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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.

35

36 **Introduction**

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

42 Microtubules endow cells with intracellular polarity, being organized as one or
43 multiple arrays of polarized tracks differently organized depending on cell types (Fig.
44 1). Microtubules are composed of heterodimers of alpha and beta-tubulin - alpha-
45 tubulin being exposed at the microtubule minus-end and beta-tubulin at the plus-end.
46 Minus ends slowly grow and are anchored to microtubule-organizing centres
47 (Hendershott and Vale, 2014), whereas microtubule plus-ends ensure their
48 elongation (Mitchison and Kirschner, 1984). Microtubules are highly dynamic
49 polymers, alternating phases of growth and shrinkage. The dynamic nature of
50 microtubules enables them to ensure exploration of the cytoplasm and fulfil multiple
51 functions. A large number of microtubule-associated proteins (MAPs) exist
52 (Bodakuntla et al., 2019), which structurally regulate the microtubule network itself,
53 but also its dynamics and functions in cellular processes. Microtubule diversity is also
54 generated owing to the existence of several post-translational modifications (PTMs)
55 of tubulin, including acetylation, phosphorylation, poly-amylation, tyrosination-
56 detyrosination, poly-glutamylated and poly-glycylation (Janke, 2014). They recently
57 became of great interest in diverse cellular processes including intracellular transport
58 (see below). Tubulin PTMs regulate the microtubule network by modifying the
59 mechanical properties of microtubules, their stability and by influencing their
60 interactions with microtubule-associated proteins (Magiera et al., 2018b; Xu et al.,
61 2017).

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

69 kinesins are plus-end directed motors, kinesin-14 family members are, on the
70 contrary, minus-end directed motors (Hirokawa et al., 2009; Kardon and Vale, 2009).
71 Here, we will focus on the role of microtubules in the organization and regulation of
72 the secretory pathway in mammalian cells. The secretory pathway ensures the
73 transport of proteins from the endoplasmic reticulum (ER) *via* the Golgi complex to
74 their destination compartment such as the cell surface, the endosomes or the
75 lysosomes (Boncompain and Weigel, 2018). Microtubules, together with molecular
76 motors, are known to accelerate long-range vesicular trafficking. However, whether
77 they are essential to ensure secretion is still debated (see below). An intimate
78 relationship between microtubules and focal adhesions do exist, playing a role in the
79 last step of the secretory pathway, the exocytosis. In this review, we will discuss the
80 contribution of microtubules to protein secretion from ER to Golgi and from Golgi to
81 the plasma membrane. We will focus on kinesin-dependent processes and on the
82 role of tubulin modifications in post-Golgi transport. We will also discuss the
83 importance of microtubule for cargo exocytosis occurring in proximity to focal
84 adhesions. Furthermore, we will put emphasis on microtubule-driven secretion in
85 non-polarized cells, but mention their importance in polarized cells such as neurons.

86

87 **Microtubules in the homeostasis of secretory pathway organelles**

88 The distribution of intracellular compartments that are necessary for protein secretion
89 is dependent on microtubules (Burkhardt et al., 1997; Vale, 1987), which vary in their
90 cellular organization depending on cell architecture (Fig. 1). The organization of the
91 organelles of the secretory pathway described below corresponds mainly to the one
92 of non-polarized cells. The ER is the organelle that constitutes the starting point of
93 the secretory pathway where proteins are translocated through the ER, either post-
94 or co-translationally (Mandon et al., 2013). The ER extends through the entire
95 cytoplasm and its distribution relies on an intact microtubule network, especially to
96 elongate ER tubules (Lee and Chen, 1988). ER tubules interact with microtubule plus
97 ends and use the force generated by microtubule polymerization to extend towards
98 the cell periphery (Grigoriev et al., 2008). Removal of microtubules increases the
99 proportion and the frequency of motion of ER sheets, which was also described as a
100 dense matrix of ER tubules (Nixon-Abell et al., 2016; Terasaki et al., 1986). The ER
101 membrane is connected to microtubules through ER-resident proteins, such as
102 cytoskeleton-associated protein 4 (CKAP4, also known as CLIMP-63) and stromal

103 interaction molecule 1 (STIM1) (Grigoriev et al., 2008; Vedrenne et al., 2005) (Fig. 2).
104 CLIMP-63 bears a microtubule-binding domain in its cytosolic part. When
105 overexpressed, CLIMP-63 leads to the re-arrangement of the microtubule network
106 (Klopfenstein et al., 1998). However, the precise function of CLIMP-63–microtubule
107 interactions remains unclear but it has been suggested to lie in anchoring the rough
108 ER to the cytoskeleton (Sandoz and van der Goot, 2015). The interaction between
109 STIM1 and growing microtubules, through the plus-end tracker end-binding protein 1
110 (EB1), has been shown to prevent Ca²⁺ overload (Chang et al., 2018).

