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NEUROSCIENCE FOREFRONT REVIEW

MOLECULAR NEUROBIOLOGY OF mTOR

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Abstract—Mammalian/mechanistic target of rapamycin (mTOR) is a serine-threonine kinase that controls several important aspects of mammalian cell function. mTOR activity is modulated by various intra- and extracellular factors; in turn, mTOR changes rates of translation, transcription, protein degradation, cell signaling, metabolism, and cytoskeleton dynamics. mTOR has been repeatedly shown to participate in neuronal development and the proper functioning of mature neurons. Changes in mTOR activity are often observed in nervous system diseases, including genetic diseases (e.g., tuberous sclerosis complex, Pten-related syndromes, neurofibromatosis, and Fragile X syndrome), epilepsy, brain tumors, and neurodegenerative disorders (Alzheimer's disease, Parkinson's disease, and Huntington's disease). Neuroscientists only recently began deciphering the molecular processes that are downstream of mTOR that participate in proper function of the nervous system. As a result, we are gaining knowledge about the ways in which aberrant changes in mTOR activity lead to various nervous system diseases. In this review, we provide a comprehensive view of mTOR in the nervous system, with a special focus on the neuronal functions of mTOR (e.g., control of translation, transcription, and autophagy) that likely underlie the contribution of mTOR to nervous system diseases.

Key words: mTOR, neuronal development, neuronal plasticity, CNS disease, rapamycin.

Contents

INTRODUCTION

Mammalian/mechanistic target of rapamycin (mTOR) is a serine-threonine kinase that controls several important aspects of mammalian cell function (Malik et al., 2013b). Its activity is modulated by various intra- and extracellular factors, and the task of mTOR is to check whether intracellular resources and the health of the cell are sufficient to respond to extracellular stimuli. In the early 2000s,

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Abbreviations: 4E-BPs, 4E-binding proteins; Atg13, autophagy-related protein 13; BDNF, brain-derived neurotrophic factor; CGG, trinucleotide; EGF, epidermal growth factor; FXS, Fragile X syndrome; Hif1α, hypoxia-inducible factor 1-α; LTD, long-term depression; LTP, long-term potentiation; MAMs, mitochondrion-associated membranes; mTOR, mammalian/mechanistic target of rapamycin; NSC, neural stem cell; p70S6K1, p70 ribosomal S6 protein kinase 1; PKC, protein kinase C; Rheb, Ras homolog enriched in brain protein; SVZ, subventricular zone; Tsc, Tuberous Sclerosis Complex; ULK1, unc-51-like kinase 1.

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when neuroscientists' interest in mTOR began, very few mTOR targets were known and confirmed in mammals. The most studied targets at the time were p70 ribosomal S6 protein kinase 1 (p70S6K1) and eukaryotic initiation factor 4E-binding proteins (4E-BPs). The major focus was on the initiation of translation by mTOR (Burnett et al., 1998). A widely accepted notion was that mTOR allows an increase in the rate of translation in response to extracellular stimuli. Long-term synaptic plasticity, learning, and memory rely on *de novo* protein synthesis. Therefore, mTOR took center stage in molecular studies of neuronal plasticity. Consequently, mTOR was shown to be critical for such forms of neuronal plasticity as long-term potentiation (LTP), long-term depression (LTD) and learning and memory (Jaworski and Sheng, 2006; Swiech et al., 2008). Soon afterward, mTOR was proven to be critical for neuronal survival, differentiation, and morphogenesis. The list of its known neuronal functions is expanding every year. Changes in mTOR activity began to be correlated with such neurological symptoms as epilepsy, mental retardation, autism, and brain tumors (Swiech et al., 2008; Garelick and Kennedy, 2011; Lipton and Sahin, 2014; Bockaert and Marin, 2015).

The list of physiological states and neuropathologies that are linked to mTOR grew very fast, but a comprehensive understanding of mTOR regulation and its cellular effectors in neurons was lagging. With the advent of transcriptomics, proteomics, and new imaging technologies, our understanding of the importance of mTOR and its cellular functions greatly expanded. Changes in mTOR activity are now known to result in increases in translation, transcription, autophagy, cell signaling, metabolism and modifications in cytoskeleton dynamics (Malik et al., 2013b). This review focuses on (*i*) new developments in downstream mTOR processes that are relevant to neuronal function, (*ii*) newly identified neuronal functions of mTOR complexes 1 and 2 (mTORC1 and mTORC2), and (*iii*) diseases that are associated with mTOR dysregulation and their underlying molecular mechanisms.

mTOR AND ITS SIGNALING NETWORK

mTOR complexes

mTOR is a large multidomain protein that exists in two distinct multiprotein complexes: mTORC1 and mTORC2 (Hay and Sonenberg, 2004; Bhaskar and Hay, 2007; Malik et al., 2013b; Fig. 1). Proteins that are present in both complexes include mTOR protein itself, mammalian lethal with SEC13 protein 8 (mLST8), DEP domain-containing mTOR-interacting protein (Deptor), Tel two-interacting protein 1 (Tti1), and telomere maintenance 2 (Tel2; Sarbassov et al., 2004; Peterson et al., 2009; Kaizuka et al., 2010). Specific to mTORC1 is regulatory-associated protein of mTOR (Raptor) and proline-rich AKT1 substrate 40 kDa (PRAS40; Kim et al., 2002; Sancak et al., 2007; Vander Haar et al., 2007). mTORC2 contains rapamycin-insensitive companion of mTOR (Rictor), mammalian stress-activated protein kinase-interacting protein 1 (mSin1), and protein observed with Rictor (Protor; Sarbassov et al., 2004; Frias et al., 2006; Pearce et al., 2007). FK506-binding protein (FKPB12) is a nonobligate mTOR-interacting protein that inhibits mTORC1 activity by blocking the catalytic domain of mTOR in the presence of the drug rapamycin (Jacinto et al., 2004; Sarbassov et al., 2006). mTORC2 was initially considered insensitive to rapamycin, but prolonged treatment with rapamycin also inhibited this complex, perhaps by sequestering mTOR kinase, which cannot form the mTORC2 complex (Sarbassov et al., 2006). In recent years, new insights into the exact structure of the mTORC1 complex have been gained, thanks to advances in cryo-electron microscopy. mTORC1 is an obligate dimer, and its dimerization is necessary for its activity and the phosphorylation of downstream targets (Yip et al., 2010; Aylett et al., 2016). The dimerization of mTORC1 complexes is regulated by newly characterized WAC protein and the TTT (Tel2, TTI1, TT12)–Reptin/Pontin complex (Kim et al., 2013; David-Morrison et al., 2016).

Regulation of mTORCs

Canonical pathways of mTORC1 activation by extracellular signals. The activation of both mTOR complexes requires the integration of specific signals, allowing the proper orchestration of multiple cellular events, and it

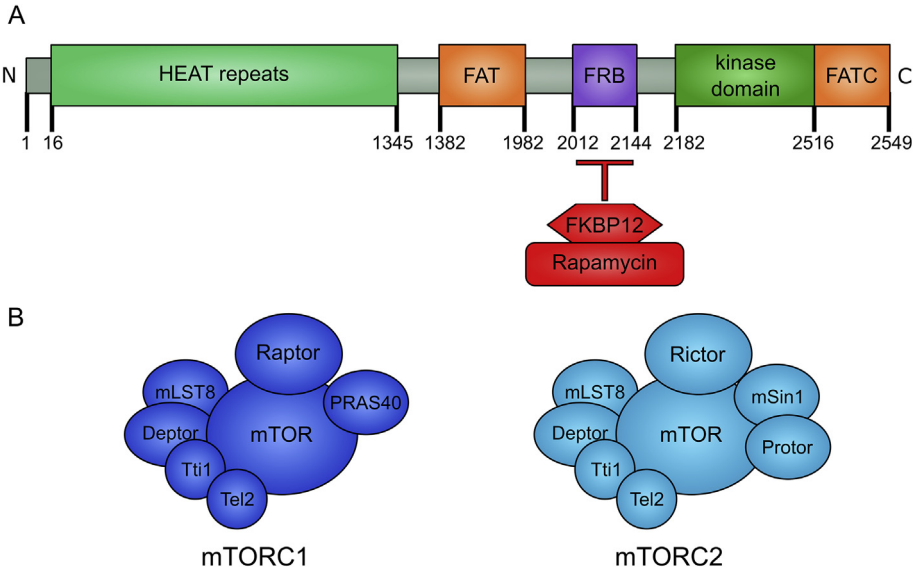

Fig. 1. mTOR and its protein complexes. (A) Protein domain composition of mTOR. HEAT – huntingtin, elongation factor 3, regulatory subunit A of PP2A, TOR1 domain, FAT – FRAP, ATM, TTRAP domain, FRB – FKBP12-rapamycin-binding domain, FATC – C-terminal domain, FKBP12 – FK506-binding protein of 12 kDa. (B) Protein composition of mTOR complexes. mTOR forms two protein complexes mTORC1 and mTORC2, which have different protein composition and non-overlapping sets of cellular substrates. See text for more details.

occurs in response to intracellular and extracellular factors (Sengupta et al., 2010; Oh and Jacinto, 2011). For mTORC1, the first group includes the availability of nutrients, especially amino acids, and the adenosine monophosphate (AMP):adenosine triphosphate (ATP) ratio, which reflects the energy state of the cell (Hahn-Windgassen et al., 2005; Malik et al., 2013b; Bar-Peled and Sabatini, 2014). Cellular stress (e.g., oxidative, genotoxic, and endoplasmic reticulum [ER] stress; Feng et al., 2005; Sofer et al., 2005; Chen et al., 2011) and the inhibition of translation (Kimball et al., 2008) also affect mTORC1 activity. Recently, Christine Holt's group presented evidence of the requirement of intact F-actin dynamics for proper mTORC1 activation in response to netrin-1 in axonal growth cones of tectal neurons (Piper et al., 2015). Among the extracellular stimuli that are important for the activation of both mTORCs in various cell types are hormones (e.g., insulin), growth factors (e.g., epidermal growth factor [EGF] and insulin-like growth factor 1 [IGF1]), the extracellular matrix, and cell adhesion molecules (Quevedo et al., 2002; Nomura et al., 2003; Saucedo et al., 2003). In neurons, mTORC1 is also induced by neuron-specific stimuli, including brain-derived neurotrophic factor (BDNF; Takei et al., 2004; Skalecka et al., 2016), reelin (Jossin and Goffinet, 2007), netrin 1 (Piper et al., 2015), and several neurotransmitters, including amino acids (e.g., glutamate, γ-aminobutyric acid [GABA]), monoamines (e.g., dopamine, serotonin), acetylcholine, and neuropeptides (e.g., orexin; Lenz and Avruch, 2005; Schicknick et al., 2008; Wang et al., 2014). In the case of glutamate, the activation of both ionotropic and metabotropic glutamate receptors (mGluRs) induces mTORC1 (Cammalleri et al., 2003; Hou and Klann, 2004; Lenz and Avruch, 2005; Macias et al., 2013; Blazejczyk et al., 2016). Not all extracellular signals activate mTOR in neurons. For example, the axonal growth chemorepellent semaphorin 3A inhibits mTORC1 activation via Tuberous Sclerosis Complex (Tsc), which leads to growth cone collapse (Nie et al., 2010). In contrast, glucocorticoids downregulate mTORC1 in hypothalamic neurons (Shimizu et al., 2010).

