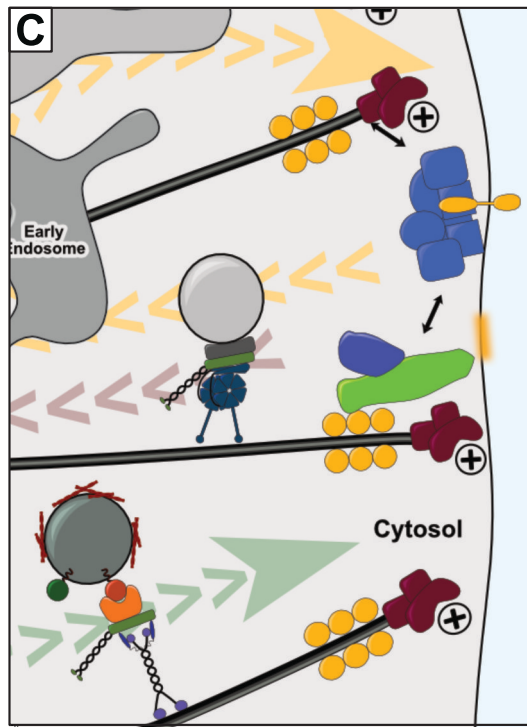
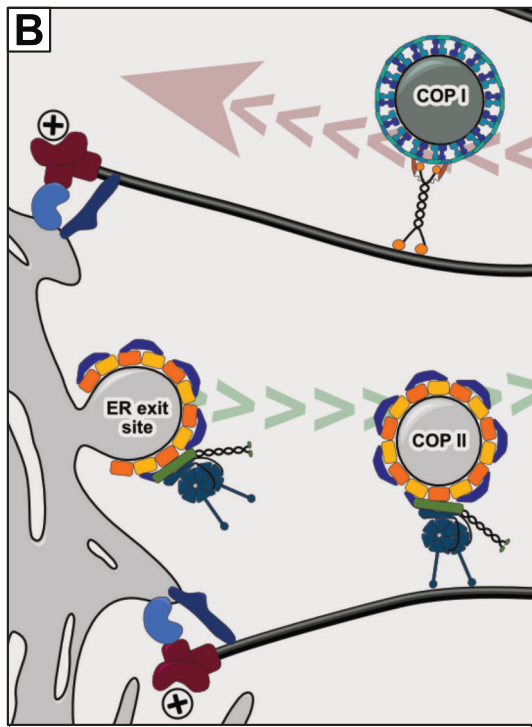
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## Legends