111 The Golgi apparatus is the next compartment in the secretory pathway, receiving
112 cargos from the ER. Functionally, it lies at the centre of the secretory pathway and
113 physically, in many cell types, at the centre of the cell (Boncompain and Perez, 2013).
114 The Golgi complex co-localizes with microtubule minus ends (Martin and Akhmanova,
115 2018). Microtubules and MAPs, such as calmodulin-regulated spectrin-associated
116 protein 2 (CAMSAP2), myomegalin, EB1, EB3 and dynein, are responsible for the
117 attachment of the Golgi apparatus to the minus ends and for its central localization
118 (Corthesy-Theulaz et al., 1992; Sandoval et al., 1984; Wang et al., 2014; Wu et al.,
119 2016; Yang et al., 2017). Dynein inhibition or microtubule depolymerization induces
120 the reversible fragmentation and the dispersion of the Golgi complex to the extent
121 that we employ Golgi morphology as a read-out for dynein activity or the efficiency of
122 dynein-inhibiting drugs like ciliobrevin (Firestone et al., 2012). Such perturbation of
123 dynein or microtubule organization induces the formation of Golgi mini-stacks that
124 display a proper *cis-to-trans* organization (Cole et al., 1996; Trucco et al., 2004),
125 indicating that microtubules are not required for the organization of the Golgi stacks
126 themselves. Nocodazole-induced Golgi mini-stacks were even shown to facilitate the
127 analysis of the intra-Golgi distribution of Golgi resident proteins or cargos by light
128 microscopy (Dejgaard et al., 2007).

129 In many cell types, the main microtubule-organizing centre is the centrosome.
130 However, conversely, microtubules can also be nucleated from Golgi membranes
131 (Chabin-Brion et al., 2001; Efimov et al., 2007). These non-centrosomal microtubules
132 are important for the perinuclear localization of the Golgi complex (Hoppeler-Lebel et
133 al., 2007), for polarized cell migration (Wu et al., 2016) and for directed post-Golgi
134 transport (Efimov et al., 2007; Miller et al., 2009) (Fig. 2A, D). Whereas a role for the
135 microtubule nucleating factor γ -tubulin in Golgi-near microtubule nucleation has been
136 reported (Chabin-Brion et al., 2001), it is Golgi-associated microtubule-binding

137 proteins such as CAMSAP2 and a-kinase anchor protein 450 kDa (AKAP450) who
138 play the dominant role in nucleation of Golgi microtubules (Wu et al., 2016). The
139 Golgi microtubule-associated protein 210 (GMAP-210) was shown to interact with γ -
140 tubulin (Infante et al., 1999; Rios et al., 2004) and several Golgi proteins physically
141 link Golgi membranes with microtubules such as Golgin-160, which binds to dynein
142 (Yadav et al., 2012). The *trans*-Golgi network (TGN), GRIP- and coiled-coil domain-
143 containing protein 185 (GCC185) was shown to interact with the microtubule-binding
144 proteins CLIP-associating proteins (CLASPs) (Efimov et al., 2007). The *cis*-Golgi
145 protein GM130 binds to AKAP450 allowing microtubule nucleation (Rivero et al.,
146 2009). In addition, the association of both CLASPs and AKAP450 with microtubule
147 cross-linking factor 1 (MTCL1) has been shown to enhance association of
148 microtubule with the Golgi complex (Sato et al., 2014). It was further demonstrated
149 that through GM130, AKAP450 and myomegalin, Golgi membranes are connected to
150 the microtubule-binding proteins CAMSAP2 (Wu et al., 2016) and EB1 an EB3 (Yang
151 et al., 2017). The proteins AKAP450, CDK5RAP2 and myomegalin bind to γ -tubulin
152 ring complexes at the *cis*-face of the Golgi apparatus (Gavilan et al., 2018; Wang et
153 al., 2010). It should be noted that several isoforms of myomegalin exist, two of them,
154 CM-MMG and EB-MMG, having different functions, promoting microtubule nucleation
155 and restricting microtubule growth, respectively (Roubin et al., 2013).

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

163

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

165 After their translocation in the ER, cargos are concentrated in specialized export
166 domains, named ER-exit sites (ERES), from where COPII-coated vesicles are
167 formed (Bannykh et al., 1996). Microtubules are not required to generate force to
168 ensure budding of COPII vesicles since they still form spontaneously in their absence
169 (Presley et al., 1997). COPII vesicles then fuse to create the ER-Golgi intermediate
170 compartment (ERGIC) (Brandizzi and Barlowe, 2013; Xu and Hay, 2004). These pre-

171 Golgi carriers have to be transported to reach the Golgi apparatus owing to the
172 separation of ER and Golgi complex in mammalian cells. Microtubule-based
173 movement of ER-to-Golgi transport carriers and the role of dynein therein were
174 demonstrated using the fluorescently tagged thermosensitive mutant of viral
175 glycoprotein tsO45 VSVG that could be tracked in pre-Golgi structures moving
176 towards the Golgi (Presley et al., 1997; Scales et al., 1997). The interaction between
177 ERES and microtubules is ensured by binding of Sec23, a component of the COPII
178 coat, and the C-terminal domain of p150glued, a component of the dynein activator
179 complex (Watson et al., 2005) (Fig. 2B). Dynein and its accessory subunits mediate
180 minus-end directed transport allowing ER-to-Golgi vesicles to reach the centrally
181 located Golgi apparatus from the dispersed ERES (Burkhardt et al., 1997; Palmer et
182 al., 2009) (Fig. 2A, B). However, kinesins are also present on pre-Golgi vesicles
183 (Lippincott-Schwartz et al., 1990) and as a consequence, the presence of both kinds
184 of motors generates opposing forces, which allow bi-directional movement of vesicles
185 on microtubules (Brown et al., 2014). More specifically, kinesins that are present on
186 pre-Golgi vesicles ensure Golgi-to-ER retrograde motility, which is important for
187 membrane recycling (Hirokawa et al., 2009).

