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Rheb in neuronal degeneration, regeneration, and connectivity

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Abstract: The small GTPase Rheb was originally detected as an immediate early response protein whose expression was induced by NMDA-dependent synaptic activity in the brain. Rheb's activity is highly regulated by its GTPase activating protein (GAP), the tuberous sclerosis complex protein, which stimulates the conversion from the active, GTP-loaded into the inactive, GDP-loaded conformation. Rheb has been established as an evolutionarily conserved molecular switch protein regulating cellular growth, cell volume, cell cycle, autophagy, and amino acid uptake. The subcellular localization of Rheb and its interacting proteins critically regulate its activity and function. In stem cells, constitutive activation of Rheb enhances differentiation at the expense of self-renewal partially explaining the adverse effects of deregulated Rheb in the mammalian brain. In the context of various cellular stress conditions such as oxidative stress, ER-stress, death factor signaling, and cellular aging, Rheb activation surprisingly enhances rather than prevents cellular degeneration. This review addresses cell type- and cell state-specific function(s) of Rheb and mainly focuses on neurons and their surrounding glial cells. Mechanisms will be discussed in the context of therapy that interferes with Rheb's activity using the antibiotic rapamycin or low molecular weight compounds.

Keywords: axonal regeneration; mTORC1; neurodegenerative diseases; neuronal protection; stem cells; tuberous sclerosis complex.

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Introduction

Small Ras-related GTP-binding proteins constitute a large superfamily of proteins that play key roles in cell proliferation and differentiation (Borasio et al., 1989), cytoskeletal organization (Luo, 2002), and protein transport (Stenmark and Olkkonen, 2001) by acting as molecular switches in these processes. Ras homolog enriched in brain (Rheb), is a member of the Ras family of small GTP-binding proteins containing Rheb1 (called from here on Rheb) and Rheb2 (Yamagata et al., 1994; Gromov et al., 1995; Tee et al., 2005; Campbell et al., 2009). Rheb and Rheb2 proteins share 51% amino acid identity (Aspuria and Tamanoi, 2004).

Rheb was found to be expressed at high basal levels in hippocampus and cerebral cortex (Yamagata et al., 1994). Other tissues that express high levels of Rheb mRNA are lung, thymus, kidney, and intestine. Rheb is biochemically activated by growth factors such as epithelial growth factors, fibroblast growth factors and brain-derived neurotrophic factors (BDNF) and is associated with cellular growth, protein synthesis, and regeneration (Yamagata et al., 1994; Saucedo et al., 2003; Takei et al., 2004). In addition, Rheb mRNA and protein levels are upregulated as an immediate early response gene after toxic insults (Yamagata et al., 1994; Karassek et al., 2010).

The role of Rheb in neuronal growth, differentiation, aging, axonal regeneration as well as energy homeostasis of the system has gained some popularity (Swiech et al., 2008; Cheng et al., 2011a; Lafourcade et al., 2013; Yang et al., 2014). Interestingly, Rheb over-expression in neurons and in secondary cell lines shows that Rheb changes its function to become an enhancer of apoptosis depending on the cellular state (Wataya-Kaneda et al., 2001; Karassek et al., 2010; Cao et al., 2013; Ehrkamp et al., 2013). Here, we focus on the role of Rheb signaling pathways in neuronal growth, differentiation, regeneration, and connectivity, as opposed to the enhancement of apoptosis and progression of neurodegenerative diseases.

Rheb signaling pathway

Growth factors such as insulin-like growth factor 1 (IGF 1) and insulin activate the receptor tyrosine kinases or G-protein coupled receptors, which trigger the lipid kinase phosphatidylinositol-3 kinase (PI3K) (Gupta and Dey, 2012; Dibble and Cantley, 2015). The serine/threonine kinase Akt is one of the key downstream mediators of the PI3K signaling (Hay and Sonenberg, 2004; Long et al., 2005; Zoncu et al., 2011; Gupta and Dey, 2012; Dibble and Cantley, 2015). Akt links to the mTOR signaling *via* the tuberous sclerosis complex protein (TSC complex) and Rheb GTPase. The TSC complex consists of TSC1 (hamartin), TSC2 (tuberin) and TBC1D7 (Dibble et al., 2012) and inhibits the activity of the small GTPase Rheb by acting as a GTPase activating protein (GAP) towards Rheb (Gao and Pan, 2001; Potter et al., 2001; Tapon et al., 2001; Inoki et al., 2002; Inoki et al., 2003; Manning and Cantley, 2003; Tee et al., 2003; Saucedo et al., 2003; Zhang et al., 2003; Aspuria and Tamanoi, 2004; Hay and Sonenberg, 2004; Li et al., 2004a,b). *In vitro*, Akt phosphorylates TSC2 at conserved consensus phosphorylation sequences and down-regulates its GAP activity (Inoki et al., 2002, 2003). Reduced GAP activity of TSC complex allows for the accumulation of GTP-bound relative to GDP-bound Rheb (Inoki et al., 2002; Zhang et al., 2003; Li et al., 2004a,b; Tee et al., 2005). Rheb exhibits a low intrinsic GTPase activity in relation to other Ras-related small G proteins (Manning and Cantley, 2003; Schöpel et al., 2017). Hence, the GTP/GDP loading state of Rheb is highly regulated by its GAP, which is controlled by the presence of growth factors (Figure 1). Rheb binds to its effector 'mammalian target of rapamycin' (mTOR) (Long et al., 2005) and GTP-loaded Rheb is required for the activation of mTOR (Inoki et al., 2003; Long et al., 2005; Menon et al., 2014; Dibble and Cantley, 2015). A guanine nucleotide exchange factor (GEF) for Rheb has been identified but the biochemical and cellular relevance is still under discussion (Rehmann et al., 2008; Schöpel et al., 2017).

mTOR (frequently quoted as 'mechanistic' target of rapamycin), is an evolutionarily conserved checkpoint protein kinase that has emerged as a major effector of cell growth and proliferation. The mTOR pathway regulates protein synthesis, autophagy and metabolism in response to secreted growth factors, cellular levels of amino acids, glucose, energy levels, and oxygen levels (Manning and Cantley, 2003; Hay and Sonenberg, 2004; Adami et al., 2007; Swiech et al., 2008; Laplante and Sabatini, 2012). The mTOR forms two distinct complexes, mTORC1 and mTORC2, which are functionally different (Loewith et al., 2002). mTORC1 consists of mTOR, regulatory-associated protein of mTOR (Raptor); and mammalian lethal with SEC13 protein 8 (mLST8), along with two endogenous inhibitors of the complex, 40 kDa Proline-rich Akt substrate (PRAS40), and DEP domain-containing mTOR-interacting protein (DEPTOR) (Loewith et al., 2002; Hay and Sonenberg, 2004; Long et al., 2005; Yang et al., 2006; Adami et al., 2007). Downstream of activated mTORC1, protein translation is promoted by phosphorylation of 70 kDa ribosomal S6 kinase (S6K) and eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1) and autophagy is suppressed via Ulk1/Atg13 (Burnett et al., 1998; Hay and Sonenberg, 2004; Ganley et al., 2009; Hosokawa et al., 2009; Jung et al., 2009; Wang et al., 2009; Sciarretta et al., 2012) (see Figure 1). Rapamycin is an immunosuppressant and anticancer agent that binds to a 12-kDa FK506-binding protein (FKBP12) with high affinity. The FKBP12-rapamycin complex then acts on mTOR to inhibit mTORC1 function and thereby inhibit cell growth (Crespo et al., 2002; Loewith et al., 2002; Hay and Sonenberg, 2004; Adami et al., 2007; Gkogkas et al., 2010). mTORC2, in addition to mTOR and mLST8, contains the essential polypeptides: rapamycin-insensitive companion of mTOR (rictor) and mammalian stress-activated map kinase-interacting protein 1 (mSin1) (Loewith et al., 2002; Sarbassov et al., 2004; Adami et al., 2007; Avruch and Long, 2009). mTORC2 may signal to the actin cytoskeleton through Rho and Rac (Jacinto et al., 2004; Sarbassov et al., 2004).

