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REVIEW

Rab-mediated trafficking role in neurite formation

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Abstract

Neuronal cells are characterized by the presence of two confined domains, which are different in their cellular properties, biochemical functions and molecular identity. The generation of asymmetric domains in neurons should logically require specialized membrane trafficking to both promote neurite outgrowth and differential distribution of components. Members of the Rab family of small GTPases are key regulators of membrane trafficking involved in transport, tethering and docking of vesicles through their effectors. RabGTPases activity is coupled to the activity of guanine nucleotide exchange factors or GEFs, and GTPase-activating proteins known as GAPs. Since the overall spatiotemporal distribution of GEFs, GAPs and Rabs governs trafficking through the secretory and endocytic pathways, affecting exocytosis, endocytosis and endosome recycling, it is likely that RabGTPases could have a major role in neurite outgrowth, elongation and polarization. In this review we summarize the evidence linking the functions of several RabGTPases to axonal and dendritic development in primary neurons, as well as neurite formation in neuronal cell lines. We focused on the role of RabGTPases from the trans-Golgi network, early/late and recycling endosomes, as well as the function of some Rab effectors in neuritogenesis. Finally, we also discuss the participation of the ADP-ribosylation factor 6, a member of the ArfGTPase family, in neurite formation since it seems to have an important cross-talk with RabGTPases.

Keywords: neuronal differentiation, neuronal polarity, rabs functions, vesicle trafficking.

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Neurons are highly polarized cells, which exhibit morphological, biochemical and functional compartmentalization, displaying axonal and dendritic domains that sustain the proper flux of information in the nervous system. Understanding the mechanisms underlying the generation and maintenance of this asymmetry during embryonic development and how it can be restored after injury or disease in the adult brain has been the focus of intensive research. In this regard, several polarization-related signaling pathways have been described (Arimura and Kaibuchi 2007; Cheng and Poo 2012), among which the regulation of cytoskeleton dynamics has been widely studied (Conde and Cáceres, 2009; Dent *et al.* 2011; Stiess and Bradke 2011).

Besides the structural changes associated with cytoskeletal rearrangement, the elongation of axons and dendrites requires an enormous amount of plasma membrane to carry out the surface expansion involved in neuritogenesis (Futerman and Banker 1996; Pfenninger 2009). Vesicle trafficking and growth cone membrane addition are emerging as important mechanisms to explain not only surface expansion required for neurite outgrowth, but also polarized transport during axo-dendrite formation (Tang 2001; Itofusa and Kamiguchi 2011). Unfortunately, the precise events and mechanisms underlying neuronal membrane trafficking during polarization still remain poorly understood.

Members of the Rab family of small GTPases are key regulators of membrane trafficking (Zerial and McBride 2001; Stenmark 2009), being able to control transport, tethering and docking of vesicles through their effectors (Grosshans *et al.* 2006). RabGTPases cycle between two states: an active state with the protein bound to GTP, which

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Abbreviations used: ARNO, ADP-ribosylation factor nucleotide-binding site opener; BICDR-1, Bicaudal-D-related protein 1; EE, early endosome; EHD1, Eps15 homology-domain containing protein 1; ER, endoplasmic reticulum; GAPs, GTPase-activating proteins; MICAL-L2, molecule interacting with CasL-like 2.

is induced by guanine nucleotide exchange factors or GEFs, and an inactive GDP-bound state induced by GTPase-activating proteins known as GAPs (Barr and Lambright 2010; Barr 2013). Since the overall spatiotemporal distribution of GEFs, GAPs and Rabs governs trafficking through the secretory and endocytic pathways, affecting exocytosis, endocytosis and endosome recycling, it is likely that RabGTPases play a major role in neurite outgrowth, elongation and polarization.

RabGTPases recruits different motor proteins to cargo vesicles, allowing polarized addition of membrane to growing neurites. This is exemplified by Rab6, which regulates membrane trafficking from Golgi apparatus to plasma membrane, through its effector Bicaudal-D (BICD), a protein that recruits kinesin-1 (Grigoriev *et al.* 2007). Rab6 also regulates COPI-independent Golgi-endoplasmic reticulum (ER) trafficking through BICD-1, a protein that associates Rab6-possitive vesicles to dynein-dynactin motor complex (Matanis *et al.* 2002). Other RabGTPases can negatively regulate cargo-motor association; for example, Rab3a inactivation is required for recruitment of kinesin-1C to amyloid precursor protein-containing vesicles, and subsequent amyloid precursor protein axonal anterograde transport (Szodorai *et al.* 2009).