188 Importantly, even if the involvement of microtubules in ER-to-Golgi transport has
189 been clearly demonstrated over the last decades, this transport still occurs in the
190 absence of microtubules (Cole et al., 1996). As indicated above, without microtubules
191 the Golgi apparatus is dispersed and Golgi mini-stacks are formed in apposition of
192 ERES (Cole et al., 1996; Presley et al., 1997). In these conditions, transport of newly
193 synthesized cargos occurs efficiently since COPII vesicles are still formed and ERES
194 and Golgi mini-stacks are in close proximity.

195 Several models that describe how cargos are transported inside the Golgi apparatus
196 have been proposed over the years; to date, the cisternal maturation model and its
197 variations appears to be prevalent one (Boncompain and Perez, 2013; Boncompain
198 and Weigel, 2018; Glick and Luini, 2011). According to this model, new Golgi
199 cisternae form by coalescence of ER-derived membranes and secretory proteins are
200 conveyed inside the cisternae. The identity of Golgi compartments is ensured by
201 constant retrograde transport of Golgi-resident proteins. To understand the role of
202 microtubules in intra-Golgi transport, it is important to monitor and quantify the
203 movement of cargos or Golgi enzymes inside the Golgi apparatus between stacks of
204 cisternae, which is a difficult endeavour. Although various cargos speeds have been

205 monitored inside the Golgi apparatus ((Beznoussenko et al., 2014) and our
206 unpublished data), it is complicated to determine if microtubules are important for
207 intra-Golgi trafficking. As described earlier, removal of microtubules strongly perturbs
208 the Golgi apparatus structure and induces the dispersion of Golgi mini-stacks
209 throughout the cytoplasm (Cole et al., 1996; Trucco et al., 2004). It is thus essential
210 to establish approaches where microtubules can be fully disassembled while
211 preventing cargo transport and subsequently monitor transport. We developed the
212 retention using selective hooks (RUSH) system to synchronize the trafficking of
213 different cargos (Boncompain et al., 2012) and monitor their transport through the
214 cisternae in absence of microtubules (Fourriere et al., 2016). Our results indicate that
215 microtubules are dispensable for intra-Golgi transport and that the role of microtubule
216 is to ensure functional maturation of the Golgi complex, but not physical movement of
217 the cargos inside and out from the Golgi apparatus (Fourriere et al., 2016).
218 Therefore, microtubules appear not to be essential for ER-to-Golgi and intra-Golgi
219 protein transport. However, microtubules, when present, are used to facilitate the
220 movement of transport carriers from the ER to the Golgi apparatus. Nonetheless, the
221 challenges to assess intra-Golgi transport, especially in the absence of microtubules,
222 limit our current understanding of the role of microtubules in intra-Golgi transport.

223

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

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

228 In physiological conditions, cargos leave the TGN inside vesicular, granular or tubular
229 structures that use microtubules to achieve a fast and directed transport (Cole and
230 Lippincott-Schwartz, 1995; Schmoranzer and Simon, 2003; Toomre et al., 1999).
231 Cargos trafficking from the TGN to downstream compartments use various kinesins
232 as molecular motors (Hirokawa et al., 2009). Reported in most mammalian cell types,
233 this kinesin-dependent transport of cargos is particularly important in highly polarized
234 cells, and KIF5B, KIF5C, KIFC3 and KIF16B have been shown to be necessary for
235 apical transport in polarized MDCK cells (Astania and Jacob, 2010; Jaulin et al.,
236 2007; Noda et al., 2001; Perez Bay et al., 2013). Microtubules are also of particular
237 importance in trafficking pathways in neurons (see Box 1, Fig. 1).

238

239 *Motors and adaptor proteins in post-Golgi transport*

240 Recruitment of kinesins to carriers is reversible and determines the final destination
241 of the cargo (Akhmanova and Hammer, 2010). As diverse kinesins are involved in
242 the transport of chosen carriers, a specific recruitment of the motors must occur.
243 Carriers can bind kinesin directly (e.g. kinesin-1 (Kamal et al., 2000)); however, most
244 of the time, adaptors and intermediates are needed. Such a function was shown for
245 the receptor-recycling retromer complex (Hunt et al., 2013; Wassmer et al., 2009), or
246 adaptor protein 1 (AP-1) (Campagne et al., 2018; Delevoye et al., 2009), which
247 enable the recruitment of kinesins on endosomal membranes. At the level of the
248 Golgi apparatus, the small GTPase RAB6 was shown to recruit KIF20A to Golgi
249 membranes to promote the fission of post-Golgi carriers (Miserey-Lenkei et al., 2017)
250 (Fig. 2D).

251 Intriguingly, different molecular motors, sometimes with opposite directionality, can
252 be found on the same carrier, leading to a bi-directional movement of the carrier
253 (Hirokawa et al., 2010; Vale, 2003) in a tug of war. Each class of motor can be
254 specifically activated and alternates between having the role of a motor or of a
255 passenger (Derr et al., 2012; Kunwar et al., 2011; Muller et al., 2008; Welte, 2004).
256 Importantly, it has been shown that several motors are activated upon binding to their
257 receptor on the carriers (Derr et al., 2012; Kunwar et al., 2011); cycles of binding and
258 release from the carriers could also be the basis of bi-directional movement. This
259 behaviour may be surprising as it appears costly for the cell and reduces the mean
260 transport speed, but it has been proposed that these cycles avoid road blocks along
261 busy microtubule tracks (Telley et al., 2009) and help in the proofreading of wrongly
262 oriented carriers (Ally et al., 2009; Jolly and Gelfand, 2011). Also, less energy may
263 be needed when loading carriers with multiple motors than assembling and
264 disassembling the full complexes each time a carrier should revert its direction (Welte,
265 2004). In the context of post-Golgi transport, loading of multiple motors on carriers
266 might require adaptors, for which little is known except the Rab6-BicaudalD1/2-KIF5B
267 pathway (see below).