Regulation of Rheb signaling pathway by subcellular localization

The localization of TSC complex, Rheb and mTORC1 at the lysosomes is critical for the regulation of mTORC1 and downstream pathway activation (Demetriades et al., 2014; Menon et al., 2014). Rheb is localized to multiple endo-membrane compartments including lysosomes due to the farnesylation of the C-terminal CAAX motif (Buerger et al., 2006). This localization of Rheb is not affected by insulin or amino acid levels (Inoki et al., 2002; Zhang et al., 2003; Li et al., 2004a,b; Tee et al., 2005; Menon et al., 2014). Although some studies suggest that Akt mediates the disassembly of the TSC complex, others suggest that Akt-mediated phosphorylation of TSC2 causes its degradation (Inoki et al., 2002; Menon et al., 2014). However, recently it was found that in the absence of insulin or growth factors, the TSC complex resides at the lysosomes in a Rheb-dependent manner and inactivates the Rheb at the lysosomes by acting as a Rheb-GAP. The TSC2 within the intact TSC complex

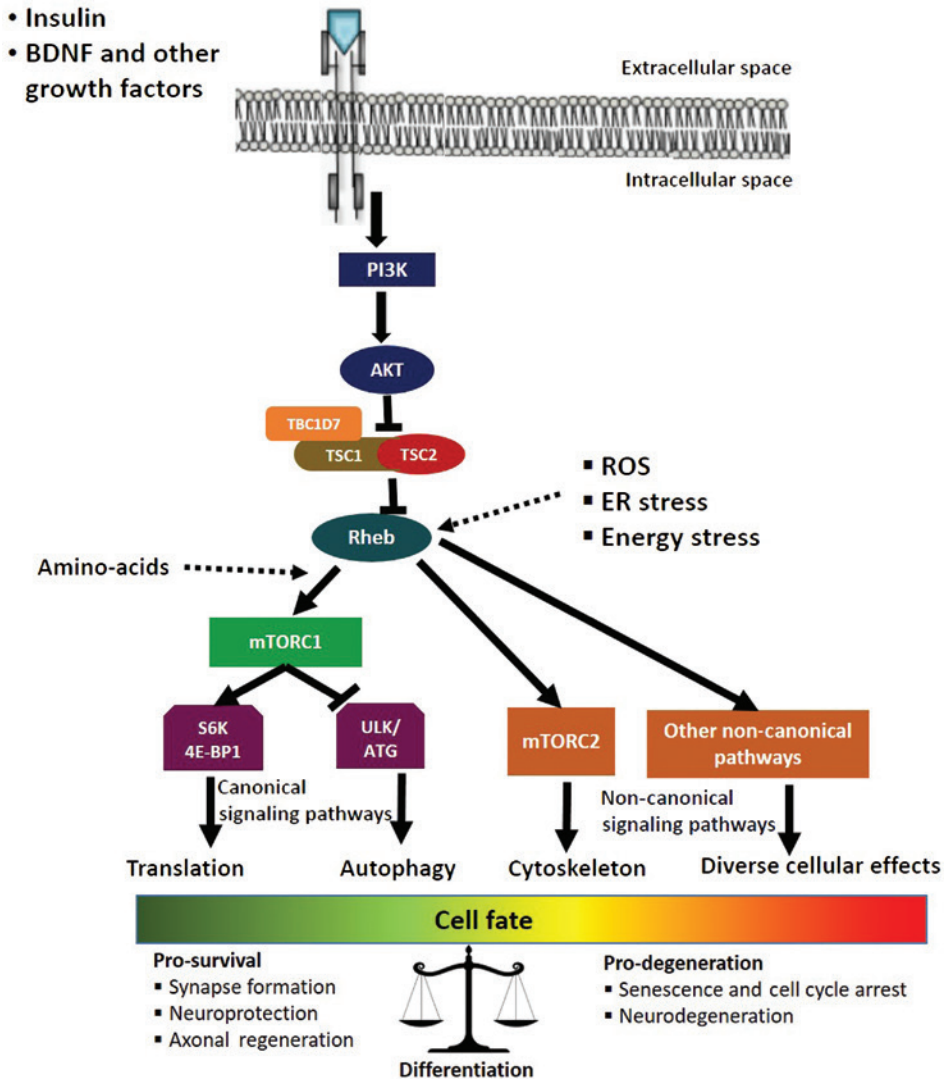

Figure 1: Rheb-mTORC1 signaling is activated in response to various intracellular and extracellular signals in the nervous system. Rheb promotes growth, regeneration and neuroprotection through the canonical mTORC1-S6K and many other non-canonical pathways. However, depending on the cellular state, cellular stress or under pathological conditions, Rheb become an enhancer of apoptosis. Canonical pathway: translation and cell cycle progression via S6K and 4E-BP1, inhibits autophagy via Ulk and ATG proteins. Non-canonical: mTORC2 regulates cytoskeleton required for axon guidance and synapse size; B-Raf regulates differentiation; Notch regulates cell fate in the external sensory organ (ESO), FKBP38 regulates apoptosis, Nix and LC3 induces mitophagy, RASSF1A enhances autophagy, dynein regulates aggresome formation, syntenin regulates spine morphogenesis, CAD regulates pyramidine synthesis, PERK inhibits protein synthesis, BACE1 in the pathogenesis of AD.

has a strong binding preference for Rheb-GDP. In the presence of insulin, Akt-mediated phosphorylation of TSC complex causes its dissociation from the lysosomes, hence promoting Rheb and mTORC1 activation (Benjamin and Hall, 2014; Menon et al., 2014; Dibble and Cantley, 2015). Interestingly, mTORC1 can also be activated at other endo-membranes including peroxisomes and Golgi complex (Flinn et al., 2010; Zhang et al., 2013).

Cellular levels of amino acids also mediate the regulation of TSC complex dissociation and activation of mTORC1 at the lysosomes (Benjamin and Hall, 2014; Demetriades et al., 2014; Dibble and Cantley, 2015), yet in a different manner involving the Rag-GTPase (Sancak et al., 2008; Groenewoud and Zwartkruis, 2013). Rag-GTPase-mediated translocation of mTORC1 to the lysosomes, brings it into close proximity of Rheb, which stimulates the kinase activity of mTOR (Sancak et al., 2008; Groenewoud and Zwartkruis, 2013; Demetriades et al., 2014). Among the amino acids that control mTORC1 activity, arginine and leucine appear to be important (Carroll et al., 2016). Furthermore, Fawal et al., identified microspherule protein 1 (MCRS1) as an essential protein whose depletion allows

the interaction of TSC complex and Rheb, hence inactivating Rheb and mTORC1 (Fawal et al., 2015).

