Review

Rab GTPases and their roles in brain neurons and glia

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ABSTRACT

The Rab family of small GTPases serves as cellular regulators of vesicular transport in all eukaryotes. Higher organisms have a vastly expanded number of Rabs. Some of these would presumably perform cell-type or tissue-specific functions. Roles for Rab proteins in several aspects of cellular physiology that are peculiar to neurons have been revealed. Rabs, in conjunction with their effectors and regulators, participate in central nervous system development (Rab23), polarized neurite growth (Rab8 and Rab11), endocytosis and axonal retrograde transport (Rab5 and Rab7). At chemical synapses, Rabs perform specific functions in synaptic vesicle exocytosis (Rab3) and postsynaptic compartment dynamics of glutamate receptors (Rab8). Much less is known about the role of Rabs in astroglia/oligodendroglia-specific processes, although Rab family members that are glia-enriched (Rab22B, Rab40C) have been reported. We summarized and discussed recent findings on the roles played by Rabs in neurons and glia in the central nervous system, and speculate on future directions.

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1. Introduction

The enormous flux of membrane traffic within a cell at any point of its existence necessitates stringent control of both the rate and specificity of traffic flow. The small GTPase family of Rab proteins plays essential roles in various aspects of membrane traffic control. The Rabs are the largest subfamily of the Ras superfamily of small GTPases, with more than 60
members encoded by the human genome, and are localized to different subcellular compartments (Pereira-Leal and Seabra, 2001; Deneka et al., 2003; Pfeffer and Aivazian, 2004; Jordens et al., 2005). Through a GDP–GTP exchange cycle, Rabs function as molecular switches to mediate vesicular transport along the cytoskeleton by engaging specific motor proteins, ensuring efficient tethering and docking of vesicles to target membranes, and timing vesicle fusion with the engagement of soluble N-ethylmaleimide sensitive factor (NSF) (Zhao et al., 2007) attachment receptor (SNARE) (Unger and Langusch, 2005; Hong, 2005) proteins. The guanine nucleotide-binding status of Rabs is modulated by three different classes of regulatory proteins, namely the guanine nucleotide exchange factors (GEFs), GTPase activating proteins (GAPs) and guanine nucleotide dissociation inhibitors (GDIs). The roles of modulators of small GTPase have been extensively delineated for Ras and other subfamilies of the Ras superfamily, such as the Rho GTPases. Modulators of Rab GTPases have also been identified, and will presumably play similar roles. Rab GEFs positively regulates small GTPases activity by promoting GDP–GTP exchange, with the binding of GEF to GTPase causing a decrease in its affinity for GDP (Horichi et al., 1997; Gournier et al., 1998; Jones et al., 2000; Lippe et al., 2001; Burton et al., 1994). Contrastingly, GAPs serve as negative regulators by increasing Rabs intrinsic GTPase activity, and accelerate the conversion of GTP-bound Rabs to the inactive, GDP-bound state (Strom et al., 1993; Clabecq et al., 2000; Will and Gallwitz, 2001; Haas et al., 2005; Sakane et al., 2006; Pan et al., 2006). The GDIs are also negative regulators of Rabs, and function by blocking GDP dissociation via interactions with the isoprenylated C-terminus tail of Rabs (Pfeffer and Aivazian, 2004; DerMarderosian and Bokoch, 2005).

Rab proteins function through their interactions with an array of effector proteins (Crosshans et al., 2006). These include actin-dependent motor adaptors (such as myosin Va, which is recruited by Rab27A to melanosomes via melanophilin; (Strom et al., 2002), microtubule-dependent motors (such as Rabkin-sin-6, which interacts with Rab6; Echard et al., 1998), tethering complexes (such as that based on early endosomal antigen 1 (EEA1); Christoforidis et al., 1999 or p115/GM130 (Allan et al., 2000), as well as other regulatory complexes (such as RIMα/ Munc13/α-liprins, which interact with Rab3A to regulate synaptic vesicle exocytosis; Schoch et al., 2002). Over the past few years, structural analyses have revealed that Rabs assume a similar general structure, but have very distinctive effector binding surfaces that permit selective recognition by diverse effector proteins. Interestingly, even highly conserved amino acid residues can contribute to structural distinctions between Rabs (Pfeffer and Aivazian, 2004).