The canonical pathway of mTORC1 activation by trophic factors begins with the activation of their respective receptor tyrosine kinases and downstream signaling (e.g., phosphoinositide-3′ kinase [PI3K]-Akt and extracellular signal-regulated kinases [ERKs]; Fig. 2a; for review, see Malik et al., 2013b). Active Akt and ERKs, in turn, phosphorylate and inactivate tuberin (Tsc2), which together with hamartin (Tsc1) and TBC1D7 forms Tsc (Fig. 1; van Slegtenhorst et al., 1998; Inoki et al., 2002; Dibble et al., 2012). Tsc2 acts as a guanosine triphosphatase (GTPase)-activating protein (GAP) toward Ras homolog enriched in brain protein (Rheb), a direct activator of mTORC1 (Inoki et al., 2003a). Thus, the inactivation of Rheb by Tsc leads to mTORC1 inhibition, which is released by trophic factor-driven Akt activation (Dan et al., 2002; Inoki et al., 2002; Manning et al., 2002).

Tsc2 can also be phosphorylated by other protein kinases that prevent its inhibition by extracellular stimulation (Fig. 2A). For example, Tsc2 is activated by AMP-activated protein kinase (AMPK; Inoki et al., 2003b), which in turn is activated by low cell energy status (signaled by an elevated AMP:ATP ratio). Thus, AMPK-dependent Tsc2 phosphorylation should counterbalance the effect of trophic factors on mTORC1 if the energy status of the cell is suboptimal. Indeed, in neurons, AMPK activity prevents the BDNF-induced activation of mTORC1 (Ishizuka et al., 2013).

Notably, Tsc is not the only heavily regulated node of the mTOR signaling pathway. Some kinases that are known to modulate Tsc2 also regulate mTORC1 directly. For example, Akt phosphorylates and inactivates PRAS40, an endogenous inhibitor of mTORC1 (Sancak et al., 2007; Vander Haar et al., 2007). AMPK phosphorylates Raptor, leading to mTORC1 inactivation through the binding of 14-3-3 protein to phospho-Raptor (Gwinn et al., 2008). In contrast, the GSK3-dependent phosphorylation of Raptor is regulated by amino acid levels and induces mTORC1 (Stretton et al., 2015). Lee et al. (2009) found that mTORC1, under low-glucose conditions, is inhibited as a result of Rheb sequestration by the glycolytic enzyme glyceraldehyde 3-phosphate dehydrogenase.

Activation of the classic mTORC1 pathway by trophic factors, morphogens, and hormones is well studied. Much less is known about the regulation of mTORC1 by neurotransmitters. The canonical mechanism described above is likely shared by some neurotransmitters (e.g., glutamate and opioids; Cammalleri et al., 2003; Lenz and Avruch, 2005; Troca-Marín et al., 2011; Xu et al., 2014). However, several examples suggest additional means by which mTORC1 is regulated by neurotransmitters. For example, Sucher et al. (2010) described the direct binding of Rheb to the NR3A subunit of the *N*-methyl-D-aspartate (NMDA) receptor. These authors speculated that NR3A sequesters synaptic Rheb, leading to mTORC1 inhibition. Orexin A stimulates mTORC1 in an Akt- and Erk-independent manner. Instead, mTORC1 activation required orexin A-induced extracellular calcium influx and depended on the activity of lysosomal vacuolar H+-ATPase (v-ATPase) and the small GTPase RagC (Wang et al., 2014).

mTORC1 activation by amino acids. The availability of amino acids is another important regulator of mTORC1 activity (Fig. 2B). The levels of amino acids are sensed by the Rag–Ragulator complex, which recruits mTORC1 to the lysosomal surface where it can be activated (for review, see Zoncu et al., 2011). Amino acids also play a role in regulating Tsc activity. The lack of amino acids recruits Tsc to the lysosome via Rags, where it can inactivate Rheb and disable the possibility of mTORC1 activation (Demetriades et al., 2014). The simultaneous deprivation of amino acids, namely arginine, and growth factors also leads to an increase in the localization of Tsc to the lysosome, which is mediated by its interaction with Rheb (Carroll et al., 2016). Additionally, the Akt-dependent phosphorylation of Tsc causes it to dissociate from the lysosome, so that it is no longer able to serve as Rheb GAP (Menon et al., 2014).

Regulation of mTORC2. The mechanisms of mTORC2 regulation are less well known. The best characterized extracellular signals that activate mTORC2 are growth factors and hormones that upregulate PI3K activity (e.g., insulin and EGF; Huang et al., 2008; Kumar et al., 2008). In yeast and cancer cells, mTORC2 associated with the ribosome upon growth factor stimulation, which was necessary for its activation (Oh et al., 2010; Zinzalla et al., 2011). Additionally, in the event of ER stress, GSK3β phosphorylates Rictor, preventing mTORC2 from phosphorylating Akt. Such a balance between the activities of GSK3β and Akt allows the cell to regulate glucose metabolism according to the metabolic state of the cell (Chen et al., 2011). Recently, Verma and Marchese (2015) described an additional level of mTORC2 regulation by means of the degradation of Deptor by the endosomal sorting complexes required for transport (ESCRT), leading to mTORC2 activation. In neuronal cells, mTORC2 is activated by neurotrophins, glutamate, NMDA, and factors that induce LTP (Huang et al., 2013).

Downstream effectors of mTORCs. In recent years, several studies, including large phosphoproteomic screens, revealed dozens of new potential targets of mTORC1 and mTORC2 (Hsu et al., 2011; Yu et al., 2011;

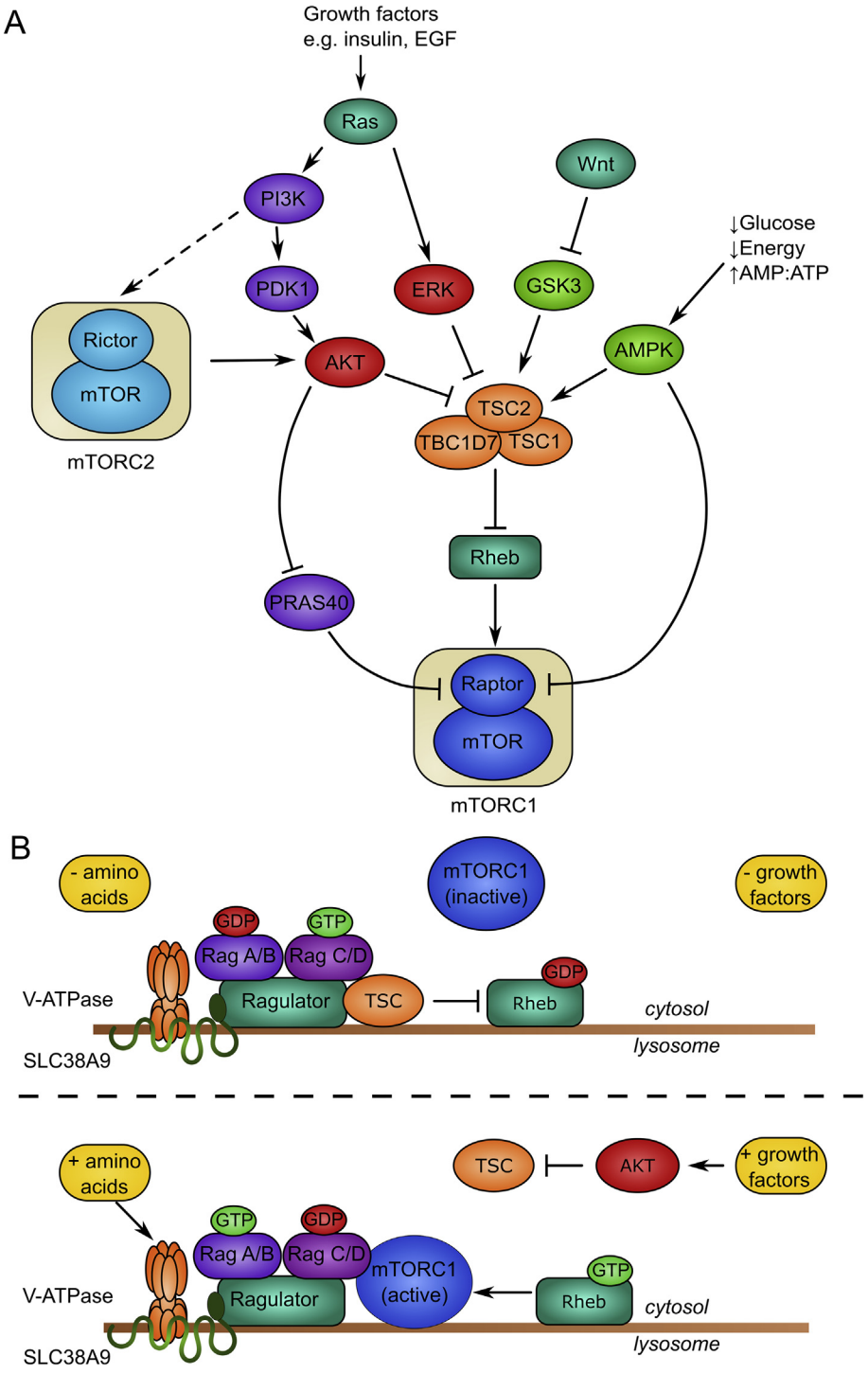

Fig. 2. Regulators of mTOR activity. (A) TSC1/2-dependent and -independent pathways of mTORCs regulation by trophic factors, glucose and cellular energy levels. The canonical pathway of mTORC1 activation by trophic factors starts with the activation of their respective receptor tyrosine kinases and subsequent increase in PI3K-Akt and ERK signaling. Akt and ERK inactivate TSC complex, which is a major inhibitor of mTORC1. However, when cellular energy level is low TSC is activated by AMPK, what prevents response to trophic factors. Some kinases can also regulate mTORC1 directly. Akt phosphorylates and inactivates PRAS40, leading to mTORC1 activation. In contrast, AMPK phosphorylates Raptor, leading to mTORC1 inactivation. Trophic factors can also activate mTORC2 via PI3K. (B) mTORC1 regulation by amino acids. The key component of mTORC1 regulation by amino acids is Rag–Ragulator complex. This complex consists of small Rag GTPases, which form heterodimers that contain RagA or RagB with RagC or RagD, and the Ragulator complex, consisting of five proteins: p18, p14, MEK partner 1 (MP1), hepatitis B virus X-interacting protein (HBXIP), and c7orf59. The RagA/B and RagC/D heterodimers are tethered to the cytoplasmic surface of the lysosome by the Ragulator scaffold. In amino acid-starved cells, RagA/B binds GDP, and RagC/D binds GTP, and the complex is unable to recruit mTORC1 to the lysosome. Upon the addition of amino acids, the dimers change their nucleotide-binding state to RagA/B-binding GTP and RagC/D-binding GDP. This "active" dimer binds Raptor and recruits mTORC1 to the lysosomal surface for subsequent activation by Rheb. The Ragulator complex serves as a scaffold for Rag GTPases, but it also acts as a guanine exchange factor (GEF) toward RagA and RagB. Ragulator senses amino acid availability via the activity of the V-ATPase and via the interaction with a newly discovered lysosomal amino acid transporter, SLC38A9.

Robitaille et al., 2013). These new substrates are too numerous to be discussed herein in detail, but their identification demonstrated functions of mTORCs beyond the control of translation initiation. In the following paragraphs, we focus on the functions of mTOR that are most relevant to neuronal physiology and pathology.

Translation. Translation is an energy-consuming process, and mammalian cells have generated complicated mechanisms to control it, depending on nutrient availability and the general condition of the cell. mTOR kinase is the main factor that links the energy state of the cell to protein translation. Canonical regulation occurs at the level of translation initiation and involves the phosphorylation of 4E-BP1 (Gingras et al., 1999) and p70S6K1 (Burnett et al., 1998); Fig. 3A). The effects of mTOR-dependent phosphorylation of these substrates were discussed elsewhere (e.g., Malik et al., 2013b) and will not be described here. Aside from its canonical roles in regulating cap-dependent translation, mTORC1 mediates additional modes of translational control. For example, mTORC1 catalyzes the cap- and eIF-4E-independent translation of IGF2 mRNA via the internal ribosomal entry site (Dai et al., 2011).