There are more than 60 RabGTPases in mammals (Klopper *et al.* 2012); however, their precise role in neurite formation or regulation of membrane trafficking in axons versus dendrites is not yet clearly understood. In this review we summarize the evidence linking some RabGTPases to axonal and dendritic development, as well as neurite formation in neuronal cell lines. Although other interesting reviews have already discussed the topic (Ng and Tang 2008; Sann *et al.* 2009; Yap and Winckler 2012), some recent work involving less-known RabGTPases requires a revision. Therefore, in this article we review recent work on the role of RabGTPases from the trans-Golgi network (TGN), early/late and recycling endosomes, as well as the function of some Rab effectors in neuritogenesis. Finally, we also discuss the participation of the ADP-ribosylation factor 6 (Arf6), a member of the ArfGTPase family, in neurite formation as it seems to have an important cross-talk with RabGTPases (Kobayashi and Fukuda 2012).

RabGTPases from the TGN

Newly synthesized transmembrane elements are transported through the secretory pathway, making the TGN an important membrane source for polarized cells (Ang and Folsch 2012).

Among the RabGTPases present at the TGN, Rab6 has been implicated in the regulation of intra-Golgi trafficking (Martinez *et al.* 1994) and exocytic transport to the plasma membrane (Grigoriev *et al.* 2007). Knockdown of Rab6A and Rab6B reduces the total neurite length in cultured hippocampal neurons; interestingly, over-expression of the Rab6 effector, Bicaudal-D-related protein 1 (BICDR-1) also reduces neurite length (Schlager *et al.* 2010). BICDR-1 negatively regulates trafficking of Rab6- positive vesicles toward the cell periphery, inhibiting neuritogenesis. The BICDR-1 inhibition on Rab6- directed transport is released when BICDR-1 expression levels decrease during development (Schlager *et al.* 2010).

Rab8 is another regulator of TGN to plasma membrane traffic (Huber *et al.* 1993); it is recruited to exocytic vesicles by Rab6 (Grigoriev *et al.* 2011). In cultured neurons antisense suppression of Rab8 arrests development, preventing initial neurite outgrowth; Rab8-suppressed neurons display a round morphology with no neurites or 1 or 2 very short processes. Additionally, TGN-derived vesicles fail to enter the axon, concentrating in the cell body (Huber *et al.* 1995). These observations emphasize the role of Rab8 in the control of neuritogenesis through the regulation of exocytic trafficking. However, Rab8 appears to regulate other aspects of membrane trafficking important for neurite formation, since it also localizes to recycling tubules, from where membrane is sent back to the plasma membrane (Hattula *et al.* 2006). This suggests that Rab8 may promote neuritogenesis through a second mechanism, based on recycling, especially in the formation of dynamic cell surface domains. In fact, constitutively active Rab8 (Q67L) and Rab8 WT over-expression generate neurite-like protrusions in fibroblast cells, relocalizing actin microfilaments toward these protrusions (Peranen *et al.* 1996), a role that depends on the recycling activity of Rab8 instead of its exocytic regulating function (Hattula *et al.* 2006). Further work is needed to elucidate if this mechanism is conserved in neurons (Figs 1 and 2).

Rab10 is a TGN RabGTPase, which participates in the regulation of exocytic trafficking, probably in cooperation with Rab8 (Schuck *et al.* 2007). Cultured neurons expressing ectopic Rab10 WT or constitutively active Rab10 (Q68L) display long and highly branched axons, whereas those expressing dominant negative Rab10 (T23N) fail to develop an axon (Wang *et al.* 2011b). Rab10 participation in neurite formation and axonal growth is promoted by Lethal (2) giant larvae homologue (Lgl1), which releases GDP dissociation inhibitor from the GTPase. In agreement with this, Lgl1 suppression also inhibits axon formation (Wang *et al.* 2011b). Rab10 exocytic vesicles, which are necessary for axon growth, are generated through the Rab10 effector Myosin Vb (Liu *et al.* 2013).