Bioinformatical analysis has revealed that Rab genes exhibit a rather strict phylogeny of homology and function. The eleven Rab proteins found in Saccharomyces cerevisiae had been grouped into 10 major subclasses (Rab5, Rab7, Rab6, Rab4, Rab28, Rab38, Rab11, Rab1, Rab8 and Rab9) (Buvelot et al., 2006). Eukaryotic Rabs can be subgrouped into eight branches in a phylogenetic tree, with members on the same branch exhibiting similar patterns of cellular localization and function (Pereira-Leal and Seabra, 2001). Although Rab proteins are themselves homologous, the GEFs and GAPs are reasonably specific for a particular Rab protein or related Rab proteins, and these share very little structural or sequence homology amongst each other. The roughly six fold increase in terms of the number of Rabs from the unicellular yeast to human is more than a simple reflection of an increase in cellular membrane traffic complexity. It is conceivable that some Rabs, enriched in particular cell or tissue types, have evolved to perform specific functions. This cell or tissue type specialization of members of a protein family also occurs for the other components of the membrane trafficking machinery, namely the coat proteins and SNAREs (Newman et al., 1995; Wang and Tang, 2006).

The exact cellular localization and tissue expression profile of many Rabs have remained unknown. To date, only a small number of Rabs, such as Rab3A, Rab8 and Rab23, have been shown to be brain-enriched and have documented roles in neurons (Geppert et al., 1997; Evans et al., 2003). We summarize and discuss in this review recent findings on the roles played by Rab GTPases, their regulatory modules and effectors in neurons and glia, particularly in concert with other Ras superfamily members (Matozaki et al., 2000), is essential for better comprehension of multiple aspects of neuron and glia physiology and pathophysiology.

2. Rab in polarized neurite growth

Neurons are polarized cells with distinct plasma membrane domains, namely the axonal and the somatodendritic domains. These domains are established upon differentiation from neural progenitor cells, and morphological changes associated with polarized outgrowth of neurites can be recapitulated and analyzed in culture (Dotti et al., 1988). Polarized outgrowth of neurites, as one would expect, involves components of the vesicular traffic machinery such as SNAREs (Martinez-Arca et al., 2000) and tethering factors such as the exocyst complex (Vega and Hsu, 2001; Tang, 2001). The involvement of some post-Golgi Rabs in neurite outgrowth is often assumed, but functional details have been somewhat lacking. Interestingly, the ER–Golgi boundary localized Rab2 has also been shown to enhance neuronal adhesion as well as neurite growth when the bacterially expressed protein is introduced into cultured cells by trituration (Ayala et al., 1990). How Rab2 functions in this context is yet unclear.

A recent study has implicated Rab13 in neurite growth associated with neuronal regeneration (Di Giovanni et al., 2006). Rab13 is expressed in cultured dorsal root ganglion (DRG) neurons, and is localized to the cortical cytoskeleton as well as growth cones along with the regeneration-associated protein, growth-associated protein-43 (GAP-43). Rab13 expression is induced in the injured spinal cord, where it is co-expressed with GAP-43 in neurons and axons. In a cell culture model, RNA interference-mediated silencing of Rab13 in nerve growth factor (NGF)-treated PC12 cells reduced neurite outgrowth. Other than the Rabs discussed above, Rab8, which was implicated in domain-specific polarized protein targeting in neurons, is also likely to play vital roles in neurite outgrowth (as discussed in the next section).
Directional membrane trafficking in neurite formation would involve not just anterograde exocytic transport from the TGN, but also membrane recycling from the endosomes. An important Rab protein that is involved in both these processes is Rab11. One primary function of Rab11 is to regulate endosomal/plasma membrane interactions by controlling membrane traffic through recycling endosomes, and dominant-negative Rab11a inhibits apical recycling and basolateral-to-apical transcytosis in polarized Madin–Darby canine kidney (MDCK) cells (Wang et al., 2000). In the brain, Rab11 is localized to the somatodendritic domain of neurons (Sheehan et al., 1996). A very recent finding now provides mechanistic insights into Rab11’s role in neurite formation through protrudin, a protein with multiple functional domains (Shirane and Nakayama, 2006). Protrudin contains an unusual Rab11 binding domain (RBD11), which enables its preferential interaction with GDP-bound form of Rab11. Over-expression of protrudin in non-neuronal HeLa cells induced the formation of long processes, and promoted neurite formation in hippocampal processes. On the other hand, silencing of protrudin by RNA interference in PC12 cells inhibited NGF-induced neurite outgrowth, and resulted in membrane extension in all directions. In the presence of NGF, protrudin, which was distributed diffusely throughout the cell body, now concentrates at the perinuclear region colocalizing with Rab11, and could eventually be found in association with vesicles at the tips of growing neurites. Interestingly, over-expression of a constitutively active (Rab11-Q70L) mutant phenocopied the morphological effects of protruding-silencing or the expression of dominant-negative Rab11 mutants disrupted rhabdomere morphogenesis, and rhodopsin trafficking to the rhabdomeres, and the protein could be found colocalizing with Rab11 in vesicles at the base of the rhabdomere. Rab11-silencing or the expression of dominant-negative Rab11 mutants disrupted rhabdomere morphogenesis, and rhodopsin-bearing vesicles accumulated within the cytosol (Sato et al., 2005). Other than rhodopsin, Rab11 is also required for the transport of transient receptor potential (TRP) gene product, another rhabdomeric protein which is a light-activated Ca\(^{2+}\) channel that serves phototransduction. Rab11 is apparently important for the formation of late endosomal multivesicular bodies in rhabdomeres, and is therefore expected to have a more general role in rhabdomeric membrane traffic. Rab11 is understandably also essential for various aspects of nervous system development. This shall be discussed further in a section below.