Taken together, the signals from both amino acids and growth factors are integrated at the cytoplasmic face of the lysosomal membrane where mTORC1 is activated. Amino acids and growth factors are necessary and act independently, but neither alone is sufficient to fully activate mTORC1 (Benjamin and Hall, 2014; Menon et al., 2014; Dibble and Cantley, 2015). More recently, it was found that in the absence of amino acids, Rag GTPase can recruit the TSC complex to the lysosomes and maintains Rheb in the GDP-bound form, hence inactivating the Rheb-mTORC1 pathway (Demetriades et al., 2014). Therefore, the spatial control of the TSC complex at the lysosomes has been associated with both amino acid levels and the growth factor stimulation. When either of the two is missing, the TSC complex re-localizes to the lysosomes (Demetriades et al., 2016). In addition, the lysosomal re-localization of TSC complex is also an important stress response to inhibit mTORC1-mediated growth and the presence of any single stress maintains TSC complex at the lysosomes (Demetriades et al., 2016).

Rheb-mTORC1 is also able to sense the cellular redox state (Patel and Tamanoi, 2006; Di Nardo et al., 2009; Yoshida et al., 2011) which occurs independent of the amino acid levels and the Rag-regulator system. In addition to TSC complex, there are other upstream regulators for Rheb. Bcl-2/adenovirus E1B 19-kDa interacting protein 3 (Bnip3) is a hypoxia-induced pro-death protein, that binds with Rheb under hypoxia condition, and inhibits its function either by blocking Rheb interaction to downstream effectors or by interfering with GTP loading of Rheb (Li et al., 2007). Under conditions of low glucose levels, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) interacts with Rheb, thereby preventing the association between Rheb and mTORC1 resulting in its inhibition (Lee et al., 2009).

Tuberous sclerosis complex

Tuberous sclerosis complex (TSC), is a rare genetic disease caused by mutation in the genes coding for TSC complex leading to the hyperactivation of the Rheb-mTORC1 pathway (Potter et al., 2001; Patel et al., 2003; Li et al., 2004a,b; Uhlmann et al., 2004; Crino et al., 2006). TSC is characterized by enhanced cell growth that affects organs such as brain, kidney, lung, heart and it leads to the development of hamartomas or benign focal malformations composed of tissue elements in a disorganized mass (Crino et al., 2006; Curatolo et al., 2008). The neurological symptoms of TSC include mental retardation, intractable epilepsy, and autism (Crino et al., 2006; Curatolo et al., 2008; Lim and Crino, 2013). Mouse models carrying mutations in the TSC1 or TSC2 genes have been extensively investigated to clarify the mechanisms of the TSC pathogenesis (Lim and Crino, 2013). Such studies emphasize that these TSC model mice exhibit impairments in neuronal morphology, dendritic spine formation, synaptic long-term depression and cognitive behaviors (Brown et al., 2012; Sugiura et al., 2015). Interestingly, in mouse models of TSC, there are both, mTORC1 dependent as well as mTORC1-independent Rheb pathways involved in the pathogenesis of TSC-associated abnormalities (Tavazoie et al., 2005; Yasuda et al., 2014; Sugiura et al., 2015)

Non-canonical Rheb pathway

Recently, several signaling pathways of TSC complex and Rheb have been described that function independently of mTORC1 (Jacinto et al., 2004; Karbowniczek et al., 2006; Zhou et al., 2009; Neuman and Henske, 2011). Hence, the activation of these non-canonical pathways cause some of the clinical manifestations of TSC that are resistant to mTORC1 inhibition by rapamycin (Yasuda et al., 2014). In order to cover a broader therapeutic spectrum, small molecules were investigated with Rheb as the primary binding target. Recent studies show that 4,4′-biphenol binds to Rheb and affects cell survival (Schöpel et al., 2013, 2017).

Some of the mTORC1-independent effects of Rheb are the suppression of aggresome formation (Zhou et al., 2009), the regulation of endocytic trafficking pathways (Saito et al., 2005) and determination of the cellular fate *via* notch signaling (Karbowniczek et al., 2010). The non-canonical mediators of Rheb signaling are as follows: B-Raf kinase (Im et al., 2002; Karbowniczek et al., 2004); FKBP38 (Ma et al., 2010); NIX, and LC3 (Melser et al., 2013); RASSF1A (Nelson and Clark, 2016); carbamoyl-phosphate synthetase 2, aspartate transcarbamoylase, and dihydroorotase (CAD) (Sato et al., 2015); β-site amyloid precursor protein (APP)-cleaving enzyme 1 (BACE1) (Shahani et al., 2014); protein kinase-like endoplasmic reticulum kinase (PERK) (Tyagi et al., 2015); and syntenin (Sugiura et al., 2015). Taken together, Rheb can regulate multiple cellular events both dependent and independent of mTORC1 and hence have roles in development, maintenance and degenerative processes. The functional significance of these interactions is summarized in Table 1.

Table 1: An overview of various proteins identified to interact with Rheb.

Proteins	Functional consequences
Proteins involved in canonical signaling pathway	
mTOR	Rheb binding to mTOR is independent of its GTP-bound state but requires GTP-bound state for activation of mTORC1, thus promoting growth, cell cycle progression and inhibition of autophagy.
TSC complex	In the absence of growth factors or insulin, the TSC complex stimulates the intrinsic GTPase activity of Rheb on the lysosomal surface and, through the conversion to Rheb-GDP, forms a complex with Rheb at the lysosomal membranes.
Bnip3	Under hypoxia condition, Bnip3 binds with Rheb and inhibits its function either by blocking Rheb interaction to downstream effectors or by interfering with Rheb GTP loading (Li et al., 2007).
PLD1	Rheb binds and activates phospholipase D1 (PLD1) in a GTP-dependent manner. Rheb indirectly activates mTORC1 by stimulating PLD1 to produce the lipid, phosphatidic acid (PA) (Sun et al., 2008).
GAPDH	In the absence of glucose, GAPDH interacts with Rheb independent of the guanyl nucleotide loaded state of Rheb.
Proteins involved in non-canonical signaling pathway	
FKBP38	FKBP38 is a member of the family of FK506-binding proteins that acts as an inhibitor of the mTOR (Bai et al., 2007). The inhibitory action of FKBP38 is antagonized by Rheb, which interacts with FKBP38 in a GTP-dependent manner and prevents its association with mTOR. Both Rheb and the anti-apoptotic protein Bcl-2 bind to the same region on FKBP38 and Rheb displaces Bcl-2 from FKBP38 facilitating the binding of Bcl-2 with pro-apoptotic proteins (Ma et al., 2010).
RASSF1	RASSF1 interacts with Rheb to promote autophagy by inhibiting mTORC1 signaling.
NIX	Upon high oxidative phosphorylation activity, Rheb is recruited to the mitochondrial outer membrane. Rheb promotes mitophagy through a physical interaction with the mitochondrial autophagic receptor Nix and the autophagosomal protein LC3-II.
LC3	Rheb physically interact with the mitochondrial autophagic receptor Nix and the autophagosomal protein LC3-II. The recruitment of Rheb to mitochondria leads to the activation of mitophagy.
Syntenin	Rheb-GDP binds syntenin to regulate spine morphogenesis.
CAD	Rheb binds with CAD at its C-terminal carbamoyl phosphate synthetase domain in a GTP dependent manner to regulate pyramidine synthesis. This interaction is dependent on the effector domain on Rheb.
B-Raf	Rheb directly interacts with, and inhibits B-Raf kinase. This direct interaction inhibits C-Raf/B-Raf heterodimerization, and Ras activation.
PERK	Under ER stress conditions, Rheb associates with PERK in a GTP-dependent manner to inhibit protein synthesis through eIF2α signaling.
BACE1	Rheb binds to BACE1 and this interaction is necessary for BACE1 degradation in a GTP-dependent manner. BACE1 initiates amyloidogenic processing of APP to generate amyloid, which is a hallmark of Alzheimer's disease (AD) pathology.