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

272 fission at the *trans*-Golgi network indeed occurs through coordinated work by the
273 actin cytoskeleton, the myosin II motor, the GTPase Rab6, Bicaudal D1/2, Dynactin
274 and the kinesin KIF20A (Hoogenraad et al., 2001; Miserey-Lenkei et al., 2017;
275 Miserey-Lenkei et al., 2010) (Fig. 2D). Rab6 has also been shown to directly interact
276 with KIF1C, which inhibits interaction of the motor with microtubules and slows down
277 the motility of post-Golgi carriers (Lee et al., 2015a). However, as noted above, our
278 observation that Golgi-to-plasma membrane traffic can occur efficiently in the
279 absence of microtubules (Fourriere et al., 2016) suggests a supportive, but not
280 essential role for kinesins in post-Golgi cargo movement.

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

285

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

287 As introduced above, tubulin is subjected to PTMs (Janke, 2014).

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

298 Interestingly, PTMs of tubulin have also been associated with defects in transport:
299 abnormally high tubulin poly-glutamylation causes defects in neuronal transport
300 (Magiera et al., 2018a). Furthermore, the drug-induced acetylation of microtubules
301 has been shown to re-establish transport in neurons in neurodegenerative diseases
302 like Charcot-Marie-Tooth and Huntington's (d'Ydewalle et al., 2011; Dompierre et al.,
303 2007), as well as for mutations in leucine-rich repeat serine/threonine-protein kinase
304 2 (LRRK2) by inhibiting aberrant interactions of mutated LRRK2 with de-acetylated

305 microtubules (Godena et al., 2014). Interestingly, it has been shown that epidermal
306 growth factor receptor (EGFR) trafficking kinetics is increased by inhibition of histone
307 deacetylase 6 (HDAC6) and microtubule acetylation (Deribe et al., 2009; Lee et al.,
308 2015b). Detyrosinated microtubules are preferentially chosen by KIF5C (Dunn et al.,
309 2008; Hammond et al., 2010) and they seem to be preferential roads for the
310 intracellular traffic in polarized cells, with a reduction of their level leading to strong
311 decrease in apical protein secretion (Zink et al., 2012).

312 Tubulin PTMs and the resulting microtubule diversity are of recent focus. At the same
313 time, several studies question the effects of tubulin PTMs on the function of motors
314 and on intracellular transport and their link to diseases (Magiera et al., 2018b; Roll-
315 Mecak, 2019). Effects of tubulin PTMs are currently questioned not only *in vitro* but in
316 living cells and in mouse models. For instance, floxed mice for genes coding for
317 cytosolic carboxypeptidases (*CCP1 and CCP6*) allowed questioning the effects of
318 hyper polyglutamylation on axonal transport (Magiera et al., 2018a). 260The
319 identification of the molecular players involved in tubulin PTMs and ways to perturb
320 them will for sure shed light on this additional level of regulation of microtubule-based
321 post-Golgi protein transport.

322

323 **Confining exocytosis using microtubules and a localized fusion machinery**

324 The final step of the secretory pathway is the arrival of cargos at the plasma
325 membrane for subsequent exocytosis. Microtubules deliver vesicles to particular
326 plasma membrane domains that are localized in the leading edge of migrating cells
327 (Schmoranzler et al., 2003). The last step of the secretory pathway occurs close to
328 focal adhesion (FAs) and microtubule-FAs interaction is required. Focal adhesions
329 (FAs) constitute an adhesive platform at the plasma membrane composed of more
330 than 180 proteins, which link actin and integrin networks to the extracellular matrix
331 (Haase et al., 2014). FAs are very dynamic and are able to adjust their shape,
332 position and composition according to the needs of the cell and to the rigidity and
333 composition of the cell substrate (Rivelino et al., 2001). Through their permanent
334 contact between the cytoskeleton and the extracellular matrix, FAs play a role in the
335 formation, maintenance and transmission of forces between the cell and the
336 environment (Chen et al., 2015). On a molecular level, focal adhesion kinase (FAK)
337 receives signals from growth factors and integrins and in turn recruits new FA

338 proteins or adaptors to the adhesive region and activates signalling cascades (mDia,
339 Rho-family complex GTPases with RhoA, Rac and Cdc-42) that will modulate the
340 actin and microtubule networks (Mitra et al., 2005). The balance of activated and
341 inactivated Rac and Rho complexes close to the FAs region is important for the
342 targeting of FAs by microtubules and for cell polarity (Small and Kaverina, 2003).