3. Rab8 is essential in several aspects of polarized neuronal transport, a function that is in line with its wider roles in plasma membrane trafficking in other polarized cell types, such as epithelial cells (van Ijzendoorn et al., 2003). In hippocampal neurons, Rab8 is localized preferentially in the somatodendritic domain, and not the axon. A role for Rab8 in dendrite-specific transport was demonstrated by examining the newly synthesized E2 glycoprotein in neurons infected by Semiliki forest virus (SFV-E2) (Huber et al., 1993). Antisense oligonucleotides corresponding to the translational initiation region of Rab8 markedly reduced transport of SFV-E2 from the neuronal cell body to the dendrites, but had no effect on axonal transport of influenza haemagglutinin (HA). Rab8 protein depletion by antisense oligonucleotide also prevented morphological maturation of hippocampal neurons in culture, while depletion of Rab3a (a neuronal specific Rab involved in synaptic vesicle exocytosis, see below) had no such effect (Huber et al., 1995). Differential interference contrast microscopy revealed a dramatic reduction in the number of vesicles undergoing anterograde transport in Rab8-depleted cells, as well as a restriction of newly formed exocytic vesicles (labeled by Bodipy-ceramide) from moving into the neurites (as they did in control cells). These early studies implicated a role for Rab8 in membrane trafficking processes that are responsible for polarized neurite outgrowth.

Other than a generalized role in neuronal traffic, Rab8 also regulates polarized transport processes in specialized neural cell types. A popular model that had been extensively investigated is the trafficking of rhodopsin in photoreceptor cells, which involves a host of different Rab proteins (Deretic et al., 1995). Early studies suggest that Rab6 (Shetty et al., 1998) and Rab8 associate with post-Golgi membranes sequentially at different stages during rhodopsin transport, and Rab8 may mediate the interaction of transport vesicles or membrane with actin filaments in rod outer segment disk morphogenesis (Deretic et al., 1995). In Xenopus, expression of the dominant-negative, GDP-restricted form of Rab8 (GFP-Rab8T22N) caused rapid retinal degeneration. In surviving peripheral rod cells, one could see an accumulation of tubulo-vesicular structures at the base of the connecting cilium, indicating that the Rab8 mutant induced a defect in vesicular docking or fusion to post-Golgi membranes in rod cells (Moritz et al., 2001).

Another Rab protein that has been implicated in rhodopsin transport is Rab11, which is essential for Drosophila eye development (Alone et al., 2005). During Drosophila larval development, Rab11 is widely expressed in photoreceptor cells and localizes to the rhabdomeres and lamina neuropil in adult eyes. In developing photoreceptors, rhodopsin traffics to the rhabdomeres, and the protein could be found colocalizing with Rab11 in vesicles at the base of the rhabdomere. Rab11-silencing or the expression of dominant-negative Rab11 mutants disrupted rhabdomere morphogenesis, and rhodopsin-bearing vesicles accumulated within the cytosol (Sato et al., 2005). Other than rhodopsin, Rab11 is also required for the transport of transient receptor potential (TRP) gene product, another rhabdomeric protein which is a light-activated Ca\(^{2+}\) channel that serves phototransduction. Rab11 is apparently important for the formation of late endosomal multivesicular bodies in rhabdomeres, and is therefore expected to have a more general role in rhabdomeric membrane traffic. Rab11 is understandably also essential for various aspects of nervous system development. This shall be discussed further in a section below.

4. Rab in axonal endocytosis and axonal retrograde transport

The endosomal Rabs, Rab5 and Rab7, are known to play important roles in regulating the various stages of endosomal trafficking, and their function in neuronal endocytosis had received much recent attention. The families of neurotrophins and neurotrophin receptors exert important activities on neuronal cells, promoting both survival and neurite outgrowth. NGF binding to its cognate TrkA receptor results in signaling activities from the cell surface. Furthermore, the ligand–receptor complexes are internalized into endosomal
compartments, but signaling activities essentially continue in structures dubbed signaling endosomes (Grimes et al., 1996; Ye et al., 2003). Activated TrkA can be found in both early endosomes and late endosomal multivesicular bodies (Saxena et al., 2005a,b). Signaling processes persist in these membranous structures as they travel en route the dynein-based axonal retrograde transport pathway towards the cell body (Barker et al., 2002, Heerssen and Segal, 2002).