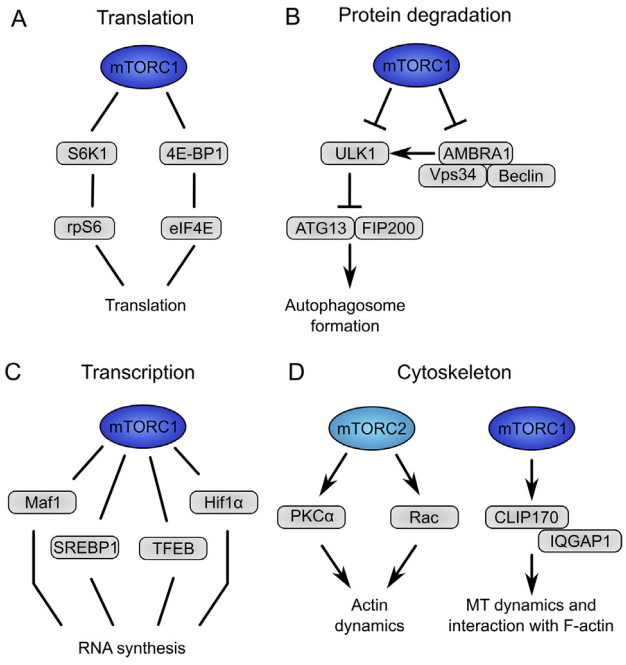

Fig. 3. mTOR targets and downstream cellular processes. (A) Translation. mTORC1 controls translation initiation acting on its best known substrates – 4E-BP1 and S6K1. (B) Autophagy. Under nutrient-rich conditions, mTORC1 interacts via Raptor with the ULK1–Atg13–FIP200 complex. Phosphorylation of ULK1 by mTORC1 prevents initiation of autophagy. When amino acids level is low, mTORC1 is released from the ULK1–Atg13–FIP200 complex and autophagy is promoted. Another way, by which autophagy is controlled by mTOR is the phosphorylation of AMBRA, a part of the Vps34-Beclin1 complex. mTOR-phosphorylated AMBRA is sequestered from the complex that it forms with TRAF6, which weakens the progression of autophagy. (C) Transcription. Several transcription factors (TFs) including MAF1, SREBP1, HIF1α and TFEB were proven to be mTORC1 targets. Phosphorylation by mTOR in some cases regulates cellular distribution of TFs (e.g., TFEB). (D) Cytoskeleton. mTORC1 controls interaction of F-actin and microtubules regulating interaction of its substrate CLIP-170 with an actin-binding protein IQGAP1. mTORC2 controls F-actin dynamics via PKCα and small GTPAse Rac1.

Since the first observation of ribosomes in axon terminals (i.e., outside neuronal pericarion) by Bodian (1965), neuroscientists have investigated the possibility that proteins can be produced on demand locally at synapses, which was eventually proven in the 1990s. Tang et al. (2002) demonstrated the influence of mTOR activity on local stimulus-driven, cap-dependent protein synthesis in dendrites. In a subsequent study, Takei et al. (2004) showed that the stimulation of hippocampal neurons by BDNF in culture induced the synthesis of dendritic Arc (activity-regulated cytoskeleton-associated protein) and CamKIIα (Ca^{2+}/calmodulin-dependent protein kinase II). This process was inhibited by rapamycin. The stimulation of NMDARs and mGluRs activates mTOR and increases dendritic protein synthesis (Hou and Klann, 2004; Gong et al., 2006), which contributes to synaptic plasticity (Jaworski and Sheng, 2006; Swiech et al., 2008).

Several follow-up studies focused on the local mTOR-dependent translation of mRNAs. Schratt et al. (2004) identified dozens of dendritic transcripts that are mTOR-dependently associated with polysomes in response to BDNF. Liao et al. (2007) then showed that upon BDNF stimulation, several hundred proteins were upregulated in synaptoneurosome preparations from cultured cortical neurons. Among the transcripts that are controlled by mTOR, some encode proteins that influence neuronal activity or the shape of dendritic spines (e.g., CamKIIα, NR1, Homer2, Limk2, GluA1, and GluA2), which likely explains the way in which mTOR-driven local translation contributes to synaptic and structural plasticity.

Despite the findings of local translation upregulation by mTOR, several studies indicate that a certain pool of locally synthesized proteins in neurons is suppressed by mTOR activity. In the case of Kv1.1 ion channels, its dendritic synthesis is repressed by active mTOR (Raab-Graham et al., 2006) via miR-129. When mTOR is rendered inactive, the RNA-binding protein HuD promotes Kv1.1 translation in dendrites (Sosanya et al., 2013). Thus, mTOR activity may upregulate the translation of a large pool of synaptic mRNA via 4E-BPs and S6K, but it suppresses another pool of mRNA via alternative mechanisms, such as miRNAs. Altogether, mTOR activity appears to regulate the local translation of transcripts that increase neuronal excitability, promote late LTP (L-LTP), and diminish mGluR-LTD. Unclear is the way in which the suppression of Kv1.1 translation at the synapse fits into this picture, although

diminished Kv1.1 expression has been suggested to increase synaptic excitability (Raab-Graham et al., 2006; Kepert and Kiebler, 2013).

Local protein synthesis that is controlled by mTOR also contributes to neuronal development. mTOR and p70S6K are needed for the local translation of Tau and CRMP2 proteins, which both participate in neuronal polarization (Morita and Sobue, 2009). Work by the Holt group has shown that mTOR-dependent local translation that occurs in navigating axonal growth cones is needed for the proper response to chemoattractants and chemorepellents (Piper et al., 2006, 2015). The requirement for mTOR-dependent local translation in axonal growth cones is not restricted to development. mTOR activation is induced by nerve injury, and the local production of syntaxin-13 was shown to play an instrumental role in the regenerative process (Cho et al., 2014). Local protein synthesis is also involved in dendritogenesis (Lee et al., 2003; Vessey et al., 2008, 2010; Perycz et al., 2011). Jaworski et al. (2005) demonstrated that mTOR activity is necessary for proper dendritic arborization in response to the activation of BDNF-PI3K-mTOR signaling and identified S6K1 and 4E-BP1 as potential downstream effectors. Nevertheless, direct evidence of the involvement of mTOR-dependent local translation in dendritogenesis awaits direct verification.

Protein degradation. Macroautophagy, commonly referred to as autophagy, allows the clearance of obsolete proteins, misfolded or overexpressed protein aggregates, and whole organelles. Autophagy begins with the envelopment of a portion of the cytoplasm that is destined for degradation with a double-membrane vesicle called an autophagosome. The autophagosome then undergoes fusion with the lysosome, and its cargo is degraded. The resulting amino acids are reused in metabolic processes (Levine and Klionsky, 2004). The first link between TOR kinase and autophagy was found in yeast by Noda and Ohsumi (1998), who demonstrated that rapamycin induces autophagy in *S. cerevisiae*. mTORC1 activity negatively controls the initiation of autophagy, and mTORC1 inhibition with either rapamycin or amino acid starvation stimulates this process. A better understanding of the molecular aspects of the involvement of mTOR kinase in autophagy was achieved only very recently when several mTORC1 substrates that are involved in this process were identified (e.g., the unc-51-like kinase 1 [ULK1]–autophagy-related protein 13 [Atg13]–FAK family kinase-interacting protein of 200 kDa [FIP200] complex and autophagy/Beclin-1 regulator 1 [AMBRA]) (Ganley et al., 2009; Hosokawa et al., 2009; Jung et al., 2009); Fig. 3B).

The basal level of autophagy is barely detectable in neuronal cells under nutrient-rich conditions (Mizushima et al., 2004). However, blocking the final steps of autophagic clearance leads to the rapid accumulation of autophagosomes, suggesting that autophagy in neurons occurs very efficiently (Boland et al., 2008). Live cell imaging of cultured dorsal root ganglion and hippocampal neurons revealed that autophagy in neuronal cells occurs constitutively and in a precisely organized manner. Autophagosomes are continuously generated at neurite tips and later transported toward the cell body while simultaneously undergoing maturation (Maday et al., 2012; Maday and Holzbaur, 2014). Accumulating evidence indicates that autophagy contributes to neuronal development and neuroplasticity. Rapamycin treatment in mouse neuroblastoma cells led to autophagy-dependent neuronal differentiation (Zeng and Zhou, 2008). Smith et al. (2014) proposed that inhibition of the autophagy pathway contributes to BDNF-mediated neuronal survival. Hernandez et al. (2012) showed that autophagy-deficient mice exhibited enhanced presynaptic neurotransmission that was rescued by rapamycin administration. The role of autophagy was also reported at postsynaptic sites. Autophagy-mediated α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor degradation occurred as a result of chemical LTD (Shehata et al., 2012), and Tang et al. (2014) further demonstrated its role in dendritic spine pruning.

mTORC1 also controls protein degradation by the ubiquitin–proteasome system. Fumagalli et al. (2015) showed that rapamycin treatment in oligodendrocyte precursor cells enhanced the interaction between E3-ubiquitin ligase murine double minute 2 (Mdm2) and G-protein-coupled receptor kinase 2 (GRK2) and the subsequent degradation of GRK2. Lower levels of GRK2 delay the desensitization of G protein-coupled receptor 17 (GPR17), which disturbs oligodendrocyte production and axonal myelination. Indeed, mTORC1 controls both processes *in vivo* (Bercury et al., 2014; Wahl et al., 2014). In addition to mTORC1, research over the last few years clearly shows that mTORC2 also regulates proteasome-driven protein degradation by acting on E3-ubiquitine ligase FBXW7 (Koo et al., 2015; Li et al., 2016). Still unknown, however, is the way in which this mTORC2-driven regulation of proteasome function contributes to neuronal function and dysfunction.

mTORC1 was recently shown to regulate the assembly of the proteasome itself. Yun et al. (2016) showed that the increased activity of mTORC1 promotes the assembly of immunoproteasomes. Immunoproteasomes differ in their subunit composition from constitutive ones and are assembled in cells in response to oxidative stress and proinflammatory cytokines (Johnston-Carey et al., 2015). The mechanism by which mTORC1 regulates proteasome formation appears to be mediated by PRAS40, which interacts with immunoproteasome subunits. These authors speculated that this response can occur in cells that present hyperactive mTORC1, in which increased protein synthesis can lead to the increased formation of misfolded, oxidized, or aggregated proteins that require clearance. Recent studies suggest that immunoproteasomes are activated in epilepsy (Mishto et al., 2011, 2015).

Transcription and RNA processing. Another cellular function of mTORC1 that is receiving more attention is the regulation of transcription and RNA processing. mTOR modulates the transcription of genes that are transcribed by all three RNA polymerases (Pol I, Pol II and Pol III). The growth factor- and rapamycin-

dependent interaction between mTOR and promoter sequences that are used by all three RNA polymerases has been reported (Mayer et al., 2004; Mayer and Grummt, 2006; Tsang et al., 2010), but the effects of mTOR on transcription have mostly been studied at the level of regulating the accessibility or activity of other transcription factors (e.g., Maf1, sterol regulatory element-binding protein 1 [SREBP1], transcription factor EB [TFEB], and hypoxia-inducible factor 1-α [Hif1α]; Hudson et al., 2002; Porstmann et al., 2008; Shor et al., 2010; Peña-Llopis et al., 2011; Fig. 3C). Additionally, recent discoveries suggest the involvement of mTOR in the post-transcriptional processing of different classes of RNA (Ye et al., 2015).