343 Microtubules are tethered at adhesion sites and influence FAs formation, maturation
344 and dissolution (Stehbens and Wittmann, 2012). Interestingly, microtubules attached
345 to FAs are more stable (Kaverina et al., 1998) and tubulin acetylation of microtubules
346 promotes FAs turnover and cell migration (Bance et al., 2019; Dubois et al., 2017).
347 Microtubules determine cellular polarity by modulating the distribution of FAs, which
348 are continuously formed and disassembled at the migration edge. Indeed,
349 depolymerisation of microtubules induces an increase in the size of FAs by
350 stimulating actomyosin- and RhoA-dependent contractility (Rafiq et al., 2019; Ren et
351 al., 1999) and inhibiting detachment of FA proteins (Chang et al., 2008). Two proteins
352 that interact with the microtubule plus end CLASP proteins are ELKS and pleckstrin
353 homology-like domain family B member 2, or LL5 β (Lansbergen et al., 2006). LL5 β is
354 important for cell migration (Astro et al., 2014) and binds phosphatidylinositol-3,4,5-
355 triphosphate (PIP3) at the plasma membrane and recruits ELKS in close proximity to
356 the FAs. The complex ELKS-LL5 β acts like a platform to recruit CLASPs who will
357 then attach and stabilize microtubules (Fig. 2C) (Lansbergen et al., 2006). It has
358 been shown that the direct recruitment of CLASPs by LL5 β supports FAs turnover
359 (Stehbens et al., 2014). Moreover, CLASPs depletion induces a decrease of
360 microtubule density and the loss of microtubules associated with the cortex (Mimori-
361 Kiyosue et al., 2005).

362 A large number of factors (e.g. microtubule plus-end tracking proteins, scaffolding
363 factors, actin-binding proteins, docking machinery components) coordinate
364 microtubule attachment at the cell membrane and localized exocytosis (Noordstra
365 and Akhmanova, 2017). The mechanisms that are responsible for such a localized
366 exocytosis are being uncovered: in particular, the Rho signalling network (comprising
367 guanine nucleotide exchange factor H1 (GEF-H1), the RhoGAP and the Rho effector
368 phospholipase C (PLC)), which regulates the activation of the protein kinase D (PKD)
369 at *trans*-Golgi network (TGN), coordinates the formation of TGN-derived Rab6-
370 positive transport carriers delivering cargo to FAs-apposed regions (Eisler et al.,

371 2018). Cargos exit the TGN at fission hotspots, the post-Golgi carriers being by Rab6
372 (Miserey-Lenkei et al., 2017) (Fig. 2D). Indeed, both Rab6 and Rab8 play a key role
373 in the attachment and fusion of vesicles to the plasma membrane and are required
374 for exocytosis events (Grigoriev et al., 2011) (Fig. 2C). The activation of RhoA in the
375 TGN induces a local PKD activation followed by the fission of Rab6 vesicles that will
376 then traffic towards the FAs (Eisler et al., 2018). Moreover, Rab6- and Rab8-positive
377 vesicles have been shown to be transported by KIF5B along microtubules toward
378 regions at the plasma membrane harbouring the protein ELKS and PIP3 that are
379 located adjacent to FAs (Grigoriev et al., 2011; Lansbergen et al., 2006). In addition,
380 α -testis anion transporter 1 (TAT1)-mediated microtubule acetylation promotes the
381 fusion of Rab6 vesicles with the plasma membrane at FAs (Bance et al., 2019).
382 Stehbens et al. described a preferential exocytosis of membrane-type matrix
383 metalloproteinase-1 (MT1-MMP) close to FAs that occurs using microtubules
384 captured by CLASPs (Stehbens et al., 2014). Additionally, we recently reported that
385 diverse cargoes follow the Rab6- and microtubule-dependent pathway sustaining
386 exocytosis close to FAs and that the carriers used a sub-population of microtubules
387 toward FAs (Fourriere et al., 2019).

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

396

397 **Conclusions and perspectives**

398 Microtubules are polarized tracks that stage-manage cell shape, mitosis and
399 intracellular trafficking. They are essential to ensure proper organelle positioning and
400 dynamics. The role of microtubules in the early steps of anterograde protein transport
401 has been established over the past two decades, demonstrating that microtubules
402 are dispensable for ER to Golgi transport of carriers but facilitate this step when

403 present. Our understanding of the role of microtubules in intra-Golgi transport is very
404 limited mainly because of the difficulty to assess this transport step. At the Golgi level,
405 microtubules do have an important role by nucleating from Golgi membranes and
406 organizing the Golgi through their minus-end dynamics. Microtubules might not be
407 essential for post-Golgi transport but clearly accelerate the transport of carriers,
408 especially in polarized cells such as neurons (see Box 1, Fig. 1C), where transport
409 over long distances is required.

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

415 Recently, microtubules were assigned a key role in the delivery of exocytic cargos
416 close to adhesion sites in cells grown in two dimensions. Microtubules are connected
417 to FAs through protein interactions and together they sustain efficient targeted
418 delivery of post-Golgi transport carriers. The role of microtubules in this transport
419 step has clear physiological and pathological implications as polarity cues and
420 proteins involved in cell migration or cancer cell invasion are delivered by protein
421 transport. An open question in the field remains the existence of targeted delivery of
422 exocytic cargos in cells grown in three dimensions, such as invading cancer cells, in
423 3D-polarized models, organoids and even in tissues. Deciphering the role of
424 microtubules and identifying molecular regulators of protein secretion in these 3D
425 models will be of particular importance and might highlight novel pathways to be
426 targeted in a pathological context.

427

428

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435

436

437 **Figure legends**

438

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

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

454

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

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

468 interaction between Golgi proteins and microtubule minus-ends stabilizing proteins
469 such as γ -TuRC complexes and CAMSAP and CLASP proteins).