Pro-growth role of Rheb

Neuronal growth and differentiation

mTORC1 activity is important in controlling organism size and survival during embryonic development (Patel et al., 2003; Zoncu et al., 2011; Laplante and Sabatini, 2012). Specific deletion of the mTORC1 component Raptor, in the neuronal progenitors, leads to decreased brain size in the developing embryos due to impaired cell cycle progression and increased apoptosis (Cloëtta et al., 2013). Moreover, Rheb knockout mice are embryonically lethal (Goorden et al., 2011, 2015). Conditional Rheb knockout in adult mice leads to limited life span of the animals indicating altogether, that Rheb is essential for embryonic as well as adult animal survival (Goorden et al., 2011, 2015).

Studies using the *Drosophila* visual system describe that hyperactivation of the insulin receptor pathway in the visual system leads to the precocious acquisition of cell fate markers and premature neuronal differentiation (Bateman and McNeill, 2004). The *Drosophila* eye is composed of a pattern of eight photoreceptors (R1–R8) called ommatidia and has been used for detailed analysis of spatial aspects of differentiation (Carthew, 2007; Morante et al., 2007). Upon loss of TSC1, the photoreceptors 1, 6, and 7 and non-neuronal cone cells differentiate prematurely. These mutants however adopt normal cell fate, but tissue organization is disrupted (Bateman and McNeill, 2004). In the visual system of *Drosophila*, Rheb regulates differentiation which occurs independent of the regulation of cell cycle and cell size (Bateman and McNeill, 2004). Such studies indicate that activation of the insulin receptor-mTOR pathway is a critical control element for the timing of neuronal differentiation (Bateman and McNeill, 2004; Gu et al., 2014). The Notch signaling pathway plays a central role in the regulation of neuronal progenitor cell (NPC) differentiation and is required to maintain stem/progenitor cells in an undifferentiated state. It was shown that Rheb regulates cell fate in the external sensory organ (ESO) of *Drosophila* independent of mTORC1 *via* notch

(Karbowniczek et al., 2010). Interestingly, the activation of Rheb leads to abnormalities in asymmetric cell division in sensory organ precursor cells of *Drosophila* (Karbowniczek et al., 2010).

Also in the mammalian system, insulin signaling plays an important role in age-dependent neuronal growth and differentiation (Cloëtta et al., 2013; Lafourcade et al., 2013). Constitutive activation of Rheb, in the NPCs and neuroblasts of subventricular zone in mouse may lead to premature neuronal differentiation and disruption in cell migration either though obstruction of the migratory path and/or release of aberrant cues (Lafourcade et al., 2013). In TSC neurons, neuronal migration defects were induced by upregulation of Cul5 expression by the hyperactivated mTORC1, leading to disruption of Reelin-Dab1 signaling (Moon et al., 2015).

Few studies using TSC knockout and Raptor knockout also show that both hyperactivation and inhibition of Rheb-mTORC1 pathway impair differentiation and can lead to premature death (Goorden et al., 2011; Angliker et al., 2015). The reduction of mTORC1 activity in specific regions of adult brain affects learning, memory and social behavior (Angliker et al., 2015; Goorden et al., 2015).

Rheb in synapse size and function

TSC complex, Rheb and mTORC1 are thought to play a key role in neuronal and synaptic plasticity and dentritic morphogenesis through regulation of protein synthesis (Kumar et al., 2005; Tavazoie et al., 2005; Swiech et al., 2008; Costa-Mattioli et al., 2009). mTORC1-mediated translation is required both at the synapse, for synaptic plasticity, and within the cell soma, for maintaining memory (Swiech et al., 2008; Gkogkas et al., 2010).

Drosophila neuromuscular junction (NMJ) was used to determine the role of TSC complex-Rheb-mTORC1 signaling on synapse assembly and function (Knox et al., 2007; Dimitroff et al., 2012; Natarajan et al., 2013). S6K regulated overall synapse growth (synapse bouton size, active zone number, and density) but not synaptic enlargement measured by the number of synaptic boutons per muscle area (Cheng et al., 2011a). However, Rheb overexpression in the motor neuron of the *Drosophila* larval stage or in NMJ did induce a doubling of the synapse size in terms of number of synaptic boutons per muscle area as well as an increase in synaptic function (Knox et al., 2007). Interestingly, rapamycin treatment reduced overall growth but did not suppress the Rheb-induced synaptic enlargement. This Rheb-induced synaptic enlargement was shown to depend upon bone morphogenetic protein (BMP) signaling mediated by wishful thinking, a BMP type II receptor (Knox et al., 2007). BMP pathways are important for normal NMJ growth in *Drosophila*. Animals bearing mutations in the *wishful thinking* gene show a very small NMJ with dramatically compromised synaptic function (Knox et al., 2007). The synaptic enlargement by over-expression of Rheb at the NMJ results in altering the balance between the number of synaptic boutons and the size of the underlying muscle which disrupts the normal growth and development (Dimitroff et al., 2012).

Recent findings show that Rheb over-expression mediated synaptic growth was morphologically and functionally different from that of the TSC mutant, indicating that TSC complex may be able to regulate NMJ synapse independent of Rheb-TORC1 pathway (Natarajan et al., 2013). TSC2 was known to regulate actin cytoskeleton through TORC2, which is critical for NMJ growth and function (Eaton et al., 2002; Luo 2002; Loewith et al., 2002; Coyle et al., 2004; Jacinto et al., 2004; Natarajan et al., 2013). Detailed studies on synaptic growth and enlargement were not yet reported in mice and more investigations in mammals into the role of Rheb and mTORC2 (Yang et al., 2006) would help to understand the regulation of brain connectivity.

Synaptic plasticity is measured as long-term potentiation (LTP) and long-term depression (LTD) in vertebrates and as long-term facilitation (LTF) in invertebrates (Gkogkas et al., 2010; Hoeffer and Klann, 2010). An interesting finding on the role of Rheb-mTORC1 canonical pathway in memory formation of the mollusk, *Aplysia californica*, was from Weatherill et al., where S6 activated by Rheb act as a critical regulator of 24 h-LTF (Weatherill et al., 2010). S6K- and 4E-BP1-mediated translation is required for synaptic plasticity and for cognitive processing in both vertebrates and invertebrates (Antion et al., 2008; Gkogkas et al., 2010; Weatherill et al., 2010).