Rab13 is another TGN protein, regulating the transport of newly synthesized membrane proteins; however, the target of Rab13-directed traffic is not the plasma membrane, but recycling endosomes (Nokes *et al.* 2008). Rab13 loss of function inhibits neuritogenesis, whereas constitutively active Rab13 (Q67L) enhances neurite elongation in nerve growth factor (NGF)-treated PC12 cells (Sakane *et al.* 2010).

Rab13 and its effector protein molecule interacting with CasL-like 2 (MICAL-L2) induce accumulation of actinin-4 at neuritic tips, promoting neurite outgrowth (Sakane *et al.* 2010). Interestingly, Rab13 transcript is up-regulated during the regeneration phase following spinal cord injury (Di Giovanni *et al.* 2005), in a transcriptional p53-dependent mechanism (Di Giovanni *et al.* 2006).

On the other hand, Rab2 is not localized in the TGN, but in the cis-Golgi and ER-Golgi intermediate compartment (ERGIC), regulate retrograde transport from the Golgi apparatus towards the ER (Tisdale and Balch 1996). Previous work has suggested that Rab2 promotes neurite formation in cultured neurons, through a yet-unidentified molecular mechanism (Ayala *et al.* 1990). Further studies are needed to verify whether this Rab displays such putative function.

RabGTPases from early/late endosomes

It is now well established that trafficking between components and cargoes in the endosomal network is regulated by RabGTPases. Since endocytosis is an important mechanism for several aspects of intracellular trafficking, including recycling and sorting of membrane receptors, sorting, signaling and maintenance of cell polarity, among others (Mukherjee *et al.* 1997; Conner and Schmid 2003; Platta and Stenmark 2011), it is likely that endosomal-associated RabGTPases are also able to control neurite formation.

Rab5 is a classic early endosome (EE) marker (Chavrier *et al.* 1990), which controls EE fusion (Gorvel *et al.* 1991). It has been shown that Rab5 WT, constitutively active Rab5 (Q79L) and Rab5 GEF Rabex5 over-expression reduce neurite elongation in PC12 cells differentiated with NGF, since the NGF-TrkA complex signaling rises under inhibition of EE fusion (Liu *et al.* 2007). Accordingly, activated TrkA induces the inactivation of Rab5 through RabGAP5 (Liu *et al.* 2007). Nevertheless, hippocampal neurons in culture Rab5 appears to promote neurite extension, since Rab5 knockdown reduces both axonal and dendritic growth, as well as axonal and dendritic branching, whereas Rabex5 *knockdown* decreases axonal and dendritic length and axonal branching (Mori *et al.* 2013). This disagreement can be explained by different models used in each case, suggesting that the neurite outgrowth signaling can differ between cells lines and neurons. In other models, such as *Drosophila melanogaster* dendritic arborization (DA) neurons, Rab5 promotes dendritic complexity and branching (Satoh *et al.* 2008).

Rab7 replaces Rab5 during endosome maturation from EE to late endosomes, a mechanism known as Rab conversion (Rink *et al.* 2005). Since Rab7 regulates the degradative endosomal route, changes in its activity can alter signaling from receptors to late endosomes. This is the case for the enhanced TrkA activity described in NGF-differentiated PC12 cells over-expressing dominant negative Rab7 (T22N). Here, T22N does not induce neuritogenesis on its own, but sensitizes the cell to NGF stimulation and consequent neurite elongation (Saxena *et al.* 2005). Interestingly, Rab7 is mutated in Charcot-Marie-Tooth type 2B disease, with the mutants behaving like constitutively active Rab7, inhibiting neurite elongation (Spinosa *et al.* 2008; Cogli *et al.* 2010) and uncoupling the TrkA signaling pathway due to reduction of extracellular signal-regulated kinase1/2 nuclear shuttling (BasuRay *et al.* 2010).

Rab21 is located in EE (Simpson *et al.* 2004) and participates in endosomal trafficking of β1-integrins (Pellinen *et al.* 2006). As some evidence indicates that Rab21 inhibits neurite outgrowth in NGF-differentiated PC12 cells (Wang *et al.* 2011a), other experiments show the opposite effect. For example, constitutively active Rab21 (Q78L) enhances neurite elongation in staurosporine-differentiated PC12 cells, whereas knockdown of Rab21 GEF Varp reduces neurite length both in PC12 cells and hippocampal neurons in