470

471

472

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

474 Neurons are polarized cells composed of a cell body, a long axon and numerous
475 branched dendrites (Fig. 1C). Their arborisation allows them to receive, process and
476 transmit information. However, neuronal organisation challenges the secretory
477 protein transport with the need to carry cargos over long distances, up to a meter in
478 motor neurons. Microtubules are key players in the organization of neurons and in
479 polarized transport (Kelliher et al., 2019). Microtubules differ in their polarity and
480 stability between axon and dendrites, with plus ends extending outward in the axon
481 when mixed anti-parallel orientation - polarized bundles with opposite orientation
482 (Tas et al., 2017) - is observed in dendrites in mammalian neurons (Yau et al., 2016),
483 which also harbour microtubule-dependent Golgi outposts (Gardiol et al., 1999;
484 Horton and Ehlers, 2003); (Fig. 1C). Dynein and kinesins are necessary to ensure
485 anterograde and retrograde movements (Bentley and Banker, 2016). In the axon,
486 dynein drives retrograde transport and kinesins drive anterograde transport, whereas
487 dynein drives bidirectional transport in dendrites (Kapitein et al., 2010). Certain
488 motors have been shown to selectively mediate cargo transport in the axon such as
489 kinesin-1, which does not enter dendrites, whereas kinesin-3 mediates transport in
490 both axon and dendrites (Tas et al., 2017). This motor selectivity might depend on
491 tubulin PTMs, such as acetylation and detyrosination for axonal selectivity of kinesin-
492 1 (Cai et al., 2009; Konishi and Setou, 2009). Kinesin-3, which is a non-selective
493 motor, has been proposed to bind to tyrosinated microtubules (Guardia et al., 2016;
494 Lipka et al., 2016). However, the preferential binding of these motors to PTMs of
495 tubulin does not fully explain axonal motor selectivity because acetylated and
496 detyrosinated microtubules are abundant in both axon and dendrites (Hammond et
497 al., 2010). In dendrites, acetylated microtubules are oriented with their minus ends
498 out, accounting for the plus end-directed kinesin-1 inefficiency to mediate transport in
499 axons (Tas et al., 2017). In addition, kinesin-3 is negatively regulated by dynamic
500 microtubules for the transport of synaptic vesicles (Guedes-Dias et al., 2019).

501 Defects in microtubule-dependent trafficking have pathological implications and
502 contribute to neurodevelopmental disorders (Lasser et al., 2018) and
503 neurodegenerative diseases (Brunden et al., 2017).

504

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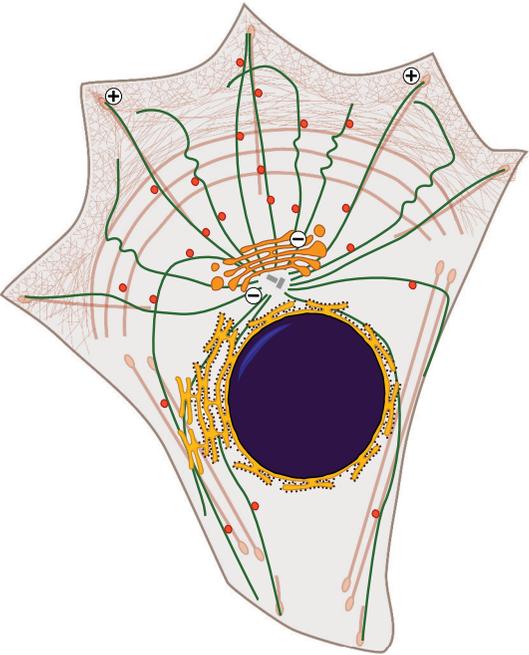
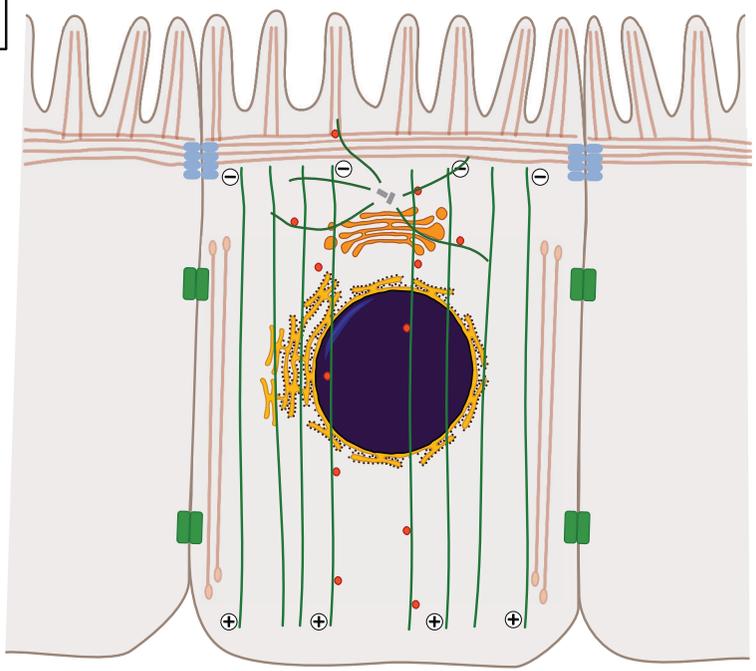
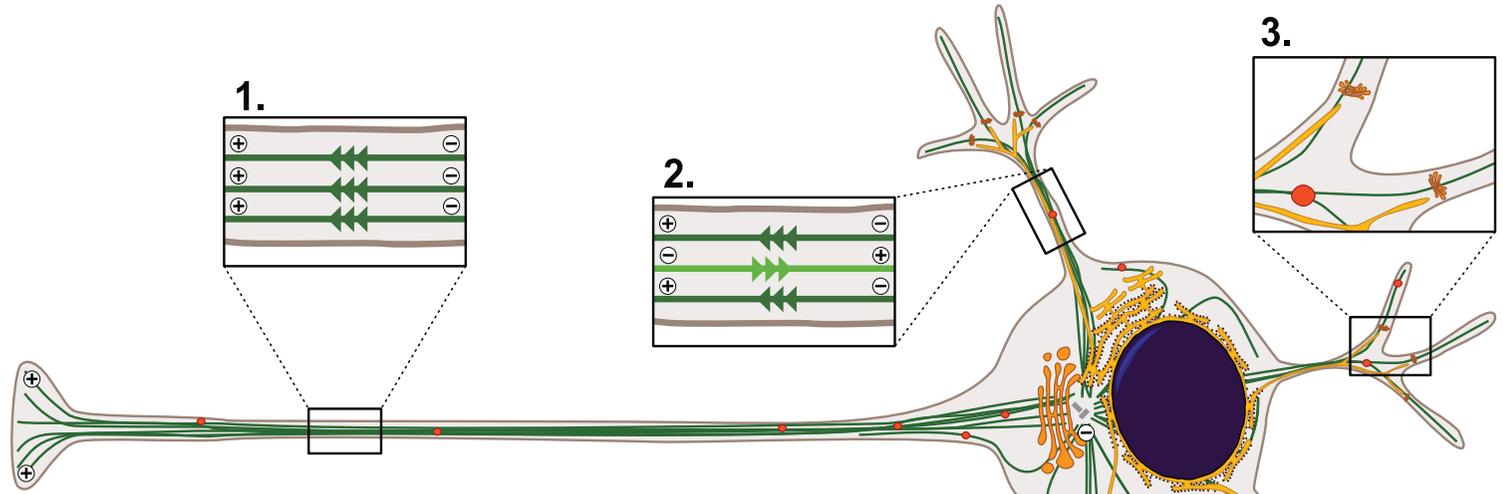
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886