The involvement of mTOR in transcription has been discussed extensively elsewhere (Malik et al., 2013b). Below we focus specifically on issues that are related to neurons. Under basal conditions, acute rapamycin treatment does not robustly affect mRNA levels in cultured hippocampal slices (Blazejczyk et al., 2016). The stimulation of these neuronal preparations with kainic acid (KA; an agonist of kainate-type ionotropic glutamate receptors) revealed some effects of mTOR inhibition on gene expression. For example, we identified a small set of genes, the KA-induced expression of which was substantially diminished by mTORC1 inhibition. The overexpression of one of these genes, *engulfment and cell motility 1*, in cultured hippocampal neurons was sufficient to increase the axonal branching pattern and change dendritic spine morphology to be more like less mature neurons (Blazejczyk et al., 2016). However, in our work, we did not identify any particular transcription factor that is needed for KA-mTOR-driven gene expression. Some transcription factors that were mentioned above (e.g., Hif1α) are important for nervous system development and neuronal plasticity. An increase in Hif1α levels promotes the dendritic arbor complexity of neurons that are postnatally born in the subventricular zone (SVZ; Zhang et al., 2016). Hif1α knockdown prevents ectopic dendrite formation that is induced by *Tsc1* knockout (KO), suggesting that Hif1α acts downstream of Tsc-mTORC1 (Feliciano et al., 2013). Unknown are the Hif1α-regulated transcripts that are needed for mTOR-driven dendritic growth. Malik et al. (2013a) screened potential modifiers of dendritic arbor growth, the transcription of which depends on mTORC1. CCN1/Cyr61 was among the positive hits. CCN1 is a matricellular protein (Malik et al., 2013a, 2015), the transcription of which is regulated by HIF1α in non-neuronal cells (Meyuhas et al., 2008). Whether Hif1α is responsible for mTORC1-dependent CCN1 transcription during dendritogenesis remains to be established, but CCN1 overexpression, similar to Hif1α overexpression, was sufficient to induce dendritic growth (Malik et al., 2013a). Other processes that are regulated by Hif1α in neurons include the formation of dendritic spines and synaptic transmission (Huang et al., 2010, 2012).

Cytoskeleton. Another aspect of mTOR activity that is being investigated in the context of proper neuronal development and physiology is control of the cytoskeleton (Fig. 3D). mTORC2 has received the most attention because it is an important modulator of the actin network (Jacinto et al., 2004; Huang et al., 2013). The way in which mTORC2 regulates actin dynamics is still debated, and new mechanisms are constantly emerging (Angliker and Rüegg, 2013; Li and Gao, 2014; Chantaravisoot et al., 2015). In neurons, two potential, although not mutually exclusive, mechanisms have been described. The Ruegg group showed that neuron-specific *Rictor* knockout (*Rictor*$^{fl/fl}$*;Nestin-Cre*) substantially reduces the phosphorylation of several isoforms of protein kinase C (PKC) and its targets that are potentially involved in the control of actin dynamics (e.g., myristoylated alanine-rich C-kinase substrate [MARCKS], growth-associated protein 43 [GAP-43], and adducins; Angliker and Rüegg, 2013; Thomanetz et al., 2013). In contrast, Huang et al. (2013) suggested that mTORC2 controls actin dynamics by interacting with T-cell lymphoma invasion and metastasis 1 (Tiam1; a Rac1 guanine nucleotide exchange factor) and subsequently activating the Rac1-Pak1-cofilin pathway. Compared with mTORC2, mTORC1 has been poorly studied in the context of the cytoskeleton, both in non-neuronal cells and in neurons. An initial observation by Choi et al. (2002) demonstrated the rapamycin-dependent phosphorylation of cytoplasmic linker protein of 170 kDa (CLIP-170; a plus-end microtubule-tracking protein). These authors also showed that rapamycin adversely affected the plus-end tracking behavior of CLIP-170. Indeed, our study confirmed that CLIP-170 is phosphorylated by mTOR, but we did not observe the detachment of CLIP-170 from microtubule plus ends upon rapamycin treatment. Instead, mTOR regulated CLIP-170 binding with IQ motif-containing GTPase-activating protein 1 (IQGAP1) and F-actin, which supported the formation of new dendritic branches (Swiech et al., 2011).

Additional functions of mTORCs. mTORC2 also plays a role in regulating cellular metabolism by controlling the activity of several downstream kinases. Among these, Akt, SGK1, and PKCα are the best studied to date, and all three play roles in neuronal morphology (see below). mTORC2 also regulates the integrity of mitochondrion-associated membranes (MAMs), which facilitate the transfer of calcium and lipids between the ER and mitochondria. Betz et al. (2013) showed that mTORC2 is translocated to the MAM upon insulin stimulation, and MAM-associated mTORC2 can phosphorylate Akt and maintain the structure and stability of MAMs. Mitochondrion-associated membranes are critical for neuronal survival, and their dysfunction appears to contribute to neurodegeneration (Hedskog et al., 2013; Guardia-Laguarta et al., 2015).

CONTRIBUTION OF mTOR TO NERVOUS SYSTEM DEVELOPMENT AND PHYSIOLOGY

mTOR is critical for proper neuronal development (e.g., during axonogenesis and axon guidance [Piper et al., 2006, 2015; Morita and Sobue, 2009; Nie et al., 2010],

dendritogenesis [Jaworski et al., 2005; Kumar et al., 2005; Urbanska et al., 2012; Skalecka et al., 2016], and the genesis of dendritic spines [Kumar et al., 2005] and neuronal physiology [e.g., learning, memory, and synaptic plasticity; for review, see Jaworski and Sheng, 2006; Swiech et al., 2008]). The involvement of mTOR has been demonstrated for different types of neurons, different parts of the nervous system, almost all kinds of neuronal plasticity (e.g., LTP, LTD, and chemical LTD), and different learning and memory processes (Jaworski and Sheng, 2006; Garelick and Kennedy, 2011; Bockaert and Marin, 2015). mTOR is also important for feeding and body weight control (Yang et al., 2012; Vinnikov et al., 2014), circadian rhythm (Cao et al., 2013), and nociception (Khoutorsky et al., 2015). mTOR activity also contributes to nervous system regeneration (Berry et al., 2016). Several of these findings are discussed above and reviewed elsewhere (Jaworski and Sheng, 2006; Swiech et al., 2008; Garelick and Kennedy, 2011; Lipton and Sahin, 2014; Bockaert and Marin, 2015). Important progress is still being made. Interesting developments in this area include the following: (*i*) final proof of the importance of mTOR in neurodevelopment *in vivo* and detailed studies of the role of mTORC1 in early steps of neurogenesis, (*ii*) a better understanding of the role of mTORC2 in the central nervous system, and (*iii*) the identification of novel functions of mTORC1 in the central nervous system beyond the control of protein synthesis. The last of these issues is discussed above. The following sections specifically focus on the first two.

Role of mTORC1 in neurodevelopment *in vivo*

Establishing proper brain morphology requires the proper temporal and spatial organization of neuron birth, migration, and differentiation. The standardization and widespread availability of *in vivo* electroporation protocols and development of conditional transgenic animal models that directly affect mTORC1 activity revealed that many of these processes *in vivo* are controlled by the mTORC1 pathway. This is an important step forward because most of our knowledge of the potential functions of mTORC1 in neuronal development was obtained using *Tsc1*, *Tsc2*, *Pten*, and *Nf1* KO mice, which serve as models of aberrant hyperactivation of mTOR in disease states (Table A.1). Although the knowledge that is gained by studying these models is greatly important, all of these proteins have effectors other than the mTORC1 signaling pathway (Trovó-Marqui and Tajara, 2006; Neuman and Henske, 2011; Alves et al., 2015).

Several recent studies concentrated on the role of mTORC1 during early steps of neurogenesis, both embryonic and postnatal. Although some differences appear to exist between these two phenomena, research suggests that tightly controlled mTORC1 activity is needed for neural stem cell (NSC) proliferation, survival, and differentiation. Cloetta et al. (2013) crossed *Rptor*$^{fl/fl}$ mice with mice that expressed CRE recombinase under the *Nestin* promoter (*Rptor*$^{fl/fl}$*; Nestin-Cre*), which allowed the removal of *Rptor* specifically in NSCs and their progeny. The resultant conditional knockout (cKO) mice, although born in a Mendelian ratio, had microcephaly and were dying just after birth. Detailed analyses revealed fewer neurons in the brain in *Rptor*$^{fl/fl}$*; Nestin-Cre* mice. The decrease in the number of neurons resulted from a combination of lower neurogenesis and an increase in apoptotic cell death of already differentiated Tuj1-positive neurons. With regard to neurogenesis, the cell cycle of *Rptor*$^{fl/fl}$*;Nestin-Cre* ventricular zone (VZ) NSCs was extended, affecting their rate of proliferation. Intriguingly, mice that also expressed a hyperactive mutant of mTOR early prenatally only in *Emx1*-expressing NSCs in the developing cortex had smaller brains because of cortical atrophy (Kassai et al., 2014). In this case, however, atrophy was attributable to the pronounced death of T-box brain protein 2 (Trb2)-positive neuronal progenitors in the embryonic SVZ. An expression analysis of Hif1α and its target, cyclin-dependent kinase inhibitor 1A, led the authors to conclude that cell death was mediated by Hif1α. The effect of mTOR hyperactivity on NSC survival can be age-dependent. Using the same active mutant of mTOR, Kassai et al. (2014) showed that the late initiation of transgene expression on postnatal day 2 (P2) did not have such a dramatic impact on cell survival. At this developmental stage, the electroporation of Hif1α to NSCs in the SVZ did not cause major neurotoxicity. Instead, the knockdown of Hif1α or its inhibition by echinomycin had deleterious effects on neuronal survival (Feliciano et al., 2013).

mTORC1 inhibition was shown to have adverse effects on the number of neurons that were born postnatally in the SVZ (Hartman et al., 2013). In this case, however, no effects of either Raptor or Rheb knockdown on the proliferation or survival rate of NSCs were reported. Instead, the inhibition of mTORC1 activity decreased the differentiation of NSCs toward transit amplifying cells (TACs), which give rise to neuroblasts that migrate toward the olfactory bulb. In the same model, mTOR hyperactivation led to the depletion of NSCs through accelerated differentiation to TACs. mTORC1-dependent protein translation that is regulated by 4E-BPs was also found to be responsible for the differentiation of NSCs (Hartman et al., 2013). At the same time, p70S6K was not involved in differentiation but rather controlled the size of the cell soma. Additional work on photoreceptor differentiation timing in *Drosophila* suggested that additional factors downstream of mTOR, such as RNA-binding protein Unkempt (Unk), may also be involved (Avet-Rochex et al., 2014). In contrast, GSK3 was identified as an upstream regulator of the mTOR pathway during the maintenance and self-renewal of neural progenitors. The inactivation of mTORC1 by rapamycin suppressed the abnormal proliferation of neural progenitors that was induced by GSK3 deletion (Ka et al., 2014). mTOR also positively regulates the generation of astrocytes and differentiation of oligodendrocytes (Bercury et al., 2014; Wahl et al., 2014).