Activation of Rheb signaling by loss of phosphatase and tensin homolog (PTEN), an important upstream negative regulator of TSC complex-Rheb-mTORC1 signaling, affects neuronal morphology and socialization behavior in mice (Kwon et al., 2006). In adult rat brains, Rheb can increase the levels of acetylcholine and total choline, which are important for cognitive functions (Jeon et al., 2015). Experiments using conditional mouse TSC1 or TSC2 knockout strains driven by synapsin promoters for neurons, emphasizes the role of these genes in epilepsy and altered cognition, behavioral phenotypes including seizures and altered social interactions (Lim and Crino, 2013).

However, in conditional adult Rheb knockout mice even though a reduction in the downstream effectors like S6K and 4E-BP1 phosphorylation was observed (Weatherill et al., 2010; Goorden et al., 2015), the hippocampal

LTP, learning and memory was not effected to a larger extend (Goorden et al., 2015). This indicates that even a mild activation of the mTORC1 signaling can bring about a drastic effect on synaptic plasticity and learning associated with various diseases (Lim and Crino, 2013), while a mild inhibition of mTORC1 appears not to affect memory functions (Goorden et al., 2015). This is in accordance with the need to use high concentrations of rapamycin for impairing learning and plasticity in mice (Gkogkas et al., 2010; Goorden et al., 2015). The relative non-responsiveness in the synaptic plasticity by reduction of Rheb may also be due to the existence of Rheb2, which also activates mTORC1 (Tee et al., 2005).

These data further assess that even though the upregulation of mTORC1 pathway is highly sensitive and leads to cognitive defects as seen in TSC, the sustained suppression of mTORC1 using drugs like rapamycin may not affect the memory and learning significantly (Goorden et al., 2015).

Studies using Rictor knockout in brain show that, mTORC2 affects the size of the soma and dentrites, the number of primary dentrites in neurons as well as the synaptic connectivity of Purkinje cells in the cerebellum (Thomanetz et al., 2013; Angliker et al., 2015). The growth defects observed in Rictor knockout brain are caused by changes in the downstream effectors of mTORC2 and not by affecting mTORC1 signaling. Hence both mTORC1 and mTORC2 control distinct function in terms of social behaviour and motor coordination (Thomanetz et al., 2013).

Rheb in spine morphology and function

TSC complex-Rheb-mTORC1 pathway is known to regulate neuronal structures and function and the neurological symptoms of TSC are, at least in some part, due to cell-autonomous synapse function alterations. More recently, excitatory spine dysmorphogenesis and reduced spine synapse density, were observed in TSC neurons of rodent models (Yasuda et al., 2014; Sugiura et al., 2015). Rheb-syntenin signaling regulates activity dependent learning and memory processes independent of mTORC1 activation. Under normal conditions, Rheb-GDP associates with syntenin, leading to its proteasomal degradation, allowing normal spine formation required for learning and memory formation. However, in TSC mutant neurons, the Rheb is maintained in its GTP-bound form, which cannot bind to syntenin anymore, leading to accumulation of syntenin thereby disrupting the interaction between syndecan-2 and calcium/calmodulin- dependent serine protein kinase. The association of these proteins is crucial for normal spine development. Therefore, in TSC models, Rheb negatively regulates spine synapse formation causing dentritic spine abnormalities (Sugiura et al., 2015).

Another interesting finding was from Tavazoie et al., where under conditions of TSC1 knockout, increased soma and increased size of dendritic spines are observed (Tavazoie et al., 2005). The cytoskeleton of dendritic spines is particularly important in the reception of input from a single axon at the synapse. TSC complex may regulate soma and spine size *via* a mTOR dependent rapamycin-sensitive pathway and spine length *via* a mTOR independent rapamycin-insensitive pathway (Tavazoie et al., 2005). TSC complex regulates the cytoskeleton *via* ERM (ezrin, radixin, and moesin) proteins, neurofilament light chain and activation of Rho family of proteins (Haddad et al., 2002). Whether this regulation also involves mTORC2 and its regulation of cytoskeleton (Jacinto et al., 2004) still needs to be investigated. TSC complex also regulate cofilin and induces alteration in the spine length (Haddad et al., 2002; Luo 2002; Tavazoie et al., 2005).

Rheb in neuronal polarity and axon guidance

The axon and dendrites constitute the cellular basis for directional flow of information within the nervous system. Neuronal polarity is defined by the elaboration of a single axon and multiple dendrites, during brain development (Bradke and Dotti, 1999; Arimura and Kaibuchi, 2007). It has long been known that PI3K-Akt signaling plays a role in neuronal polarization *in vitro* (Arimura and Kaibuchi, 2007). Previous investigations show that TSC complex-Rheb-mTORC1 components are localized along growth cones of axons of cortical neurons in culture (Haddad et al., 2002; Choi et al., 2008). The presence of TSC complex and Rheb-mTORC1 pathway components as well as the activation of local translation of proteins that are required for axonal growth, together support the axon outgrowth in response to extracellular signals like Ephrins (Nie et al., 2010; Gracias et al., 2014). Rheb and mTORC1 control neuronal polarity by acting as upstream regulators of Rap1B GTPases. The restriction of Rap1B to a single neurite in turn ensures the extension of a single axon (Li et al., 2008).

Axon targeting has been studied extensively using the *Drosophila* visual system (Tayler and Garrity, 2003; Knox et al., 2007; Dimitroff et al., 2012). A study on the role of Rheb in the mushroom bodies of *Drosophila* shows that over-expression of Rheb in the mushroom bodies results in enlarged axonal lobes (Brown et al., 2012). Rheb over-expression produced cell autonomous defects in photoreceptor axon guidance in the *Drosophila* visual system, but did not produce motor-neuron axon misrouting and the

motor-neuron axon follows the normal trajectory and synapses at the correct location on muscle (Knox et al., 2007; Dimitroff et al., 2012).

Regulation of actin is essential for axon guidance in the motor neuron and in the visual system and disruption of actin regulation may be the underlying cause of molecular deficits in axon guidance in the *Drosophila* visual system (Liebl et al., 2000; Luo 2002; Coyle et al., 2004). However, the axon guidance in photoreceptors may not be completely dependent on Rheb-mTORC1 growth signaling but also on TSC complex-mTORC2 regulation of actin cytoskeleton (Knox et al., 2007; Dimitroff et al., 2012).

Altogether, Rheb-mTORC1 and possibly mTORC2 are involved in the establishment of neuronal polarity in both, *Drosophila* and mammals. However, there may be differences in the detailed mechanisms involved.

Rheb in neuroprotection and axon regeneration

Several studies were conducted in analyzing the neuroprotective roles of Rheb by axon regeneration or increased survival in different neuron types (Jeon and Kim, 2015; Nam et al., 2015; Wu et al., 2016) (Figure 1). Interestingly, the fact that the adult mammalian central nervous system (CNS) is incapable of an axonal regenerative response after injury has been challenged by the recent work by Park et al. showing that constitutive activation of Akt-mTORC1 signaling in adult retinal ganglion neurons prior to optic nerve injury promoted regeneration of their axons (Park et al., 2008; Kim et al., 2012). Irrevocably, Akt and Rheb play an important role in protection and regeneration of axons not only during the period of acute axon injury but also 3 weeks post-lesion, by which time the degenerative process has run its course and ceased (Kim et al., 2011, 2012).