The importance of endosome-associated Rab7 in TrkA trafficking is illustrated by the fact that Rab7 could be co-immunoprecipitated with TrkA (Saxena et al., 2005a,b). Inhibiting Rab7 by the dominant-negative Rab7T22N mutant resulted in endosomal accumulation of TrkA, and prolonged persistence of internalized TrkA. This led to an enhancement of TrkA signaling in PC12 cells exposed to a limited stimulation (a brief 10 min pulse) with NGF, as assessed by phosphorylation of TrkA, Erk1/2 activation, neurite outgrowth, as well as the expression of regeneration marker GAP-43. In another report, a strategy involving membrane carrier purification (with tetanus toxin heavy chain, TeNT HC-conjugated to paramagnetic beads) identified Rab7 as a functional marker of axonal retrograde carriers transporting neurotrophins and their receptors. Consistent with the known sequential action of Rab5 and Rab7 in carriers transporting neurotrophins and their receptors. Consistent with the known sequential action of Rab5 and Rab7 in endocytosis, it was revealed that although Rab5 is essential for an early TeNT HC-sorting step that precedes axonal transport, this Rab is absent from vesicles undergoing retrograde axonal transport (Deinhardt et al., 2006). Accordingly, GFP-tagged Rab5 is found largely in TeNT HC-containing vesicles that exhibit oscillatory, short-range bidirectional movements, but not those undergoing long-range retrograde transports. The latter type of vesicles contains Rab7, and axonal transport of TeNT HC was blocked by Rab7N125I expression. The above findings have important clinical implications, as mutations of Rab7 are found in patients suffering from Charcot–Marie–Tooth type 2B neuropathy (Verhoeven et al., 2003) and ulceromutilating neuropathy (Houlden et al., 2004). Rab7 dysfunction may therefore cause neurodegeneration by affecting the trafficking of neurotrophin receptors, and is a potential strategic target for the development of treatment for such hereditary neuropathies.

5. Rab7s functioning at the synapse

The Rab3 subfamily (Rab3A, Rab3B, Rab3C, and Rab3D) is arguably the most extensively investigated Rab in terms of synaptic functions. The Rab3 proteins are expressed in brain as well as endocrine tissues and, in conjunction with their effectors such as rabphilin (Shiratani et al., 1993; Li et al., 1994) and Rim (Wang et al., 1997), function in regulated exocytosis and neurotransmitter release (Geppert et al., 1994; Darchen and Goud, 2000). However, the reported function of Rab3 proteins in exocytosis has ranged from docking (Martelli et al., 2000) and fusion (Geppert et al., 1997; Schluter et al., 2002) to vesicle recycling, and some of the findings are at variance with each other (Darchen and Goud, 2000; Weimer and Jorgensen, 2003). Targeted deletion of Rab3A (Geppert et al., 1994) or Rab3D (Riedel et al., 2002) in mice and Rab3 mutation in Caenorhabditis elegans (Nonet et al., 1997) are non-lethal, and the resulting neural phenotypes are relatively mild. The regulation of synaptic vesicle exocytosis is abnormal in Rab3A-deleted mice, but exocytosis per se is not impaired (Geppert et al., 1997). Rab3D knockout mice exhibit a significant increase in the size of secretory granules in both exocrine pancreas and parotid gland, but there is also no alteration in overall exocytosis (Riedel et al., 2002). Analysis of rabphilin knockout mice also revealed no abnormalities in synaptic transmission or plasticity. Interestingly, those synaptic properties that are impaired in Rab3A knockout mice were unchanged in rabphilin knockout mice (Schluter et al., 1999).

Südhof et al. (Schüter et al., 2004) have since generated multiple knockouts for the Rab3 isoforms, and found that all single and double Rab3 knock out mice are viable and fertile. A knockout of Rab3A in combination with two other Rab3s results in lethality, but triple knockout mice that lack Rab3B, Rab3C, and Rab3D (BCD−/− mice) were fully viable, even when the remaining Rab3A gene was a heterozygous (+/−) mutant. Quadruple knock out mice appeared to develop normally but die shortly after birth due to respiratory failure. These displayed no apparent changes in synaptic structure or brain composition other than a loss of rabphilin, and there was also no alteration in the properties of spontaneous neurotransmitter release. Excitatory postsynaptic current (EPSC) in cultured hippocampal neurons from these mice, however, was about 30% smaller than control mice, caused apparently by a marked decline in synaptic vesicle release probabilities. Rab3 proteins appear therefore to be essential for the normal regulation of Ca2+-triggered synaptic vesicle exocytosis, but not for synaptic vesicle exocytosis per se. This notion is in line with the finding that both Rab3A and Rim1α are required for mossy fibre long-term potentiation (LTP) in the hippocampus (Lonart et al., 1998; Castillo et al., 1997, 2002). Although a detailed molecular sequence of action involving Rab3 in exocytosis at the presynaptic compartment is not yet available, the GTP-dependent binding of Rab3 to RIM1α/2αa appears to allow it to regulate a Rim-based macromolecular complex (consisting of cytomatrix proteins, Munc13, Rim-binding proteins and α-liprins), that in turn engages the SNAREs (syntaxin and SNAP-25) directly mediating synaptic vesicle fusion (Sudhof, 2004).