Several examples suggest that mTORC1 is also involved in the control of late neurogenesis, which follows neuron birth and initial lineage specification. Kassai et al. (2014) electroporated hyperactive mTOR in

embryonic day 14 (E14) brains, which halted cell migration from the VZ to the pial surface in the intermediate zone of the forming cerebral cortex. The break in migration was released by either rapamycin treatment or the co-electroporation of shRNAs that targeted Raptor or S6K1 mRNA. The aberrant migration of neurons through the hyperactivation of mTOR was not seen solely in the developing cortex. The Bordey group showed that the constitutive activation of Rheb in neural progenitor stem cells of the SVZ increased mTOR activity and led to disruptions in their neuronal migration to the olfactory bulb and abnormal neuron morphology and circuit formation (Lafourcade et al., 2013). These results are consistent with migration phenotypes that were described previously in *Tsc1* KO mice, suggesting that hyperactive mTORC1 might be a major contributor to defects in migration that are observed in TSC patients. Nonetheless, much less is known about the influence of lower mTORC1 activity on neuronal migration. Our recent study did not directly focus on migration analysis *per se*, but it showed that neuroblasts in *mTOR*$^{fl/fl}$ mice that were electroporated with a plasmid that constitutively expressed CRE migrated from the SVZ through the rostral migratory stream (RMS) to the olfactory bulb and differentiated into neurons (Skalecka et al., 2016).

mTOR is known to control axon and dendrite development in neurons that are cultured *in vitro* and in organotypic slice preparations (Swiech et al., 2008). Gong et al. (2015) recently showed that the activation of mTORC1 *in vivo* by means of the *in utero* electroporation of E15 mice with a plasmid that encoded constitutively active Rheb substantially increased axon growth in layer 2/3 cortical neurons. This effect was mediated by the mTORC1 canonical effectors 4E-BP1/2 and S6K1/2. However, S6Ks were suggested to regulate microtubules by inhibiting the IRS1-PI3K-Akt pathway and subsequently activating GSK3 rather than by controlling protein synthesis (Gong et al., 2015). mTORC1 also has an impact on proper axon myelination in both the central and peripheral nervous systems (Bercury et al., 2014; Lebrun-Julien et al., 2014; Norrmén et al., 2014). More detailed studies of the peripheral nervous system showed that this process requires the Raptor-dependent regulation of sterol synthesis in Schwann cells (Norrmén et al., 2014). Several studies in *Tsc1*, *Tsc2*, and *Pten* KO mice suggested that increased mTOR activation leads to excessive and improper dendritic arborization (Table A.1). However, the requirement of mTOR for physiologically occurring dendritic arborization *in vivo* was proven only recently. Using neonatal *mTOR*$^{fl/fl}$ mice that were electroporated with CRE, Skalecka et al. (2016) showed that the removal of mTOR from NSCs in the SVZ on P0-P1 profoundly simplified both apical and basal dendritic arbors of descendant neurons, which reached and differentiated in the olfactory bulb.

Role of mTORC2 in the nervous system

Much less is known about the function of mTORC2 in the nervous system. This is likely because rapamycin is used as a tool to study mTOR in neurons. The first report of the role of mTORC2 in neurons came from a study of dendritogenesis in *Drosophila*. In class IV dendrite arborization (*da*) sensory neurons, TORC2, together with tricornered kinase, was shown to control dendritic tiling. During this process, dendrites of the same neuronal class actively avoided crossing each other and formed non-overlapping dendritic fields (Koike-Kumagai et al., 2009). Soon afterward, the importance of mTORC2 in dendritogenesis was also described in cultured mammalian hippocampal neurons *in vitro* (Urbanska et al., 2012; Thomanetz et al., 2013). Urbanska et al. (2012) showed that Rictor knockdown led to the simplification of dendritic tree morphology through the inhibition of new dendritic branch formation. The lack of Rictor also affected the BDNF-induced dendritic branching of SVZ-derived neurons that were cultured *in vitro* (Skalecka et al., 2016). At least two mechanisms by which mTORC2 controls dendritogenesis have been proposed. Results from the Ruegg laboratory show that the mTORC2-dependent control of actin dynamics is needed for normal dendritic growth (Angliker and Rüegg, 2013; Thomanetz et al., 2013). Our team identified Akt-mTORC1 as a downstream effector of mTORC2, which controls dendritic growth. Rictor knockdown decreased Akt phosphorylation and simplified dendritic trees, which could be rescued by Akt or S6K1 overexpression (Urbanska et al., 2012).

Mouse models that lack Rictor expression in specific areas of the brain have been generated only recently, accelerating studies of the role of mTORC2 in the nervous system in mammals *in vivo*. Carson et al. (2013) reported reductions of brain size in mice with *Rictor* KO selectively in the dorsal cortex (*Rictor*$^{fl/fl}$*;Emx1-Cre*). However, this decrease was proportional to an overall reduction of body size. A non-proportional decrease was achieved by conditionally deleting *Rictor* in the entire central nervous system by crossing *Rictor*$^{fl/fl}$ mice with *Nestin-Cre* mice. These mice had a 40% reduction of brain size at birth (Thomanetz et al., 2013). The reduction was uniform throughout all of the analyzed brain areas (i.e., cortex, hippocampus, striatum, and olfactory bulb). More detailed analyses of hippocampal CA1 pyramidal and cerebellar Purkinje neurons revealed that the observed microcephaly was not an effect of a decrease in proliferation or dramatic increase in cell death but rather stemmed from a reduction of neuronal cell soma size and fewer dendritic arbors. An additional observation in Purkinje cells was that these neurons, which normally develop single apical primary dendrites, grew multiple dendrites in the absence of Rictor (Thomanetz et al., 2013). Electrophysiology revealed that the lack of Rictor also resulted in a decrease in both excitatory and inhibitory basal synaptic transmission (Thomanetz et al., 2013). These changes in the cerebellum led to clear deficits in motor skills in *Rictor* KO mice in the rotarod test. The use of Purkinje neuron-specific *Rictor* KO (*Rictor*$^{fl/fl}$*; L7/Pcp-2-Cre*) allowed deciphering the deficits that are intrinsic to Rictor's actions in Purkinje cells and the deficits that stem from whole-cerebellar network dysfunction. Such KO produced morphological defects but not synaptic defects (Thomanetz et al., 2013).

More detailed studies of the role of Rictor in synaptic transmission were performed using conditional, postnatal forebrain-specific *Rictor* KO (*Rictor*$^{fl/fl}$*;CaMKIIα-Cre*) mice (Huang et al., 2013). Such *Rictor* KO resulted in the inhibition of L-LTP but not early LTP (E-LTP) in hippocampal slices. These authors also showed that *Rictor*$^{fl/fl}$*;CaMKIIα-Cre* mice formed significantly fewer dendritic spines and suffered from deficits in the formation of both contextual and cued long-term memory (Huang et al., 2013). These deficits were likely attributable to disturbances in actin dynamics, and F-actin-stabilizing drugs rescued both the electrophysiological and cognitive deficits. Furthermore, these authors showed that A-443654, a drug that induces mTORC2 activity, enhanced both L-LTP and long-term memory in wildtype mice (Huang et al., 2013). This observation may have further practical implications in the context of a recent report that demonstrated that mTORC2 activity and long-term memory both decrease with age, and A-443654 restored performance in aged mice to the level of young mice (Johnson et al., 2015).

In addition to classic learning and memory paradigms, *Rictor* KO mice were tested in a wide variety of behavioral tests. One of the first findings regarding the function of mTORC2 in the mammalian brain was that mice with neuron-specific conditional *Rictor* KO (*Rictor*$^{fl:fl}$*;Nestin-Cre*) exhibited lower levels of Akt phosphorylation, leading to the disruption of dopaminergic signaling and neurochemical and behavioral phenotypes associated with schizophrenia and anxiety (Siuta et al., 2010). Prepulse inhibition was disrupted in *Rictor* KO mice. In *Emx1-Cre*-induced *Rictor* KO mice, Carson et al. (2013) reported that the lack of Rictor in only one part of the cortex increased locomotor activity and novelty-induced insomnia and decreased anxiety-like behavior. Additionally, the contribution of mTORC2 to reward-seeking behavior was demonstrated using local *Rictor* KO and local active Rictor (T1135A) overexpression in ventral tegmental area neurons. Using these experimental models, Mazei-Robison et al. (2011) showed that Rictor KO decreased morphine-induced conditioned place preference, and Rictor (T1135A) overexpression led to the opposite response.

mTOR-RELATED DISEASES OF THE NERVOUS SYSTEM

Genetic diseases

Mutations in the genes that encode components of the mTOR signaling pathway result in genetic diseases with severe neurological symptoms. Although these genetic syndromes have their own unique features, they also have several similarities, including epilepsy, benign tumor formation, autism, and intellectual disability. Data from existing animal models (Table A.1) strongly support a causal link between mTOR dysregulation and these clinical manifestations. Below we discuss a few genetic disorders for which the involvement of mTOR has been the most intensively investigated.

Tuberous sclerosis complex. Tuberous sclerosis complex is a multi-system autosomal dominant disorder and the best studied mTORopathy to date. Primary manifestations of TSC are benign tumors and lesions in various organs of the body, with cortical tubers, subependymal nodules (SENs), subependymal giant cell astrocytomas (SEGAs), retinal hamartomas, and cerebral white matter migration tracts in the nervous system. Recent progress in brain imaging revealed additional manifestations of TSC in the nervous system, including cerebellar tubers (Ertan et al., 2010), hypomyelination in some brain areas (e.g., the frontal lobe, pons, and corpus callosum; Arulrajah et al., 2009; Simao et al., 2010), and a tendency toward a decrease in the diameter of the optic nerve (Gialloreti et al., 2015). The most common clinical neurological symptoms of TSC include epilepsy (in 80–90% of affected individuals), intellectual disability (60%), and autism spectrum disorder (ASD; 25–50%; Crino et al., 2006; Curatolo et al., 2008, 2015). Some patients also suffer from other neuropsychiatric disorders, including anxiety, depression, attention-deficit/hyperactivity disorder (ADHD), and aggressive/disruptive behavior (Muzykewicz et al., 2007).

The majority (80–85%) of TSC cases diagnosed to date are caused by mutations in either the *TSC1* or *TSC2* gene (Fig. 4). A large number of mutations have been reported for both *TSC* genes (>200 for *TSC1* and >700 for *TSC2*; Dabora et al., 2001; Sancak et al., 2005). Other unknown TSC genes have also been suggested. Mutations in *TCB1D7* were recently reported, but the clinical presentation in these patients appeared to be different from TSC (Capo-Chichi et al., 2013; Alfaiz et al., 2014). Moreover, in the majority of patients in whom standard gene sequencing identifies no mutation, more comprehensive studies can detect mosaic or intronic mutations in either the *TSC1* or *TSC2* gene, indicating that the existence of other TSC genes is less likely (Tyburczy et al., 2015). Establishing a genotype–phenotype association in TSC has been difficult because of the large number of mutations and mosaicism. To date, most studies suggest that TSC symptoms are generally more severe in patients with *TSC2* mutations than in patients with *TSC1* mutations (Dabora et al., 2001; Curatolo et al., 2015).