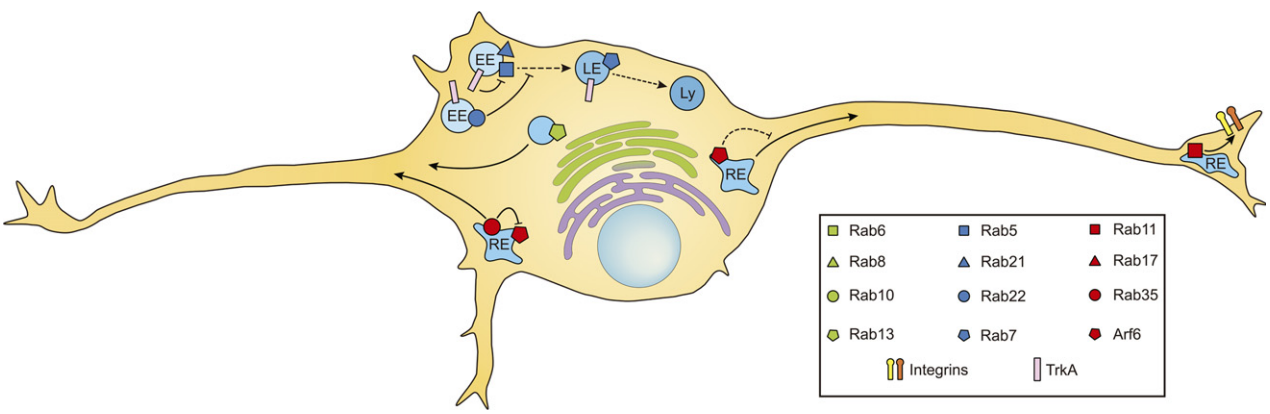

Fig. 1 Membrane trafficking contribution to neurite elongation in neuroblastoma. RabGTPases from TGN derived vesicles (green), early/late endosomes (blue) and recycling endosomes (red) are represented in a neuroblastoma cell line. The identity of several Rabs involved in membrane trafficking is illustrated by triangles, squares, circles and pentagons. The vesicle routes promoting neurite outgrowth are represented by continued lines, whereas inhibitory activities are shown as dashed lines.