Other than Rab3, only members of the Rab11 family (Rab11A, Rab11B, and Rab25) impaired Ca2+-induced exocytosis in PC12 cells when over-expressed (Khvotchev et al., 2003), with the brain-enriched Rab11B having the strongest effect. Rab11, like Rab3, co-fractionates with other secretory vesicle proteins of PC12 cells, and can be found enriched in mature synaptic vesicles of rat brain. Both the GDP-restricted and GTP-restricted Rab11B inhibited Ca2+-induced exocytosis, but the inhibitions likely occur via different mechanisms. The GDP-restricted Rab11B stimulated constitutive secretion of human growth hormone (hGH) exogenously expressed in PC12 cells, and depleted hGH stores in secretory vesicles. On the other hand, GTP-restricted Rab11B affects constitutive secretion only moderately, and had no effect on vesicular hGH stores. Therefore, while GTP-restricted Rab11B could directly impaired Ca2+-triggered exocytosis, the GDP-restricted Rab11B may act in a more indirect manner. In non-neuronal cells, both Rab11B mutants inhibited constitutive exocytosis of hGH. Rab11 thus appear to have a specific role in neuronal and neuroendocrine cells as a GTP-dependent switch that could shunt certain cargoes between the regulated and constitutive secretory pathways.
Rab5 is localized both to synaptic vesicles and endosomal intermediates involved in synaptic vesicle recycling (Fischer von Mollard et al., 1994). Studies in Drosophila had indicated that, contrary to earlier expectation, Rab5 was not involved in the endocytic reformation of synaptic vesicles. The dominant-negative mutant of Rab5, Rab5-N142I, however induces a peculiar phenotype of enlarged synaptic vesicles in fly photoreceptor cells (Shimizu et al., 2003). This enlargement could be suppressed by enhancing synaptic vesicle recycling. Not only are they larger, synaptic vesicles prepared from Rab5N142I-expressing flies also exhibited enhanced homotypic fusion in vitro. Rab5 may therefore function to keep the size of synaptic vesicles uniform by preventing their homotypic fusion via mechanisms that are yet unclear.

The somatodendritic location of Rab8 in neurons would also suggest that it mediates dendritic specific transport processes. Indeed, Rab8 appears to have a critical role in the delivery of a-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)-type glutamatergic receptors into the postsynaptic compartment. Although mainly trans-Golgi network (TGN)-localized, Rab8 is found also in close proximity to the postsynaptic plasma membrane and the postsynaptic density (Gerges et al., 2004). Electrophysiological measurements indicated that Rab8 functions in the synaptic delivery of AMPA receptors during LTD and during constitutive receptor cycling. In this context, Rab8 appears to function at a rather specific step(s) in the local delivery of AMPA receptors into synapses. Although it is required for AMPA receptor delivery into the synapse at the surface of dendritic spines, it is not essential for a preceding step of AMPA receptor transport from the dendritic shaft into the spine compartment, nor for their delivery to the general dendritic membrane surface.

Neuronal activity-dependent insertion or removal of AMPA receptors from the postsynaptic plasma membrane underlies the phenomenon of both LTP and long-term depression (LTD), which are experimental manifestations of synaptic plasticity (Malinow and Malenka, 2002). In the case of LTD, AMPA receptors are transported from recycling endosomes to the plasma membrane (Park et al., 2004). Both AMPA receptor recycling and LTD could be blocked by the dominant-negative Rab11A mutant Rab11-S25N (but not by a corresponding mutant of the Golgi-localized Rab6, Rab6-T27N). As discussed above, Rab8 also has a role in AMPA receptor insertion during LTD, but the relationship between the pathway mediated by Rab8 and that by Rab11 is not yet clear.