To better understand the mechanisms of disease progression and study cellular and molecular defects in TSC, several animal models have been established (Table A.1), including rats (Ecker rats), mice, zebrafish, and *Drosophila*. The complete deletion of either *Tsc1* or *Tsc2* results in embryonic death in mice. Thus, studies are mostly performed in heterozygous or cKO animals with *Tsc* genes that are selectively inactivated in a chosen cell type or brain region. Using this strategy, mice have been developed that lack either *Tsc1* or *Tsc2* in NSCs, neuroblasts, different subtypes of developing or adult neurons (e.g., excitatory or inhibitory), astrocytes, and oligodendrocytes (Table A.1). Mouse models are also available in which *Tsc* gene KO can be induced by either doxycycline or tamoxifen. To date, however, no single transgenic or KO mouse can fully recapitulate the complicated phenotype of TSC in

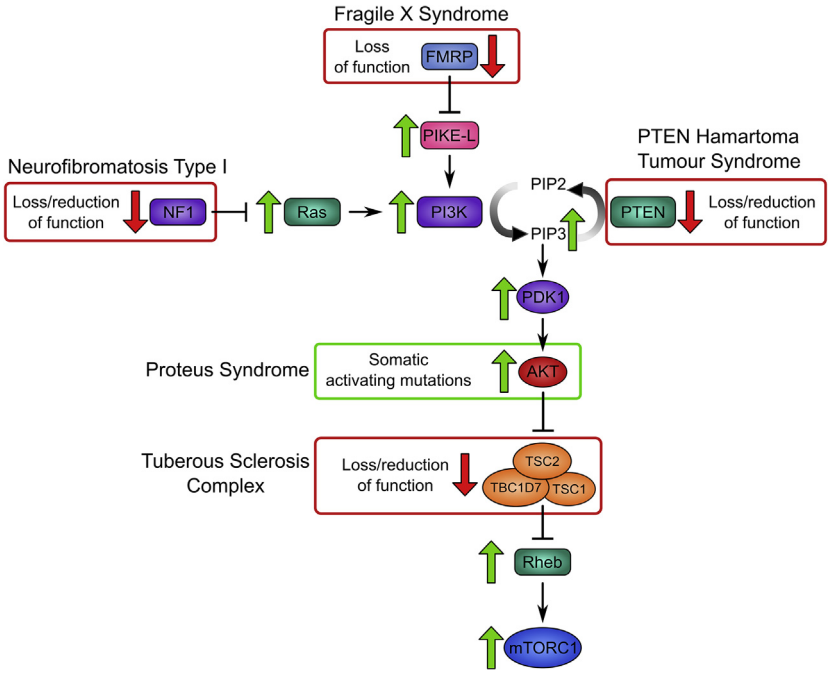

Fig. 4. Cell signaling dysfunction in genetic diseases with changed mTOR activity caused by mutations in *TSC1*, *TSC2*, *PTEN*, *AKT*, *NF1* and *FXR1*. See text for more details.

humans. See Table A.1 for a detailed description of phenotypes that are found in "TSC mice." Generally, heterozygous mice survive normally, do not display typical anatomical changes that are observed in patients' brains (e.g., tubers, SENs, SEGAs, and white matter changes), and are mostly used in studies that evaluate behavioral dysfunction in TSC (Table A.1). A lack of spontaneous seizures was considered one disadvantage of using *Tsc1*$^{+/-}$ and *Tsc2*$^{+/-}$ mice as models of TSC. Recently, however, early postnatal intracortical electroencephalography (EEG) in *Tsc1*$^{+/-}$ mice revealed transient seizures between P9 and P18 in approximately 80% of the animals (Lozovaya et al., 2014). Mice with early gene deletion that is restricted to selected cell populations (e.g., NSCs and developing neurons) are generally excluded from behavioral studies because of their poor survival. Instead, such animal models are mostly exploited to study cellular and anatomical abnormalities (e.g., the appearance of enlarged neurons, balloon-like vacuolized cells, and cells that simultaneously express neuronal and glial markers) and seizure progression. Only a few studies reproduced more complex abnormalities that resemble cortical tubers, SENs, and SEGAs (Table A.1). Some phenotypes (e.g., those that exhibit a decrease in the caliber of the optic nerve) have not yet been reported in mammalian models but are observed in zebrafish (Fig. 5).

Clinical observations, extensive studies of animal models of TSC, and biochemical analyses of the TSC1–TSC2 protein complex undoubtedly point to mTORC1 hyperactivity as a molecular cause of TSC. Nevertheless, recent studies suggest additional contributions of decreases in mTORC2 activity, non-mTORC1-related effectors of TSC, and individual cellular functions of TSC1 and TSC2 to TSC (Switon et al., 2016). Increases in mTORC1 activity are repeatedly reported in pathologically altered cells that are found within tubers, SENs, and SEGAs in TSC patients (Baybis et al., 2004; Chan et al., 2004). The potential contribution of mTORC1 to TSC pathology is further corroborated by observations that several pathogenic mutations in *Tsc1* or *Tsc2* lead to an increase in mTORC1 activity in cultured cells (Hoogeveen-Westerveld et al., 2012, 2013). However, causal relationships between particular TSC symptoms in humans and mTORC1 activity are not always unequivocally proven. The inhibition of mTORC1 with rapalogs prevents SEGA growth (Kotulska et al., 2013; Krueger et al., 2013; Franz et al., 2015). Some examples also exist for the inhibitory effects of rapalogs on epilepsy progression and the rapamycin-induced reduction of ASD and specific neurocognitive symptoms (Kotulska et al., 2013; Franz et al., 2015; Hwang et al., 2016). Most of our knowledge concerning the direct role of mTORC1 in TSC comes from animal models. Some studies reported that stronger mTORC1 activation is correlated with more severe phenotypes. For example, Zeng et al. (2011) observed higher phosphorylation of rpS6 protein, earlier death, and more severe seizures in *Tsc2*$^{fl/fl}$*;GFAP-Cre* KO mice than in *Tsc1*$^{fl/fl}$*;GFAP-Cre* KO mice. Kwiatkowski and coworkers who investigated the effect of Tsc2 dosage on TSC outcome, generated mice with different amounts of remaining tuberin (20% and 35% of physiological Tsc2 levels). Using these mice, Yuan et al. (2012) showed that this 15% difference in tuberin levels was sufficient to lower mTORC1 activation and improve the outcome of "mouse TSC" with regard to both neuroanatomical and behavioral abnormalities. Nonetheless, the strongest evidence of the involvement of mTOR in TSC pathology comes from studies that used rapamycin or its derivatives. As listed in Table A.1, rapamycin treatment in many TSC models extends the life span, reduces seizures, decreases the number of cytomegalic cells, rescues lamination defects, prevents astrogliosis, improves myelination, and reverses behavioral deficits. However, some aspects of TSC pathology appear to be independent of mTORC1. For example, Magri et al. (2011) showed that rapamycin was ineffective in preventing the apoptotic cell death of *Tsc1*$^{-/-}$ neurons that differentiated *in vitro*. The self-renewal of *Tsc1*$^{+/-}$ embryonic NSCs appeared to occur independently from mTORC1 hyperactivation. Alves et al. (2015) showed that Pak2 acts downstream of the TSC complex and is responsible for migration deficits, at least in fibroblasts.

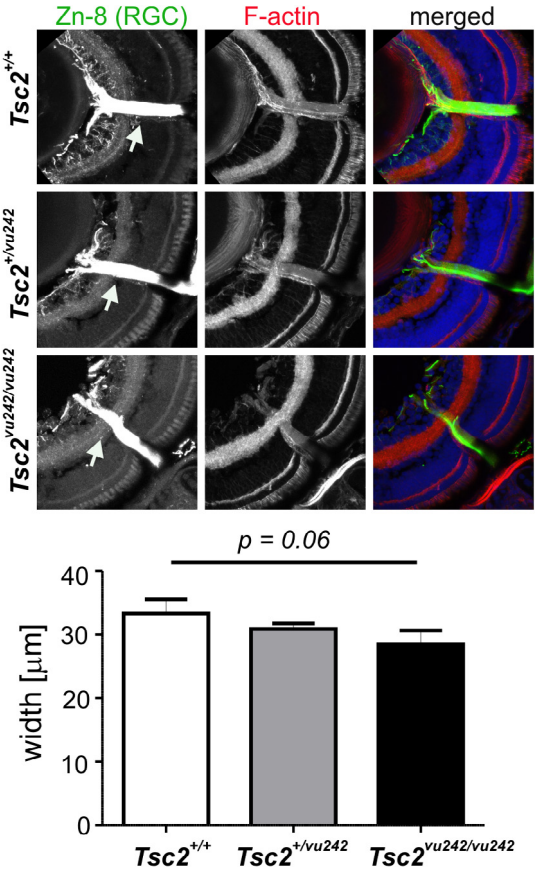

Fig. 5. Optic nerve diameter changes in zebrafish model of TSC. Representative images of developing zebrafish (genotypes as indicated; *Tsc2*^{vu242} – functional KO of Tsc2; (Kim et al., 2011)) retina stained for retinal ganglion cell marker (Zn-8) to visualize optic nerve and F-actin (left panel). Quantification of optic nerve diamiter.

PTEN hamartoma tumor syndrome and Proteus syndrome. Phosphatase and tensin homolog deleted on chromosome ten (PTEN) hamartoma tumor syndrome (PHTS) is a spectrum of disorders that is characterized by loss-of-function mutations in the *PTEN* gene. PTEN is a phosphatase that negatively regulates the PI3K/Akt/mTOR pathway by dephosphorylating PIP3 and preventing Akt stimulation (Song et al., 2012; Fig. 4). It also acts as a tumor suppressor in cancers of the human brain, breast, and prostate (Li et al., 1997). Disorders that are caused by *PTEN* mutations include Cowden syndrome, Lhermitte–Duclos disease, Bannayan–Riley–Ruvalcaba syndrome, and various cancers (Endersby and Baker, 2008; Pilarski et al., 2013). Until very recently, Proteus syndrome was believed to be caused by a *PTEN* mutation. Subsequent studies showed that an underlying cause in many patients with Proteus syndrome is a mutation in *Akt1*, which encodes a protein that is inhibited by PTEN (Lindhurst et al., 2011). *PTEN* mutation disorders, as well as Proteus syndrome, have overlapping characteristics. Because the same mutations in the *PTEN* gene can cause any of these syndromes, they may in fact be one disorder (Zori et al., 1998; Marsh et al., 1999). Secondary factors and mutations may also play a role in establishing specific phenotypes (Pilarski et al., 2013). The vast majority of patients with different disorders that are associated with *PTEN* mutations exhibits macrocephaly and a higher risk of benign or malignant tumors. Cowden disease is a rare disease that is characterized by macrocephaly, a higher risk of malignant cancers of the breast, thyroid gland, and endometrium, and benign hamartomas in various organs of the body (Liaw et al., 1997). Lhermitte–Duclos disease is characterized by benign neuronal overgrowth in the cerebellum, which results in an increase in intracranial pressure, ataxia, and seizures. Dysplastic neurons express only the mutant allele of *PTEN* or they have no *PTEN* expression at all, which results in elevated Akt levels (Zhou et al., 2003). Bannayan–Riley–Ruvalcaba syndrome is a disorder with primary clinical features that include macrocephaly, developmental delay, hemangiomas, spotted pigmentation of the skin, scoliosis, and hamartomatous intestinal polyps (Lynch et al., 2009). *PTEN* mutations that do not fully abrogate its phosphatase activity are also found in patients with ASD (Rodriguez-Viciana et al., 1997; Varga et al., 2009). However, *PTEN* mutations were described in only 1% of human ASD patients.