A**B****C****Legends**

ER/Nucleus



Golgi Apparatus



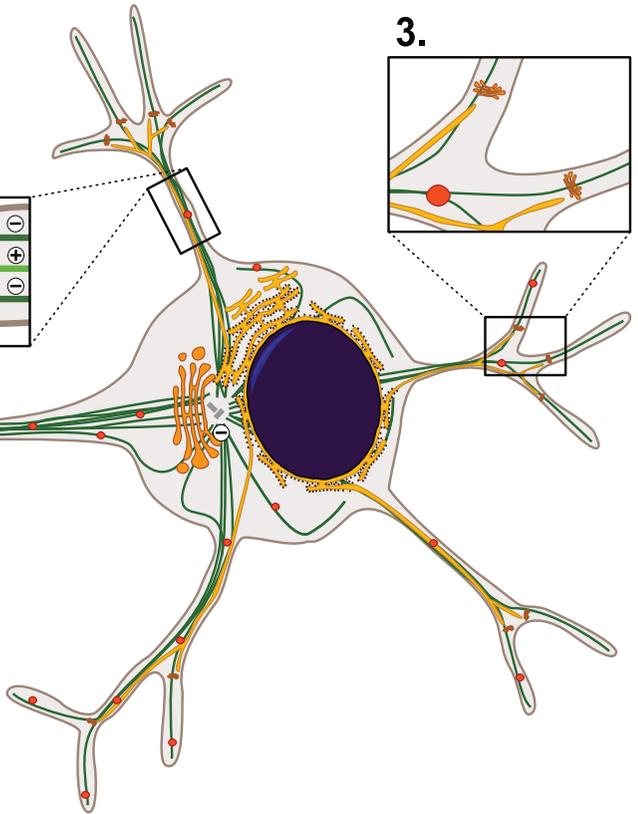
Transport Intermediates

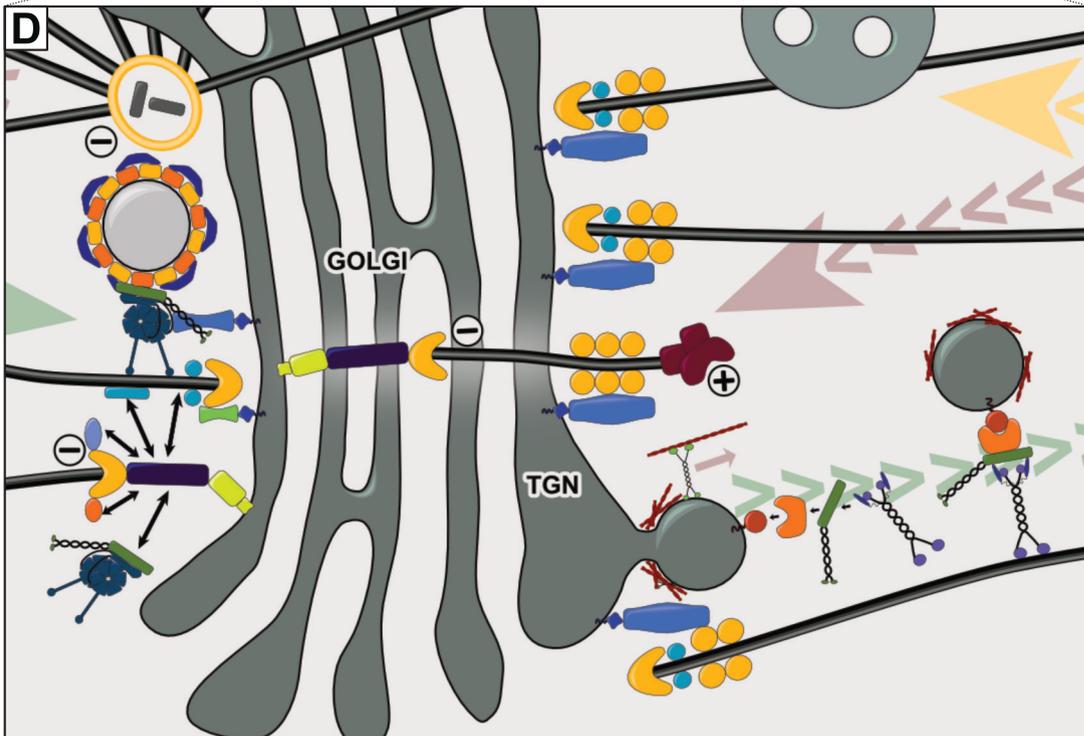
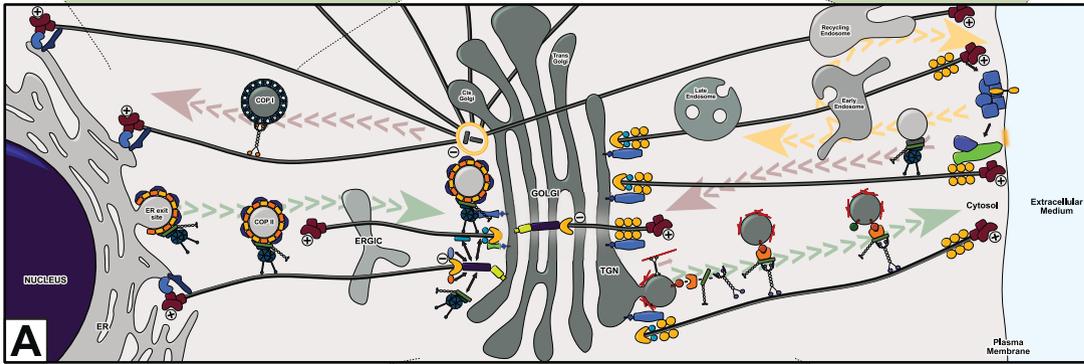
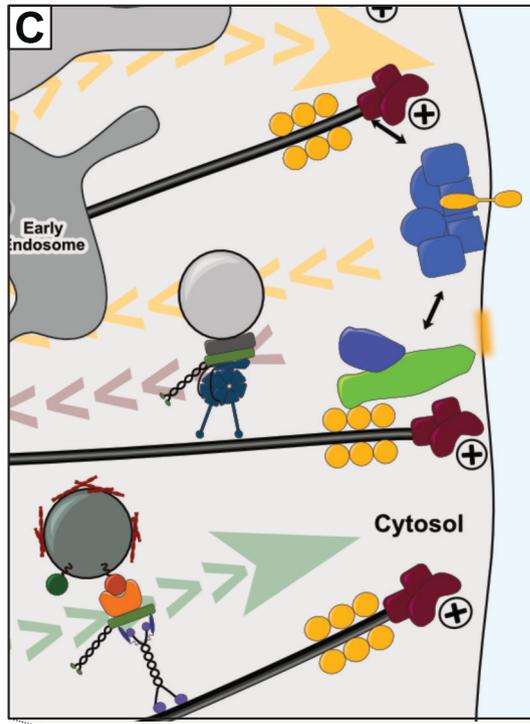
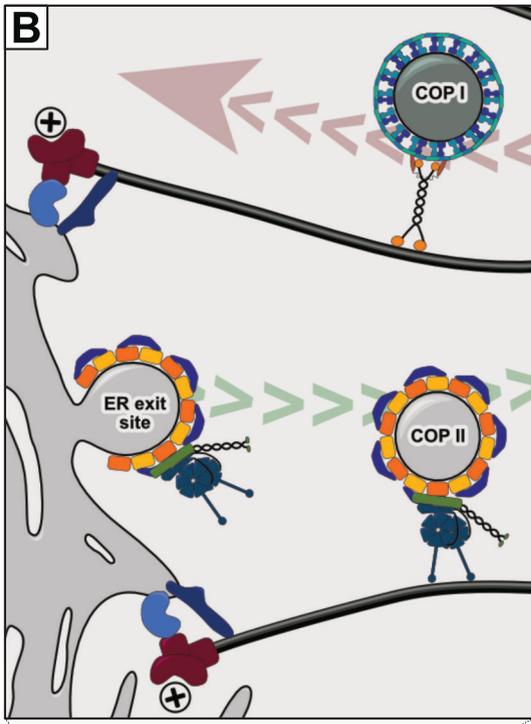
Actin/
Focal Adhesions

Microtubules



Tight Junctions

Adherens
Junctions



Legends

-  Microtubules
-  Centrosome
-  γ-Turc complex
-  Plus-Tips proteins
-  STIM1
-  CLIMP-63
-  COP I complex
-  Kinesins (KIF5, KIF3, KIF1C)
-  Sec23/24
-  Sec13/31
-  Dynactin (with p150glued)
-  Dynein 1
-  Arf1/Golgin160
-  GMAP210/Arf1
-  AKAP450
-  GRASP65/GM130
-  Myomegalin
-  CDK5RAP2
-  MTCL1
-  CAMSAPs
-  CLASPS (1 and 2)
-  Arf1/GCC185
-  Acto-myosin complexes
-  Rab6
-  Rab8
-  Bicaudal D1/2
-  Kinesin (KIF5, KIF13A, KIF20A)
-  Dynein adaptors
-  PIP3
-  LL5b
-  ELKS
-  Focal adhesion
-  Retrograde traffic
-  Anterograde traffic
-  Endosome recycling transport