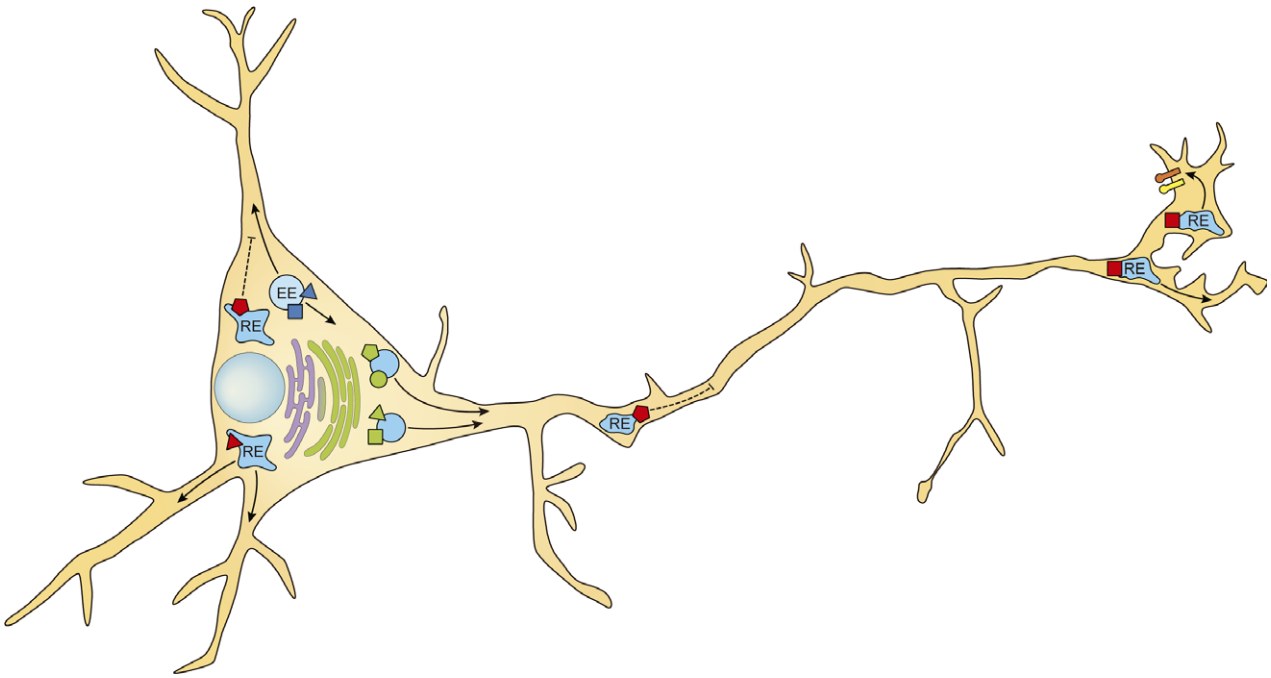

Fig. 2 Membrane trafficking contribution to dendrite and axon development in primary neurons. RabGTPases from TGN derived vesicles (green), early/late endosomes (blue) and recycling endosomes (red) are represented in polarized neurons showing an axon and several dendrites. The identity of several Rabs involved in membrane trafficking is illustrated by triangles, squares, circles and pentagons. The vesicle routes promoting dendrite or axon elongation are represented by continued lines, whereas inhibitory mechanisms are shown as dashed lines. The main differences between primary neuron and differentiated cell lines are found within early endosome (EE)/late endosomes (LE) Rabs.

culture (Burgo *et al.* 2009). Again, the discordance between reports seems to be due to the different experimental models employed.

Rab22 localizes in EE, regulates inter-endosomal trafficking (Kauppi *et al.* 2002) and promotes the sorting of some ligands towards the recycling route (Magadan *et al.* 2006). Rab22 and its effector Rabex5 increase neurite elongation in PC12 cells differentiated with NGF, whereas dominant negative Rab22 (S19N) or Rab22 knockdown reduces p-TrkA endocytosis and neurite outgrowth (Wang *et al.* 2011a).

RabGTPases from recycling endosomes

Like TGN, recycling endosomes (RE) are a source of plasma membrane for elongating neurites (Li and DiFiglia 2012). They also regulate the availability of endocytosed receptors in the plasma membrane, the fine-tuning of their distribution and the associated signaling in different subcellular compartments (Platta and Stenmark 2011; Yap and Winckler 2012). These features of RE suggest that they may be part of key nodes that locally regulate neurite extension (Table 1).

Rab11 is a classic RE marker, regulating recycling towards the plasma membrane (Ullrich *et al.* 1996). Previously, Rab11-GDP was shown to promote neuritogenesis both in hippocampal neurons in culture and in PC12 cells differentiated with NGF through its interaction with protrudin (Shirane and Nakayama 2006). Later experiments showed that Rab11-GTP is the form that promotes neurite extension. In fact, Rab11 WT and constitutively active Rab11 (Q70L) increase axon outgrowth in hippocampal neurons in culture, whereas Rab11 knockdown reduces axonal length, in a pathway dependent on lemur kinase (LMTK1) and Cdk5 (Takano *et al.* 2012). Additionally, Rab11 over-expression increases neurite elongation in NGF-treated PC12 cells and dorsal root ganglia, whereas Rab11 knockdown decreases neurite length and reduces the levels of α9 and β1 integrins in the growth cone (Eva *et al.* 2010). Together, these experiments suggest that active Rab11 promotes neurite elongation. In later stages, Rab11 increases brain-derived neurotrophic factor (BDNF)-induced dendritic branching, as BDNF receptor TrkB is transported to dendrites in Rab11-positive vesicles. Rab11 Q70L is sufficient to enhance dendritic branching, sensitizing neurons to BDNF (Lazo *et al.* 2013).

Rab11 can also recruit actin nucleators Spire1, Spire2 and Formin-2 to the surface of Rab11-positive vesicles, thereby promoting an actin network assembly, suggesting that this subpopulation of vesicles may act as nodes connecting actin microfilament and plasma membrane. In such a way transport of Rab11-positive vesicles may be facilitated by

Table 1 RabGTPases: their subcellular localization and effect on neuronal differentiation