Since removal of AMPA receptors from the postsynaptic plasma membrane in LTD would involve endocytosis, the endosomal Rab5 protein is therefore predictably a prime regulator of at least part of this process. Rab5 is ultrastructurally localized to CA1 hippocampal synapses, and has indeed been shown to link the signaling cascades triggered by LTD induction and the endocytic machinery that executes the activity-dependent surface removal of AMPA receptors (Brown et al., 2005). Rab5 activation drives the specific internalization of synaptic AMPA receptors in a clathrin-dependent manner, and this activity is required for LTD as a dominant-negative Rab5 inhibits LTD induction. N-methyl d-acetate (NMDA) receptor-dependent LTD induction (experimentally induced by a brief application of NMDA to hippocampal slices) produces a rapid and transient burst of Rab5 activation (as indicated by an increase in Rab5-GTP affinity precipitated by its effector protein Rabaptin-5). Rab5 does not, however, participate in the constitutive cycling of AMPA receptors. Although the details remained elusive, the emerging rough picture is that synaptic activity activates Rab5, which in turn drives the removal of AMPA receptors from synapses into endosomes.

6. Rabs in nervous system development

As key regulators of membrane transport, Rabs are presumably important for developmental processes. However, knowledge in the requirement of rab proteins during the development of the nervous system is scarce. As mentioned above, Rab11 is required for Drosophila eye development (Alone et al., 2005), and it is essential for Drosophila embryogenesis as a whole because Rab11 endosomes are key trafficking intermediates that control vesicle exocytosis and membrane growth during the process of cellularization (Pelissier et al., 2003). The Rab11 family-interacting protein 4 (Rab11-FIP4) have also been recently shown to be expressed predominantly in neural tissues, and are important for retinal development in both zebra fish (Muto et al., 2006) and mouse (Muto et al., 2007).

In the Drosophila nervous system, trafficking of Delta, the ligand of Notch, in sensory organ precursor cells derived from asymmetric cell division, is different in the two daughter cell types, named pIIa and pIIb (Emery et al., 2005). For pIIb, Delta traversed through the Rab11-containing recycling endosome. pIIa, however, appear to lack recycling endosomes, apparently as a result of the failure by its centrosome to recruit the Rab11 binding partner Nuclear fallout (Nuf), which is essential for recycling endosome formation. It was shown that trafficking through recycling endosomes is essential for the signaling capacity of the mouse Delta homolog, Delta-like-1 (Dll1). The Drosophila Beige and Chediak-Higashi (BEACH) domain protein Blue cheese (Bchs), which is evolutionarily conserved and neurally expressed, acts during development as an antagonist of Rab11 (Khodosh et al., 2006). Loss of one or both copies of bchs alleles strongly enhanced the viability of Rab11 hypomorphic rab11<sup>rb11<sub>232</sub>/rb11<sup>232</sup> flies, and suppressed bristle defects. The Bchs protein is associated with vesicles and colocalized extensively with Rab11 at the neuromuscular junction (NMJ). At the Drosophila NMJ, Rab11 is important for synaptic morphogenesis, as Rab11 hypomorphs have increased synaptic bouton density and branching. These NMJ defects are also suppressed by a loss of Bchs.

Another prominent example of a Rab protein with a specific function during mammalian development is Rab23. Sonic hedgehog (Shh) functions as a morphogen at the early neural tube to specify the expression of ventral cell markers. open brain (opb) was first identified as a natural mouse mutation resulting in severe defects in the developing neural tube (Gunther et al., 1994). Phenotypically, the <i>opb</i> neural patterning defects appeared opposite to that resulting from a loss of <i>shh</i>. <i>opb</i> also appears to act downstream of <i>shh</i>, as the <i>shh</i> phenotype is at least partially rescued in <i>opb</i> mutant background. For example, ventral cell types, including the floor plate, are absent in <i>shh</i> mutants, but are present in <i>shh opb</i> double mutants. Positional cloning revealed that <i>opb</i> encodes Rab23 (Eggeschwiler et al., 2001). Recent findings indicate that several nonsense mutations
of human Rab23 underlie the autosomal recessive disorder Carpenter’s syndrome (Jenkins et al., 2007), with patients exhibiting some developmental congenital deformities. The exact role of Rab23 in antagonizing Shh signaling is unclear at the moment. Although it is conceivable that Rab23 may regulate the cellular dynamics of components of the Shh pathway, it does not appear to act directly on either its transmembrane receptor Patched1, or the downstream effector Smoothened (Wang et al., 2006). Rab23 may also have a function in neurons beyond Shh signaling during development, as it is also expressed in adult neurons (Guo et al., 2006).

7. Rabs in glial cells

The glial cells made up 90% of cells in the brain. Recent findings indicate that glial cells do not simply hold the architecture of the human brain together, but play essential roles in brain development (Mori et al., 2005; Peres et al., 2007) and the modulation of synaptic transmission (Araque et al., 2000). There are three main types of glia cells in the central nervous system. The astrocytes are the most abundant glia. Astrocytic processes known as end-feet are in close-contact with neuronal processes serving in neurotransmitters re-uptake (Hertz and Zielke, 2004), and these may also wrap around blood vessels to form the blood-brain barrier (Abbott, 2002). Oligodendrocytes, on the other hand, wrap their processes around axons to form myelin sheaths (Simons and Trajkovic, 2006). The microglia are the equivalence of immune cells in the CNS, playing roles in CNS inflammatory responses and serving in phagocytotic clearing of dead cells and debris (Mallat et al., 2005).