Brain-derived neurotrophic factor (BDNF) and glial cell-derived neurotrophic factor (GDNF) are required for regulating the development, maintenance function and plasticity of mature neurons and hence are important in protection of neurons in Parkinson's diseases (PD), which is characterized by progressive degeneration of dopaminergic (DA) neurons (Kim et al., 2012; Jeon and Kim, 2015; Nam et al., 2015). Transduction of DA neurons with adeno-associated virus constructs containing a constitutively active form of Rheb (AAV-hRheb S16H), provided protection of DA neurons from 1-methyl-4-phenylpyridine (MPP) induced degeneration in adult brain. This neuroprotective effect is mediated by the induction of BDNF or GDNF expression and signaling through activation of mTORC1-CREB pathway (Nam et al., 2015).

Similar results were also observed in the case of mouse models of Alzheimer's disease (AD) where hRheb (S16H) expression in the hippocampal neurons induced the sustained production of BDNF *via* mTORC1-dependent pathway, contributing to neuroprotection in the adult brain (Jeon et al., 2015).

Akt-Rheb-mTORC1 signaling has the ability to enhance many features of axon growth, including not only axon length, but also axon numbers per neuron and number of neurons having multiple axons (Choi et al., 2008). Rheb was found to promote activation of intrinsic sensory axon regrowth in dorsal root ganglia (DRG) across the dorsal root entry zone (DREZ) after inhibitory molecules were digested with chondroitinsulfate-ABC endolyase (ChABC) or in combination with Neurotrophin-3 (Liu et al., 2016; Wu et al., 2016). Both processes depend on the activation of Rheb-mTORC1 pathway supporting that developmental re-growth shares common molecular mechanism with regeneration following injury (Yaniv et al., 2012).

Even though inhibition of autophagy leads to cell death and neurodegeneration (Rubinsztein, 2006; Sarkar and Rubinsztein, 2008; Wang et al., 2009), excessive autophagy by inhibition of Rheb-mTORC1 pathway can result in axon degeneration following acute injury (Cheng et al., 2011b). Increased Akt-mTORC1 dependent macroautophagy induces retrograde axon degeneration in dopaminergic neurons after acute chemotoxic injury (Cheng et al., 2011b; Wang et al., 2012). Therefore, the ability of Akt or Rheb to protect axons from retrograde degeneration is likely to be due to its ability to suppress autophagy through mTORC1 signaling (Cheng et al., 2011b).

Altogether, the studies on the regulation of mTOR signaling on neural and behavioral development show that different inputs to mTOR signaling have distinct activities in the nervous system development, yet non-canonical mechanisms may have to be considered as well (Dimitroff et al., 2012; Yasuda et al., 2014; Sugiura et al., 2015).

Pro-apoptotic role of Rheb

Role in oxidative stress and aging

Cellular stress plays a significant role in the susceptibility, progress, and actual outcome of neuronal damage (Esch et al., 2002). Free radicals and reactive oxygen species (ROS) have been reported for their contribution to neuronal loss in cerebral ischemia, seizure disorders, schizophrenia, PD and AD (Floyd and Carney, 1992; Good et al.,

1996; Christen, 2000; Barja, 2004; Hinerfeld et al., 2004). A growing body of evidences implicates oxidative stress as being involved in the propagation of cellular injury that leads to neuropathology in these various conditions (Good et al., 1996; Christen, 2000; Andersen, 2004).

Neurons are considered to be more susceptible to oxidative damage as compared to cells in other tissues (Barja, 2004; Gille et al., 2004; Hinerfeld et al., 2004). ROS can initiate a cascade of reactions that leads to neuronal cell death (Floyd and Carney, 1992; Berlett and Stadtmann, 1997; Uttara et al., 2009). It was found that TSC neurons have increased accumulation of ROS and have oxidative stress (Di Nardo et al., 2009). Over-expressing Rheb was ubiquitously found to sensitize adult *Drosophila* to oxidative stress (Patel and Tamanoi, 2006). However, selective Rheb over-expression in neurons and fat bodies did not sensitize the whole *Drosophila* to the oxidative stress response. Nevertheless, the failure of flies with increased Rheb-TOR-S6K signaling to directly elicit a stress response could result in the accumulation of oxidative damage and thus may serve as a model for age-related diseases. Early senescence of locomotor activity was observed in flies that over-express Rheb (Patel and Tamanoi, 2006).

The mTORC1 pathway has been discussed to be involved in cellular aging *via* its role in growth, stress response and protein metabolism, which affects the susceptibility of the organism to neuronal degeneration and death (Esch et al., 2002; Rubinsztein, 2006; Sarkar and Rubinsztein, 2008; Laplante and Sabatini, 2012; Nixon, 2013; Signer and Morrison, 2013). Hence inhibiting mTORC1-mediated translation results in protection and life span extension (Zoncu et al., 2011; Laplante and Sabatini, 2012; Signer and Morrison, 2013). In Rheb loss-of-function clones, the expression of two important cell fate markers, Prospero and Bar, is delayed, supporting that differentiation and senescence both are controlled by the TSC complex-Rheb-mTORC1 signaling pathway (Bateman and McNeill, 2004; Patel and Tamanoi, 2006).

Activation of TSC2 and repression of Rheb-mTORC1 by peroxisomal ROS induces autophagy which is an important response protecting from stress (Zhang et al., 2013). Hence, activation of Rheb-mTORC1 leads to a defective stress response and degeneration. Pathological protein aggregation and age-dependent neuronal vulnerability may act together to induce neurodegeneration (Yankner et al., 2008). Therefore early senescence caused by oxidative stress could be one of the pre-disposing factors for neurodegeneration (Floyd and Carney, 1992; Raina et al., 2001; Barja, 2004; Yankner et al., 2008; Uttara et al., 2009; Bao and Ohlemiller, 2010).

In summary, the effect of Rheb-mTORC1 pathway inhibition using rapamycin is different depending on the cellular state, which is changing during development and aging (Patel and Tamanoi, 2006; Lamming, 2016).

Response to other stresses

Rheb-mTORC1-S6K signaling also affects the response to various forms of stress such as UV, ER etc. (Patel and Tamanoi, 2006; Karassek et al., 2010). Karassek and colleagues have shown that Rheb plays a role in enhancing the apoptosis induced by UV, tumor necrosis factor α (TNF α) and tunicamycin in neuronal cells *via* apoptosis signal-regulating kinase 1 (ASK1), which is also activated under oxidative stress or ER stress (Ichijo 1997; Nishitoh et al., 2002; Karassek et al., 2010; Soga et al., 2012).

Prolonged inhibition of autophagy by Rheb-mTORC1 pathway induces protein stress (Zhou et al., 2009; Sciarretta et al., 2012). Studies have also shown that abrogated autophagy can induce apoptosis in response to misfolded protein stress (Zhou et al., 2009; Kang et al., 2011). In addition, Rheb inhibits aggresome formation independent of mTORC1 and plays a role in apoptosis induction (Wataya-Kaneda et al., 2001; Zhou et al., 2009). Furthermore, Ozcan et al. describes that the loss of the TSC1 and TSC2 genes causes ER stress and activates the unfolded protein responses (UPR) dependent on Rheb and mTORC1 (Ozcan et al., 2008). These cells are prone to apoptosis due to ER stress and UPR (Marciniak and Ron, 2006; Ozcan et al., 2008). Evidences generated from TSC brain lesions *in vivo*, also identified a role for the TSC complex in the neuronal stress response (Floyd and Carney, 1992; Berlett and Stadtmann, 1997; Di Nardo et al., 2009; Uttara et al., 2009). Di Nardo et al. demonstrated that lack of a functional TSC complex and activation of the downstream translational pathway of Rheb-mTORC1, resulted in increased vulnerability to ER stress and to neuronal damage (Di Nardo et al., 2009). In *Drosophila*, prolonged expression of Rheb in fly photoreceptors results in degeneration of rhabdomeres due to the loss of autophagy (Wang et al., 2009).