Several mouse models of Pten deficiency have been developed using conditional *Pten* KO in different neuronal populations or the viral delivery of short-hairpin RNAs (shRNAs) against *Pten* mRNA (Table A.1). These mice exhibit numerous abnormalities at the cellular, anatomical, physiological, and behavioral levels. Changes in mTOR pathway activity have also been repeatedly reported (Kwon et al., 2003, 2006; Amiri et al., 2012; Pun et al., 2012; Sperow et al., 2012; Zhu et al., 2012; Lugo et al., 2013; Chen et al., 2015; Nguyen et al., 2015). Nonetheless, the causal role of mTOR in *Pten* KO phenotypes has been studied in only a few cases. The first described mouse model that lacked *Pten* was *Pten*$^{fl/fl}$*;GFAP-Cre* (also known as NS-*Pten* KO). In this model, *Pten* was deleted in several NeuN-positive cells (Backman et al., 2001; Kwon et al., 2001; Nguyen et al., 2015). These mice were characterized by a shortened lifespan, severe epilepsy, and neuronal hypertrophy in the hippocampus and cerebellum. Chronic rapalog (CCI-779) treatment decreased death and seizure frequency in these mice. The average diameter of neurons also decreased, although the effects on cerebellar neurons required higher doses of CCI-779 (Kwon et al., 2003). Ljungberg et al. (2009) showed that even relatively short rapamycin treatment for 2 weeks *vs*. 6 weeks (Kwon et al., 2003) prior to seizure development was sufficient to prevent activation of the mTOR pathway, cortical neuron hypertrophy, and the development of severe epilepsy. Importantly, the antiepileptogenic effect lasted for 3 weeks after rapamycin discontinuation. Sunnen et al. (2011) subsequently found that even a single dose of rapamycin that was administered in NS-*Pten* KO mice early during the epileptogenic process (~4 weeks of age) blocked seizure recurrence and aberrant mossy fiber sprouting for a few weeks. Additional doses of rapamycin at later time points (11 and 16 weeks) delayed *Pten* KO-related pathology even further. Finally, the ability of rapamycin to inhibit seizures and increase the rate of survival

in NS-*Pten* KO mice with very advanced pathology was recently reported (Nguyen et al., 2015). Rapamycin also efficiently reversed astrogliosis and microgliosis (Nguyen et al., 2015). In other models, rapalogs also effectively prevented neuropathology that was related to a lack of *Pten*. For example, in *Pten*$^{fl/fl}$*;Nex-Cre* KO mice that lack *Pten* selectively in excitatory neurons of the forebrain, rapamycin administration at birth substantially increased the rate of survival (Kazdoba et al., 2012). Interesting insights into the role of mTOR in *Pten* KO-related pathology have come from studies that used animals in which *Pten* was selectively removed from postnatally born neurons in the dentate gyrus of the hippocampus and SVZ (*Pten*$^{fl/fl}$*;Nestin-Cre-ERT2* and *Pten*$^{fl/fl}$*;Gli-Cre-ERT2*, respectively; (Pun et al., 2012; Zhu et al., 2012). In *Pten*$^{fl/fl}$*;Nestin-Cre-ERT2* mice, gene KO was induced on P30, resulting in the premature differentiation of neuroblasts and subsequent SVZ expansion. This effect was prevented by rapamycin, underscoring the importance of mTORC1 silencing during the physiological migration of neurons. Pun et al. (2012) specifically removed *Pten* in adult-born granule cells of the dentate gyrus, resulting in rapamycin-sensitive, progressive epilepsy and the aberrant sprouting of mossy fiber axons.

Although the findings discussed above reveal the contribution of mTOR to *PTEN*-related syndromes, recent work by Chen et al. (2015) suggests that this might be the case only when selective mutation leads to a >50% decrease in Pten activity. Supporting this hypothesis, the authors found that heterozygous *Pten* KO mice (*Pten*$^{+/-}$ or *Pten*$^{fl/+}$*;Emx1-Cre*) did not exhibit increases in mTORC1 activity, although cortical hypertrophy was evident. This hypertrophy stemmed from β-catenin pathway-induced hyperplasia, and neither *mTOR* nor *Rptor* KO was able to reverse it. However, when a second copy of *Pten* was removed (*Pten*$^{fl/fl}$*;Emx1-Cre*), the mice displayed more substantial hypertrophy of the cortex. This augmented increase in cortex size coincided with mTORC1 pathway hyperactivation and cellular overgrowth. These results suggest that similar brain phenotypes (i.e., brain hypertrophy) can occur, depending on the degree of *Pten* inactivation, but the underlying molecular mechanisms differ. This also raises the question of whether autistic-like behaviors that are observed in *Pten*$^{+/-}$ mice are mTOR-dependent.

Malformations of cortical development. Malformations of cortical development (MCD) comprise a group of diseases with different origins, the common feature of which is abnormal development of the cerebral cortex, either globally or locally. Malformations of cortical development include megalencephaly, hemimegalencephaly, and focal cortical dysplasia (FCD). Megalencephaly is characterized by symmetric enlargement of the cortex. Hemimegalencephaly affects only one hemisphere. In FCD, only localized abnormalities are found throughout the cortex. Focal cortical dysplasia can be further subdivided into three types: Type I, Type II, and Type III. Type II is characterized by changes in cortical architecture and cellular abnormalities, including dysmorphic neurons. The presence of balloon cells causes further subdivision of FCDII into a (w/o) and b (with) subclasses. FCDIIb presents several similarities to pathological changes that are found in the cortex in TSC patients. Patients with MCD often suffer from intractable epilepsy, which can be treated with surgery. The similarities between MCD and TSC prompted researchers to investigate mTOR pathway activation in brain samples from MCD patients. Subsequently, increases in mTOR signaling were repeatedly reported in pathologically altered cells (i.e., cytomegalic neurons and balloon cells).

Progress in sequencing and sequencing data analysis greatly advanced the search for the genetic causes of MCD. Several studies that involved dozens of patients revealed that pathogenic mutations in megalencephaly, hemimegalencephaly, and FCDII often occur only somatically in a small part of neuronal cells and are found in genes that encode proteins of the PI3K-Akt-mTOR pathway or its regulators, including *DEPDC5*, *PIK3CA*, *AKT3, LYK5* (*STRADA*), and *mTOR* itself (Table 1). *DEPDC5* encodes DEP domain-containing protein 5, which is a component of the GATOR complex. Mutations in *DEPDC5* lead to mTOR activation. *LYK5* encodes STE20-related kinase adaptor protein α, which is required for the activity of LKB1 kinase, which in turn inhibits mTORC1 via AMPK. PIK3CA and AKT3 encode activators of the mTOR pathway, mutations of which increase their activity. Finally, the majority of mTOR mutations that have been identified in the brain to date are gain-of-function mutations that increase mTORC1 activity in dysplastic cells in patient specimens and *in vitro* cultured cells (Table 1). These mutations typically map to the FAT or kinase domains (Fig. 1). Analyses of the available mTOR structure imply that these mutations increase the accessibility of the active site for the substrates. Another possible mechanism of mTOR activation in the case of some mutations that are found in the FAT domain is loss of the interaction between mTOR and Deptor, an inhibitory component of mTORC1.

Altogether, the above findings suggest that changes in mTOR signaling underlie MCD. However, this hypothesis has been difficult to prove. First, although the aforementioned mutations were not found in control samples from non-MCD patients, no mutations in these genes were found in a substantial number of patients with MCD. Second, although mTOR activity increased in cells that contained the mutation, it also often increased in pathologically altered cells that lacked it. Third, until only recently, no relevant animal models were available. Nevertheless, some recent findings may shed light on the potential link between mTOR and MCD and the use of mTOR inhibitors as a treatment strategy. Correlations between the levels of mTOR mutation in samples that were obtained during parietotemporal lobectomy and intraoperative electrocorticography revealed that the highest mutation frequency was in the epicenter of epilepsy and dropped to zero at the edges of the resected area (Mirzaa et al., 2016). The authors also correlated the level of mTORC1 activation that was caused by particular mutations with the observed phenotypes.

Table 1. Brain pathology-related mTOR mutations

mTOR mutation	Disease phenotype	mTOR activity	Rapa effects	Refs.
Cys1483Tyr	HME/CD	mTORC1 up	Yes	Lee et al. (2012), D'Gama et al. (2015), Mirzaa et al. (2016)
Ser2215Tyr	FCDIIb; FCDIIa	mTORC1 up	Yes	Nakashima et al. (2015), Mirzaa et al. (2016)
Ser2215Phe	FCDIIb; FCDIIa	mTORC1 up	Yes	Lim et al. (2015), Nakashima et al. (2015), Mirzaa et al. (2016)
Ala1459Asp	FCDIIb	mTORC1 up	Not analyzed	Nakashima et al. (2015)
Leu1460Pro	FCDIIb; FCDIIa	mTORC1 up	Yes	Nakashima et al. (2015), Mirzaa et al. (2016)
A1669S	HME	Not analyzed	Not analyzed	D'Gama et al. (2015)
W1456G	FCDIIa	mTORC1 up	Not analyzed	Leventer et al. (2015)
Leu2427Pro	FCDIIa	mTORC1 up	Yes	Lim et al. (2015)
Arg2193Cys	FCDIIa	Not analyzed	Not analyzed	Lim et al. (2015)
Arg624His	FCDIIa	Not analyzed	Not analyzed	Lim et al. (2015)
Arg1709His	FCDIIa	Not analyzed	Not analyzed	Lim et al. (2015)
Leu2427Gln	FCDIIb	mTORC1 up	Not analyzed	Lim et al. (2015)
Thr1977Lys	FCDIIb	Not analyzed	Not analyzed	Lim et al. (2015)
Thr1977Ile	ME	mTORC1 up	Yes	Mirzaa et al. (2016)
Tyr1450Asp	FCDIIb	Not analyzed	Not analyzed	Lim et al. (2015)
Cys1483Arg	FCDIIb/ME_HME	mTORC1 up	Not analyzed	Lim et al. (2015)
Glu1799Lys	ME	mTORC1 up	Yes	Baynam et al. (2015), Mroske et al. (2015), Mirzaa et al. (2016)

ME – megalencephaly, HME – hemimegalencephaly, FCD – focal cortical dysplasia, CD – cortical dysplasia.

They then classified the patients into three groups based on genotype–phenotype correlations. The first *MTOR* group consisted of patients with FCDIIa and intractable seizures who carried mutations in either position 1460 (Leu to Pro) or 2215 (Ser to Phe or Tyr). The second *MTOR* group presented correlations with a Thr1997Ile mutation. These patients were characterized by asymmetric megalencephaly with polymicrogyria. Two of three patients displayed seizures. The third *MTOR* group had diffuse megalencephaly and carried a Glu1799Lys amino acid substitution. An analysis of the effects of the *MTOR* mutation on mTORC1 activity showed that the most and least active mutants correlated with FCDIIa and megalencephaly/hemimegalencephaly, respectively.

Additional insights into the role of mTOR in MCD have come from recent animal models. Lim et al. (2015) used *in utero* electroporation to introduce mTOR(Leu2427Pro) to cortical neuroprogenitors. This resulted in the appearance of electroporated cytomegalic neurons in the cortex and spontaneous seizures 3 weeks after birth. Animals that developed seizures were then given rapamycin, which resulted in seizure cessation and the shrinkage of cell bodies of electroporated neurons. The effectiveness of rapamycin in treating the FCDII-like phenotype was also confirmed in another study, in which Bordey and colleagues (Hsieh et al., 2016) developed a new experimental model of FCDII. They employed *in utero* electroporation to introduce constitutively active Rheb ($Rheb^{CA}$) specifically to the medial prefrontal cortex. By 2 months of age, these mice developed several characteristic features of FCD2, including cell mispositioning, white matter heterotopia, the appearance of dysmorphic neurons with greater mTORC1 activity, astrogliosis, and most importantly severe seizures. When $Rheb^{CA}$ was introduced to the somatosensory cortex, the animals developed no seizures, suggesting that pathological changes need to occur in a particular brain area. Another important observation of this study was that postnatal rapamycin treatment prevented changes in both FCD cortical architecture and seizure development. Rapamycin discontinuation resulted in recurrent seizures in more than 50% of the mice, although cortical lamination remained intact. These observations corroborate the findings of some TSC models, in which mTOR hyperactivity-related seizures may occur in the absence of cortical malformations. The results also indicate the necessity of lifelong rapamycin treatment in mTOR hyperactivity-related epilepsies.