Knowledge about the role of Rabs in glia specific functions are scarce compared to that of neurons. Ayala et al. (1989), were the first to report the expression of Rab3 in astrocytes. The various isoforms of Rab3 were in fact differentially expressed in oligodendrocytes (Rab3A and Rab3C) and astrocytes (Rab3B) (Madison et al., 1996). Rab22B was cloned from a rat oligodendrocyte library (Burcelin et al., 1997). Its transcripts are found in abundance in oligodendrocytes, and to a lesser extent, in astrocytes and microglia. Our recent analysis with Rab22B specific antibodies had however revealed the presence of the protein in radial glia and adult astrocytes, colocalizing with both RC2 and the glial fibrillary acid protein (GFAP), respectively (Ng et al., unpublished data). Rab22B’s function in astrocytes is still unclear at the moment, but various trafficking assays performed in HeLa cells, such as anterograde traffic of the vesicular stomatitis viral glycoprotein (VSVG), suggested that Rab22B might function in exocytosis from the TGN (Ng et al., 2007). Our observations concurred with previous observations that tubulovesicular transport vesicles containing Rab22B exiting the TGN to the plasma membrane via the early endosomes (Rodriguez-Gabin et al., 2001). Therefore, it is possible that Rab22B may play unknown roles perhaps in regulating calcium-dependent exocytotic release of glutamate that is dependent on SNAREs (Araque et al., 2000; Kreft et al., 2004), or regulating the surface expression of astrocytic glutamate transporters (Fournier and Robinson, 2006).

Coincidentally, another Rab protein, Rab40C, was also cloned from an oligodendrocyte cDNA library (Rodriguez-Gabin et al., 2004). Its transcript was found to be enriched in oligodendrocytes, oligodendrocyte progenitors, microglial and astrocytes. However, Rab40C transcript levels appear to be upregulated in mature oligodendrocytes. Localization experiments done with EGFP-tagged Rab40C in HeLa cells revealed it to be localized to the perinuclear recycling endosomes, a compartment which acts as a transit for receptors and other cellular components which may be targeted back to the cell surface or the lysosomes.

Table 1 – A summary of known Rab-associated specific functions in neurons and glia

<table>
<thead>
<tr>
<th>Rab</th>
<th>Cellular/subcellular membrane association</th>
<th>Implicated neuronal/glial specific function</th>
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<tbody>
<tr>
<td>Rab2</td>
<td>ER-Golgi boundary</td>
<td>Over-expression increased neuronal cell adhesion and neurite growth in vitro (Ayala et al., 1990).</td>
</tr>
<tr>
<td>Rab3 (A–D)</td>
<td>Synaptic vesicles, presynaptic plasma membrane</td>
<td>Synaptic vesicle exocytosis. Possibly exocytosis in astrocytes and oligodendrocytes as well.</td>
</tr>
<tr>
<td>Rab5</td>
<td>Early endosome</td>
<td>Uniformity of synaptic vesicle size (Shimizu et al., 2003); AMPA receptor endocytosis (Brown et al., 2005); endosomal sorting and axonal retrograde transport (Deinhardt et al., 2006).</td>
</tr>
<tr>
<td>Rab6</td>
<td>Golgi-trans-Golgi network</td>
<td>Rhodopsin transport in retinal photoreceptors (Shetty et al., 1998).</td>
</tr>
<tr>
<td>Rab7</td>
<td>Endosome</td>
<td>Endosomal sorting and axonal retrograde transport (Saxena et al., 2005a,b; Deinhardt et al., 2006).</td>
</tr>
<tr>
<td>Rab8</td>
<td>Golgi, vesicles and plasma membrane ruffles</td>
<td>Polarized dendritic transport (Huber et al., 1993); rhodopsin transport in retinal photoreceptors (Deretic et al., 1995; Moritz et al., 2001); AMPA receptor trafficking (Gerges et al., 2004).</td>
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<tr>
<td>Rab11</td>
<td>Endosome, synapse</td>
<td>Regulated secretion (Kvotchev et al., 2003); rhodopsin transport in retinal photoreceptors (Satoh et al., 2005); polarized membrane traffic in neurons (Shirane and Nakayama, 2006).</td>
</tr>
<tr>
<td>Rab13</td>
<td>Cytoplasmic vesicles, tight junction in polarized epithelial cells</td>
<td>Possible regulation of neurite outgrowth (Di Giovanni et al., 2006).</td>
</tr>
<tr>
<td>Rab22B/Rab31</td>
<td>Golgi/TGN</td>
<td>Oligodendrocyte transport (Rodriguez-Gabin et al., 2001).</td>
</tr>
<tr>
<td>Rab23</td>
<td>Plasma membrane, endosome</td>
<td>CNS development (Eggenhuis et al., 2001), mutations in humans results in Carpenter’s syndrome (Jenkins et al., 2007).</td>
</tr>
<tr>
<td>Rab24</td>
<td>ER-Golgi boundary, autophagosomes, nucleus</td>
<td>Increase transcript expression in injured motor neurons (Egami et al., 2005).</td>
</tr>
<tr>
<td>Rab40C</td>
<td>Perinuclear compartment</td>
<td>Possible role in vesicular transport in oligodendrocytes (Rodriguez-Gabin et al., 2004).</td>
</tr>
</tbody>
</table>
for degradation (Mellman, 1996). As an oligodendrocyte could simultaneously wrap myelin around several axons, the perinuclear recycling endosomes may thus play a crucial role by allowing cellular components to be targeted to multiple domains. Rab40C’s role in the CNS is unclear, but its enrichment in oligodendrocytes and localization in the perinuclear recycling compartments suggest that it may function in myelination.