Protein	Localization	Effect on neurite elongation	Experimental models	References
Rab6	TGN	Promotes elongation through exocytotic trafficking	Primary neurons	Schlager et al. (2010)
Rab8	TGN, RE	Promotes outgrowth through polarized transport and actin relocalization	Primary neurons, fibroblasts	Huber et al. (1995) and Hattula et al. (2006)
Rab10	TGN	Positive regulator of secretory vesicles transport and neurite growth	Primary neurons	Wang et al. (2011b)
Rab13	TGN	Enhances outgrowth via exocytotic transport and actinin-4 relocalization	DRG, PC12 cells	Sakane et al. (2010) and Di Giovanni et al. (2005)
Rab5	EE	Inhibition and promotion have been described	Primary neurons, PC12 cells	Liu et al. (2007) and Mori et al. (2013)
Rab7	LE	Reduces TrkA signaling in endosomes, inhibiting neurite outgrowth	PC12 cells	Saxena et al. (2005)
Rab21	EE	Both positive and negative effects have been reported	Primary neurons, PC12 cells	Wang et al. (2011a) and Burgo et al. (2009)
Rab22	EE	Increases elongation through its effector Rabex5	PC12 cells	Wang et al. (2011a)
Rab11	RE	Promotes elongation through membrane trafficking and integrin recycling	Primary neurons, DRG, PC12 cells	Takano et al. (2012) and Eva et al. (2010)
Rab17	RE, EE	Enhances dendritic growth and branching	Primary neurons	Mori et al. (2012)
Rab35	RE	Promotes elongation through Arf6 inactivation and EHD1 recruitment to RE	PC12 and N1E-115 cells	Chevallier et al. (2009) and Kobayashi and Fukuda (2013)
Arf6	RE	Inhibits dendritic growth and branching, the axonal effect is not clear, although it seems to be also a negative regulator	Primary and retinal neurons, PC12 and N1E-115 cells	Hernandez-Deviez et al. (2002, 2004)

The table summarizes the contribution of all Rabs playing a role in neurite outgrowth in immortalized cell lines and in axonal and dendrite elongation in primary neurons. Rabs were classified in TGN-derived (green), early/late endosomes (blue) and recycling endosomes (red), as in the figures.

myosin-Vb, and independent of microtubule motor proteins (Schuh 2011). The implication of this staggering mechanism, if conserved in neurons, could be of great relevance, given the central role of actin dynamics during axon elongation, and could imply an additional mechanism for cargo movement in neuronal compartments, such as the growth cone P-domain, where F-actin is more abundant than microtubules.

Rab17 regulates membrane recycling and transcytosis in epithelial cells (Hunziker and Peters 1998; Zacchi *et al.* 1998). Among all the RabGTPases screened, Rab17 is the only one showing an exclusively dendritic localization. Constitutively active Rab17 (Q77L) increases both dendritic length and branching, whereas Rab17 knockdown reduces total dendritic length and branching, without affecting axonal parameters (Mori *et al.* 2012). Rabex5 (a putative Rab17-GEF) is necessary for Rab17 translocation to dendrites (Mori *et al.* 2013).

Rab35 participates in membrane recycling from RE (Kouranti *et al.* 2006), and rapid recycling routes from tubular EE (Walseng *et al.* 2008). Rab35 WT and constitutively active Rab35 (Q67L) induce neuritogenesis in N1E-115 neuroblastoma cell line, possibly through the activation of Cdc42 (Chevallier *et al.* 2009). This role was confirmed in NGF-treated PC12 cells, where Rab35 also increased neurite elongation (Kanno *et al.* 2010).

The mechanism described in PC12 cells involves recruitment of two Rab35 effectors: MICAL-L1 and centaurin-β2 (ACAP2, Arf GAP With Coiled Coil, ANK Repeat And PH Domains 2). MICAL-L1 functions as a scaffold for the recruitment of Eps15 homology-domain containing protein 1 (EHD1) (Kobayashi and Fukuda 2013), which regulates vesicles exiting from endosomes (Lasiecka *et al.* 2010) and that is necessary for NGF-induced PC12 neurite extension (Kobayashi and Fukuda 2013). The other Rab35 effector is centaurin-β2, an Arf6 GAP, which inactivates Arf6 (Kobayashi and Fukuda 2012), a step required for EHD1 recruitment to Arf6-positive endosomes.

Like Rab8 and Rab11, Rab35 is able to control membrane transport and actin dynamics, activating Cdc42 through a yet-unknown mechanism (Chevallier *et al.* 2009) and inducing filopodia protrusion through fascin, a Cdc42 downstream effector (Zhang *et al.* 2009). This dual role of Rab35 makes it an interesting candidate to regulate a possible crosstalk between trafficking and cytoskeleton dynamics during neurite elongation. In this regard, Rab35 effector MICAL-L1 localizes Rab8 and EHD1 in tubular endosomes (Sharma *et al.* 2009), which can be interpreted as key Rabs conferring robustness to membrane transport pathways.