Although in general, Rheb-mTORC1 signaling promotes protein synthesis, recently, it was reported that Rheb inhibits protein synthesis by mediating phosphorylation of eukaryotic initiation factor 2α (eiF2α) *via* PERK independent of mTORC1 signaling (Tyagi et al., 2015). Rheb-PERK signaling is activated under ER stress leading to inhibition of protein synthesis, indicating that Rheb may function as a molecular switch between stimulation and inhibition of protein synthesis under physiological as well as pathological conditions (Tyagi et al., 2015).

Human age-related macular degeneration (AMD) is one of the major sight-threatening diseases where, irreversible neuron death including photoreceptor atrophy and retina ganglion cell (RGC) apoptosis are the major features. Light-induced retina degeneration in rodents has been widely used as models of AMD. Studies using rodent model of AMD supported that Rheb enhances the cell death induced by other stress signals (Shu et al., 2014).

In conclusion, cells failing to elicit a proper stress response are more prone to cell death as compared to 'healthy cells'. In addition, activation of Rheb-mTORC1 under stress and during aging increase the organism's susceptibility to neurodegeneration (Patel and Tamanoi, 2006; Ozcan et al., 2008; Di Nardo et al., 2009; Karassek et al., 2010; Kang et al., 2011; Lamming 2016).

Role in neurodegenerative diseases

Many lines of evidences suggest that intracellular protein degradation pathways such as autophagy and the ubiquitin-proteasome system are deregulated in neurodegenerative diseases and may play key roles in the etiology of these pathologies (Rubinsztein, 2006; Sarkar and Rubinsztein, 2008; Wang et al., 2009; Laplante and Sabatini, 2012; Nixon, 2013). The inhibiton of autophagy in TSC mutant or under Rheb over-expression leads to accumulation of toxic aggregate-prone proteins such as ataxin, Tau, and α-synuclein, which are linked to diseases such as spinocerebral ataxia, AD and PD, respectively (reviewed in Rubinsztein, 2006; Sarkar and Rubinsztein, 2008; Swiech et al., 2008). Therefore, inhibition of mTORC1 with rapamycin and small molecules enhances the autophagic degradation of aggregate-prone proteins *in vitro* and inhibition of protein synthesis *in vivo* reduces the severity of neurodegeneration (Sarkar and Rubinsztein, 2008).

Huntington's disease (HD) is a neuro degenerative disease where the protein huntingtin (Htt) has an expanded polyglutamine (Poly-Q) tract and is characterized by early loss of medium spiny neurons in the striatum, which impairs motor and cognitive functions. In mouse models of HD, it was found that Htt might also interact with Rheb and cause abnormally high activation of mTORC1 that may contribute to disease progression through its roles in protein translation and autophagy (Pryor et al., 2014).

However, recently, it was found that mTORC1 activity is reduced in striatum tissues of HD patients and consequently mTORC1 activation by introducing the constitutively active form of Rheb could alleviate metabolic and degenerative phenotypes in HD brain (Lee et al., 2015). This was surprising since mTORC1 inhibits autophagy and it is possible that autophagy is regulated by other pathways, for example by the proteasomal degeradation, independent of mTORC1 (Sarkar and Rubinsztein, 2008). Protective effects may also result from the ability of mTORC1 to promote the lipogenic gene expression *via* sterol regulatory element-binding proteins (SREBPs) mediated transactivation (Zhang and Manning, 2015). There were also reports that Rhes, a striatum enriched protein, was able to improve neuropathological and motor phenotypes in HD mice (Lee et al., 2015). This effect of Rhes is mediated by promotion of autophagy independent of mTORC1 activation by binding to Beclin-1, a protein critical for the induction of autophagy (Mealer et al., 2014).

Moreover, Rheb levels were found to be downregulated in the AD brain, consistent with increased β-Site APP-cleaving enzyme 1 (BACE1) levels. BACE1 is the rate-limiting and principal enzyme responsible for Aβ generation in neurons of AD brain (Shahani et al., 2014). Rheb interacts with BACE1 in a GTP-dependent manner and promotes its degradation. Such data explain how the activity of Rheb could be linked to AD and HD specific processes.

Role in neurological deficits

TSC patients exhibit a spectrum of neurological deficits including autism, intellectual disability, and intractable epilepsy (Crino et al., 2006). The altered developmental effects in brain of TSC patients is reflective of Rheb overexpression (Lim and Crino, 2013). Rheb activation using constitutively active Rheb, in newborn NPCs and neuroblasts leads to TSC-like lesions, ectopic and premature neuronal differentiation and integration, micronodule formation, and hypertrophic neuronal morphogenesis (Lafourcade et al., 2013). Moreover some of the symptoms of TSC such as seizures are assumed to arise as a consequence of neural cell death and neurodegeneration (Wang et al., 2009). Impaired Rheb signaling may be associated with cognitive deficits seen in TSC, defective learning and memory processes. The altered cognition may be associated with the role of Rheb in both mTORC1-mediated long-lasting synaptic plasticity and memory as well as mTORC1 independent effects on spine and synapse development (Brown et al., 2012; Yasuda et al., 2014; Sugiura et al., 2015). Only a part of TSC complex-mediated changes in dendritic morphology of hippocampal neurons in organotypic cultures were suppressed by rapamycin treatment, indicating that such changes could also occur independent of mTORC1 activation (Knox et al., 2007; Nixon, 2013).

Interestingly, increased phosphorylation of the human TSC2 has been found in the frontal cortex of AD and PD patients indicating that damage to neurons may accumulate with persistent Rheb-mTORC1 pathway upregulation (Brown et al., 2012). The activation of mTOR is associated with many diseases other than TSC, collectively termed 'mTORopathies' characterized by epilepsy, intractable seizures, a spectrum of developmental delay, altered cortical architecture and evidence of activated mTOR signaling (Lim and Crino, 2013). mTORC1 has also been found to be aberrantly activated in many benign tumors such as Lhermitte-Duclos disease as well as in malignant tumors such as glioblastoma multiforme and primary central nervous system lymphomas (PCNSL) (Nitta et al., 2016). Rheb has been found to be highly expressed in most but not all cases of PCNSL. Nevertheless, the downstream effectors S6K and 4E-BP1 were activated indicating that other upstream signaling pathways such as MAPK, AMPK, WNT, RalB signaling may be involved (Martin et al., 2015; Nitta et al., 2016).

Role of Rheb in neural stem cells

Stem cells persist throughout life in numerous mammalian tissues, allowing the replacement of cells during homeostatic turnover in healthy tissues and cells lost during disease. The embryonic development and differentiation of neural stem cells (NSCs) or NPCs requires mTORC1 activation by Rheb (Bateman and McNeill, 2004; Goorden et al., 2011; Hartman et al., 2013; Lafourcade et al., 2013). The deletion of Rheb in the NSCs leads to impaired postnatal growth and reduced body weight in mice and eventual death (Yang et al., 2014). During development, NPCs differentiate into neurons and glia and mTORC1 is crucial for glial differentiation *via* its ability to phosphorylate the transcription factor, Stat3 (Cloëtta et al., 2013).