Relatively little is known about microglia-specific Rabs. However, unlike Rab6A and A’ which are ubiquitously expressed, a parologue of these Golgi Rabs, Rab6B, was found to be enriched in the brain and expressed in the microglia, pericytes and Purkinje neurons (Opdam et al., 2000). In a recent study, Rab6B is shown to be involved in retrograde trafficking in SK-N-SH neuroblastoma cells via interaction with Bicaudal-D1, a large coiled-coil protein known to bind to the dynein/dynactin complex (Wanschers et al., 2007). As mammalian Bicaudal-Ds also interact with Rab6A, this is unlikely to be a unique feature in the microglia.

8. Epilogue

The Rab family’s role in neurons and glia are only beginning to be explored and understood. A summary of known Rab-associated specific functions in neurons and glia are tabulated in Table 1 and schematically depicted in Fig. 1. Even for neuronally enriched Rabs that had been known for some years now, their modulators and effectors, as well as the exact trafficking process they function in, had remained largely elusive. The exact trafficking process that requires Rab23 during neural tube closure, for example, remains unknown (Wang et al., 2006), and the exact step in which Rab3A functions in synaptic vesicle exocytosis is still being debated. We know much less about the role of Rabs in glial specific processes, although it is clear that astrocytic exocytosis and oligodendrocyte-mediated myelination are all processes that would require Rabs. These areas should be fertile grounds for future studies.

Of all the Rab family members, only Rab27 has been firmly linked to human diseases (Menasche et al., 2003). Knowledge of Rab mutations associated hereditary CNS dysfunctions are at present limited to Charcot-Marie-Tooth type 2B neuropathy (Verhoeven et al., 2003) and ulcero-mutilating neuropathy (Houlden et al., 2004) associated with Rab7 mutations, and cranial-suture defects in Rab23 mutants (Carpenter’s syndrome) (Jenkins et al., 2007). In addition, mutations in human Rab GDI1 are associated with X-linked non-specific mental retardation (D’Adamo et al., 1998). Rab-mediated membrane traffic processes in neurons and glia may be sensitive to certain pathological factors. An interesting recent example pertains to the finding that ER–Golgi traffic in yeast is inhibited by wild type and mutant

Fig. 1 – A schematic diagram depicting the localization and function of Rab GTPases in diverse neuronal processes (polarized neurite outgrowth/regeneration, axonal retrograde transport, postsynaptic glutamate receptor trafficking and synaptic vesicle exocytosis) in relation to astrocytes and oligodendrocytes. Microglia are not shown.
ao-synuclein, accumulation of the misfolded product of which is a pathological hallmark of Parkinson’s disease in humans (Cooper et al., 2006). Intriguingly, Rab1 over-expression could rescue dopaminergic neuron loss resulting from a-synuclein-induced toxicity. The pathological basis of idiopathic, late-onset Alzheimer’s disease (AD) is unclear, but could be associated with aging-related changes in neuronal trafficking components (including Rab) that mediate the traffic itineraries of the amyloid precursor protein (APP) and the beta-site APP cleaving enzyme (BACE1) (Small and Gandy, 2006). These changes could conceivably result in an increased production of amyloid-beta (Ab), and consequently neuronal toxicity through both amyloid plaques as well as soluble oligomeric Ab.

One could expect more associations between Rab and neuron or glial dysfunctions to be revealed in the near future. Understanding Rab regulated membrane trafficking networks via a systems biology-type approach has already been grounded in furthering our understanding of the mechanisms underlying various neuropathological conditions.

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