Arf6 is a class III member of ADP-ribosylation factor (ARF) GTPases family, with important roles in membrane

trafficking and actin dynamics and involved in several processes related to neuronal development (Jaworski 2007). Even though Arf6 is not a RabGTPase family member, we included it in this review as its pathways often crosstalk with Rabs, and because Arf6 is necessary for a comprehensive description of membrane trafficking and neuritogenesis.

Dominant negative Arf6 (T22N) expression or inactivation of the Arf6 GEF ADP-ribosylation factor nucleotide-binding site opener (ARNO) increases axonal length and branching in hippocampal neurons in culture, a mechanism independent of Arf6 effector Rac1, but on phosphatidylinositol-4-phosphate 5-kinase (PI4P5 kinase), inducing a depletion of the actin-binding protein Mena, at the axonal growth cone (Hernandez-Deviez *et al.* 2004). The Arf6 inhibitory role in axon growth, axonal branching and dendritic elongation was also described to be dependent on its effector JIP3 (Suzuki *et al.* 2010). Additionally, Arf6 GEFs EFA6 and ARNO reduce neurite elongation in NGF-treated PC12 cells, whereas Arf6 loss of function increases β1 recycling to the cell surface and integrins anterograde movement (Eva *et al.* 2012). Altogether, these results argue in favour of Arf6 being a negative regulator of axonal elongation.

However, there is evidence indicating that in other experimental models, Arf6 may promote axon elongation. For example, Arf6 WT is necessary for proper neurite extension in cultured avian retinal neurons (Albertinazzi *et al.* 2003). In rat cortical neurons in culture, the adaptor protein Fe65 activates Arf6, which in turn activates Rac1, promoting neurite outgrowth (Cheung *et al.* 2014). Finally, in N1E-115 cells, either ARNO or Arf6 knockdown inhibits valproic acid-induced neuritogenesis, since ARNO interaction with actinin-1 raises Arf6-GTP levels and promotes neurite outgrowth (Yamauchi *et al.* 2009; Torii *et al.* 2012). Arf6 effect on axonal growth should be analyzed carefully, taking into account the different experimental models and specific effectors used to differentiate cell lines.

The contribution of Arf6 to dendritic growth has been more consistent. Inactivation of either ARNO or EFA6A, as well as loss of Arf6 function, increases dendritic growth through a Rac1 pathway (Hernandez-Deviez *et al.* 2002; Sakagami *et al.* 2004). It has been proposed that by decreasing endocytosis of the transmembrane protein vezatin, Arf6-GTP reduces susbtrate adhesion and dendritic elongation (Sanda *et al.* 2010).

There is evidence that Arf6 may also regulate spine formation; however, this issue is far from clear since one study described Arf6 as a spine promoter (Choi *et al.* 2006), whereas the other as an inhibitor factor (Miyazaki *et al.* 2005). Further work is required to resolve the discrepancy.

Conclusions

Throughout this review, we have described how RabGTPases regulate axonal and dendritic elongation. Nevertheless, it is necessary to point out that sometimes Rabs behave differently when compared between models, something to be kept in mind when analyzing Rab effects in cell lines, cultured neurons or during in situ development.

As regards the source of plasma membrane that sustains the surface expansion needed for neurite elongation, it is clear that membrane can be sorted from more than one compartment, given the high number of Rabs participating in the process, with some of them residing in the TGN, whereas others in the endosome network. However, the role of Rabs in neurite outgrowth is not limited to membrane trafficking, as some Rabs are able to control the temporal dynamic of transmembrane receptor signaling in endosomes (the case of Rab5 and Rab7 in PC12 cells), and others can also modulate actin dynamics (Rab8, Rab11 and Rab35).

The high number of Rabs affecting either neuritogenesis or neurite outgrowth suggests some degree of redundancy among them, and also crosstalks between transport routes and signaling pathways, which in turn is supported by shared GEFs/GAPs and effectors (e.g. Rab5 and Rab17 can be activated by Rabex5; Rab35 and Rab8 interact with MICAL-L1).

Finally, given that many RabGTPases have not been studied in terms of their participation in neurite formation, it is very likely that more of them will be added to the already large list of neurite outgrowth regulators.

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