Self-renewal and differentiation of the NSCs are important processes at different stages of brain development. In neonatal NSC, hyperactive mTORC1 induces NSC differentiation at the expense of self-renewal, while genetically decreasing mTORC1 activity prevents their differentiation, leading to reduced neuron production (Hartman et al., 2013).

In addition, constitutive activation of Rheb in the NPCs also affects neuroblast migration, probably due to its role in Reelin-Dab signaling (Moon et al., 2015). Taken together, Rheb is essential for the differentiation of NPCs, although its aberrant activation may cause abnormal differentiation.

Role of Rheb in glial cells

Astrocytes, as the major glial cell population in the brain, provide metabolic and trophic support for neurons, and modulate synaptic transmission and plasticity (Zhao and Flavin, 2000; Sofroniew and Vinters, 2010). Astrocytes are sensitive to infection and injury, and induce and amplify an immune reaction (Saijo et al., 2009; Sofroniew and Vinters, 2010). Astrocytes respond to tissue damage in CNS by becoming reactive (Ridet et al., 1997; Faulkner et al., 2004). This transformation into reactive astrocytes is promoted by upregulation of epidermal growth factor (EGF) receptor expression (Ridet et al., 1997; Faulkner et al., 2004; Codeluppi et al., 2009).

Codeluppi et al. have demonstrated that the Rheb-mTORC1 pathway is upregulated in reactive astrocytes of the injured spinal cord, whereas inhibition of mTORC1 by rapamycin can reverse activation of astrocytes (Codeluppi et al., 2009). Recent findings suggest that the cell size abnormalities seen in the brains from individuals affected with TSC may reflect the Rheb-S6K pathway regulation by the TSC complex in astrocytes (Uhlmann et al., 2004).

Role in glial cell regulated axon regeneration

Recent investigations have shown that the EGF activated Rheb-mTORC1 pathway inhibits the regeneration of damaged axons by enhancing astrocyte proliferation leading to glial scar formation by astrocytes (Codeluppi et al., 2009). This in turn suggest that even though rapamycin is an inhibitor of growth promoted by the Rheb-mTORC1, it could be used to control astrocytic responses in the damaged nervous system to promote a more permissive environment for axon regeneration (Codeluppi et al., 2009). However, the formation of a glial barrier around a lesion site is also an advantage, because it isolates the still intact CNS tissue from secondary lesions (Sofroniew and Vinters, 2010).

Nevertheless, in certain conditions, reactive astrocytes may provide a permissive substratum to support axonal regrowth. This is possible by the expression of several adhesion and synergistic factors by reactive astrocytes (Ridet et al., 1997). Whether the Rheb-mTORC1 pathway is involved in the expression of such molecules that enable astrocyte–neuron interactions in promoting axonal regeneration is yet to be investigated.

Myelin is a fatty substance that wraps around nerve fibers and serves to increase the speed of electrical communication between neurons. A dynamic turnover of myelin membranes is occurring throughout the lifespan

and depends on the populations of adult myelinating oligodendrocyte and Schwann cells. Rheb-mediated mTORC1 activation is critical for differentiation of the oligodendrocyte precursor cells (OPCs) to mature oligodendrocytes. mTORC1-mediated activation of several transcription factors regulate the oligodendrocyte differentiation and the differentiation of the OPC is also likely through the control of cell-cycle exit by mTORC1 (Lebrun-Julien et al., 2014; Zou et al., 2014).

While the role of mTORC1 signaling for maintaining integrity of myelinated fibers is still under discussion (Lebrun-Julien et al., 2014; Norrmén et al., 2014; Zou et al., 2014), it has been shown that mTORC1 signaling in the Schwann cells is required for myelin formation by regulating lipid synthesis (Norrmén et al., 2014).

mTORC2-mediated signaling is a minor but important contributor too, as Rictor knockout led to hypomyelination. Therefore, a balanced and strictly regulated activation of mTORC1 and mTORC2 in oligodendrocytes is required for myelination and lipogenesis (Lebrun-Julien et al., 2014).

Role in glial cell regulated neuronal degeneration

Neuroinflammatory mechanisms involving microglial activation, astrogliosis, and lymphocyte infiltration probably also contribute to the cascade of events leading to neuronal degeneration (Carson et al., 2006; Hirsch and Hunot, 2009; More et al., 2013) in several neurodegenerative disorders, such as AD, PD, HD, amyotrophic lateral sclerosis (ALS) and progressive supranuclear palsy (Hirsch and Hunot, 2009; Wright et al., 2013). Exposure to lipopolysaccharides induces neuroinflammation in mice and dopaminergic neuronal cultures, which further leads to dopaminergic neuronal death and accumulation of insoluble cytoplasmic inclusions of α-synuclein in nigral neurons. Activated astrocytes express a broad array of neurotoxic molecules, including pro-inflammatory cytokines, chemokines, ROS and nitrogen species, which were found to play crucial roles in neuron inflammatory process and death (Hirsch and Hunot, 2009).

The Rheb-mTORC1 pathway enhances glial scar formation by astrocytes, and might play a role in regulating neuronal apoptosis in response to injury or stress (Codeluppi et al., 2009). Cao et al. investigated temporal-spatial patterns of Rheb expression after lipopolysaccharide (LPS) lateral ventral injection (Cao et al., 2013). It was found that Rheb was highly expressed and mostly located in neurons and reactive astrocytes in the brain cortex after LPS treatment. This Rheb expression in response to inflammation after LPS treatment induces neuronal apoptosis and therefore, Rheb may be associated with physiological and pathological process in neuroinflammation (Cao et al., 2013). Altogether, astrocytes can play an important role in neural cell damage by either inhibiting axon regeneration or by inducing neuronal apoptosis.

Conclusion

Rheb is emerging as an important component of the signaling pathway associated with various diseases such as TSC, tumors, metabolic diseases, and several neurodegenerative diseases. Studies on the role of Rheb and its associated upstream and downstream effectors in the neurons suggest that Rheb is involved in neuronal plasticity and differentiation, synapse growth and function and in the regeneration of axons as well as in neurodegeneration in response to injury and stress. However, corresponding *in vivo* experiments are still missing to validate the basic molecular mechanisms involved in the mammalian species. Some of the aspects of neuronal morphology such as synapse growth and axon guidance may be mediated *via* the mTORC2 pathway. More research on the Rheb-mTORC2 regulation may be needed, to establish the relationship between the two components and their role in actin cytoskeleton mediated regulation of synapse growth and axon guidance. Moreover, the activation of Rheb-mTORC1 pathway in astrocytes plays an important role in both neuroprotection and apoptosis evolving from inflammation as well as in inhibiting axon regeneration from damaged neurons. However, the switch in function of Rheb from a growth promoter to an enhancer of apoptosis in response to cellular and physiological stress indicates that Rheb could have a role in the pathogenesis of several neurodegenerative diseases and aging. In order to design drugs for treating diseases associated with Rheb signaling, future studies should take into consideration the cellular redox state, the cell type specific signaling in brain and in stem cells as well as in cellular senescence and aging.

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