Neurofibromatosis. Neurofibromatosis type I (NF1) is a relatively common neurocutaneous syndrome that occurs in 1 in 2,500 in the general population. It is characterized by the development of benign and malignant tumors of the central and peripheral nervous systems and abnormalities in the skin, bones, and kidneys. Many patients also suffer from learning disabilities, ADHD, and anxiety (Johannessen et al., 2005; Diggs-Andrews and Gutmann, 2013). Some patients develop epilepsy, although this is relatively rare. NF1 is caused by mutations in the gene that encodes neurofibromin 1, which acts as a GTPase-activating protein for the protooncogene Ras, suppressing its activity (Fig. 4). Consequently, cells with mutations in *NF1* have high levels of activation of the mitogen-activated protein kinase and PI3K pathways (Lau et al., 2000). Subsequently, mutations in *NF1* were shown to increase the activation of mTORC1 in human samples of NF1-associated pilocytic astrocytomas and neurofibromatosis 1 patients' malignant peripheral nerve sheath tumor (MPNST) cells cultured *in vitro* (Dasgupta et al., 2005; Johannessen et al., 2005). In the latter case, rapamycin treatment effectively reduced rpS6 phosphorylation and the rate of cell proliferation. A causal link between upregulation of the mTORC1 pathway and the partial or complete loss of *Nf1* was further confirmed in various cell cultures and animal models. One of the intriguing observations was that *Nf1* KO-dependent mTORC1 activation in cultured astrocytes required Akt but not TSC2 or Rheb

(Banerjee et al., 2011a). The mTORC1-dependent increase in the proliferation of *Nf1*$^{-/-}$ astrocytes depended on Akt but not Rheb. According to Johannessen et al. (2005), the activation of mTORC1 in *Nf1*$^{-/-}$ mouse embryonic fibroblasts (MEFs) required Tsc2. The reasons for this discrepancy are not clear because different models were used, and the molecular mechanisms that underlie Tsc2/Rheb-independent regulation in astrocytes are not fully understood.

As in the case of *Tsc1*, *Tsc2*, and *Pten*, several mouse models that involve heterozygous, mosaic, or conditional loss of *Nf1* have been developed (Table A.1). Heterozygous *Nf1*$^{+/-}$ mice did not develop tumors of the nervous system that are specific to NF1 patients. Cichowski et al. (1999) developed two new mouse models, *Nf1*$^{+/+}$*:Nf1*$^{-/-}$ chimeric mice and *Nf1*$^{+/-}$*:p53*$^{+/-}$ mutants, that develop neurofibromas and MPNST, respectively. The latter model was used to demonstrate an increase in mTORC1 activation in MPNST cells and the effectiveness of rapamycin in inhibiting the growth of this highly malignant tumor and consequently prolonging mouse survival (Johannessen et al., 2008). However, similar to the case of the several models described so far, the tumor regrew after rapamycin discontinuation. The beneficial effects of mTOR inhibition were also evident in *Nf1*$^{fl/fl}$*;GFAP-Cre* mice, which developed optic glioma (Hegedus et al., 2008; Banerjee et al., 2011b). However, recent studies suggest that not only mTORC1 contributes to glioma formation in NF1. Using an NSC proliferation assay, Lee et al. (2010) showed that the loss of *Nf1* increases the proliferation of NSCs in the brainstem but not neocortex. This was attributed to the higher expression of Rictor in *Nf1*$^{-/-}$ NSCs in the brainstem than in the neocortex and the subsequent activation of mTORC2-Akt and degradation of p27, a cyclin-dependent kinase inhibitor. Clinical trials have evaluated mTOR inhibitors for the treatment of neurofibromas in NF1 patients. In contrast to TSC, mTOR inhibitors did not exert any clear beneficial effects on the clinical symptoms of NF1.

Fragile X syndrome. Fragile X syndrome (FXS) is a leading genetic cause of inherited mental retardation in males and relatively frequent cause of autism. Additional neurological FXS symptoms include hyperactivity, sleep problems, epileptic seizures, and anxiety. One cause for the disease is trinucleotide (CGG) repeat expansion in the 5′ untranslated region of the fragile X mental retardation gene. The fully developed phenotype occurs once the number of repeats reaches 200. The consequence of this mutation is the loss of FMRP (fragile X protein), an RNA-binding protein that is involved in the translational silencing of mRNAs to be locally translated in dendrites and postsynaptic sites. The loss of FMRP expression subsequently leads to aberrant increases in the translation of several proteins, several of which contribute to synaptic and structural plasticity (e.g., GluA2, PSD95, RhoA, Rac1, and matrix metalloproteinase 9; Fernández et al., 2013). Postmortem analyses of patients' brain samples revealed several abnormalities in neuronal morphology, including substantial changes in dendritic spine shape (Irwin et al., 2001).

The excessive translation of mRNAs that are typically bound by FMRP is considered a molecular cause of FXS. The potential contribution of mTOR to FXS pathology and therapeutically targeting mTORC1 have been considered. Fragile X-related mTOR activation was first described in KO mice (Sharma et al., 2010; Bhattacharya et al., 2012; Amiri et al., 2014). Subsequently, Hoeffer et al. (2012) showed that mTOR pathway activity is upregulated in both patient-derived lymphocytes and brain tissue from FXS patients. Joint work by the Zukin and Klann laboratories showed that KO of the FMRP-encoding gene disinhibited PIKE-L, an upstream activator of the PI3K-Akt-mTORC1 pathway (Fig. 4). However, in this study, the short-term application of rapamycin, similar to the protein synthesis inhibitor anisomycin, failed to inhibit the increase in mGluR-driven LTD, which is a hallmark of FXS (Sharma et al., 2010). This argues against the involvement of mTORC1 in this FXS-related synaptic pathology. One consideration, however, is that protein synthesis is already saturated in this FXS model, and the relatively short-term inhibition of *de novo* protein synthesis may be insufficient to reverse FXS-related synaptic defects. This hypothesis was corroborated by the observation that the lack of S6K1 in FXS KO mice, which leads to long-term effects on translation, reversed synaptic, dendritic spine, and behavioral defects that were caused by the lack of FMRP (Bhattacharya et al., 2012).

Down syndrome. Down syndrome is the most common chromosomopathy in humans. It is caused by the presence of an additional, third copy of chromosome 21 (or a part of the chromosome). The disease is characterized by several neurological abnormalities, the most common of which is intellectual disability. Down syndrome patients can also suffer from autism and have a higher risk of epilepsy, including infantile spasm. Older Down syndrome patients (~40 years of age) develop symptoms of Alzheimer's disease and have a high risk of stroke. Two independent studies revealed greater activation of the mTORC1 pathway in brain tissue from Down syndrome patients (Iyer et al., 2014; Perluigi et al., 2014). mTORC1 hyperactivity appeared early in development and steadily increased until adulthood. Perluigi et al. (2014) observed an increase in mTOR activity in Down syndrome patients both with and without Alzheimer's disease pathology. Iyer et al. (2014) observed very strong activation of mTORC1 in cells with characteristic Alzheimer's disease features (e.g., neurofibrillary tangles), suggesting that increased mTORC1 activity to some extent contributes to the neurodegeneration that is observed in Down syndrome patients, similar to Alzheimer's disease (see below). However, studies with mTOR inhibitors in animal models of Down syndrome are needed to further confirm the causal role of mTOR in this disease.

Neurodegenerative disorders

Major neurodegenerative disorders (e.g., Huntington's disease, Parkinson's disease, and Alzheimer's disease), non-Alzheimer tauopathies, spinocerebral ataxia type 3,

amyotrophic lateral sclerosis, and retinal degeneration are characterized by the accumulation of syndrome-specific protein aggregates. The involvement of mTOR in the control of both translation and autophagy prompted several research teams to investigate mTOR activity and the therapeutic potential of rapamycin in various animal models (e.g., in mice, rats, and *Drosophila*; Bove et al., 2011; Lipton and Sahin, 2014; Bockaert and Marin, 2015). Indeed, increased mTORC1 activation was reported in patients with Alzheimer's disease (An et al., 2003; Li et al., 2005; Tramutola et al., 2015). Moreover, rapamycin treatment or the partial genetic reduction of mTOR levels improved learning and memory deficits in different models of Alzheimer's disease (Caccamo et al., 2010, 2014; Spilman et al., 2010). Beneficial effects of mTOR inhibition with rapalogs (e.g., a reduction of cell death and improvements in behavioral deficits) were observed in most studies that involved animal models of other neurodegenerative disorders (Bove et al., 2011; Lipton and Sahin, 2014; Bockaert and Marin, 2015). Nevertheless, the inhibition of mTORC1 might not always be beneficial as a treatment option for neurodegenerative disorders. For example, in mice with a G93A mutation in *SOD1*, which serves as a model of amyotrophic lateral sclerosis, rapamycin accelerated motor neuron degeneration and shortened the animals' lifespan (Zhang et al., 2011).

Non-syndromic/cryptogenic epilepsy

As mentioned above, several genetic disorders show a strong causal link between mTORC1 activation and epilepsy. Several animal models of temporal lobe epilepsy (TLE) establish a more general role for mTORC1 in epileptogenesis. Strong mTORC1 activation in the brain was reported immediately upon KA- or pilocarpine-induced status epilepticus and during subsequent days when epileptogenesis occurred (Buckmaster et al., 2009; Zeng et al., 2009; Hartman et al., 2012; Sha et al., 2012; Macias et al., 2013; Shima et al., 2015). Acute pentylenetetrazol-induced seizures result in immediate but transient mTORC1 activation (Zhang and Wong, 2012). mTORC1-induced increases in signaling were described in epilepsy that was evoked by electric stimulation of the amygdala and angular bundle (Sliwa et al., 2012; van Vliet et al., 2012). mTOR activation was observed in both neuronal cells and glia but at different times post-status epilepticus. Macias et al. (2013) showed that after KA treatment, neuronal mTORC1 activation preceded astrocytic activation by several hours. Shima et al. (2015) reported a second wave of mTORC1 activation in dentate gyrus neurons but as late as 21 days after KA application. Sha et al. (2012) reported mTORC1 activation in astrocytes and oligodendrocytes at later timepoints post-KA in already pathologically altered hippocampi.

Although mTOR is generally accepted to be activated in various models of TLE, unknown are the specific processes associated with epileptogenesis that actually require mTOR (e.g., seizure severity, neuronal cell death, neurogenesis, astrogliosis, axonal sprouting of dentate gyrus cells, dispersion of dentate gyrus granular neurons, synaptogenesis, blood–brain barrier leakage, and behavioral changes). The most beneficial effects of mTOR inhibition were described in KA-induced epilepsy. In a pioneering study by Zeng et al. (2009), prolonged rapamycin treatment post-KA efficiently inhibited KA-induced cell death, neurogenesis, axonal sprouting, and spontaneous seizure development. Other groups reported less significant effects of rapamycin treatment that was administered post-KA. For example, Shima et al. (2015) reported the effects of rapamycin on dentate gyrus cell dispersion, mossy fiber sprouting, and astrogliosis but no effects on the inhibition of cell death or spontaneous seizure development. As opposed to rapamycin treatment during epileptogenesis, many studies reported no benefits or even adverse effects of rapamycin treatment prior to KA administration. Six-hour or 3-day pretreatment had no effect on the severity of KA-induced status epilepticus in mice (Hartman et al., 2012). One-week rapamycin pretreatment shortened the seizure onset time and increased the number of rats that exhibited status epilepticus in the KA model (Macias et al., 2013). Four-week pretreatment additionally increased mortality upon KA administration (Macias et al., 2013).

In a pilocarpine model, rapamycin treatment after the epileptogenic stimulus appeared to impact seizure-induced anatomical changes. Similar to the KA model, rapamycin inhibited mossy fiber sprouting (Buckmaster et al., 2009; Buckmaster and Lew, 2011; Tang et al., 2012; Hester et al., 2016). The inhibitory effects of rapamycin on excitatory synapse formation on proximal dendrites in dentate gyrus granule neurons were recently reported (Yamawaki et al., 2015). The beneficial effects of rapamycin were also demonstrated with regard to moderating aggressive behavior that stemmed from pilocarpine-induced epilepsy. However, similar to the KA model, there is disagreement about whether mTORC1 inhibition suppresses both anatomical changes and recurrent seizures or only the former (Buckmaster and Lew, 2011; Tang et al., 2012).

With regard to models of TLE that is induced by electrical stimulation, rapamycin treatment after epileptogenic stimulation of the angular bundle inhibited recurrent seizures and reduced blood–brain barrier leakage in rats (van Vliet et al., 2012; Drion et al., 2016). Drug pretreatment was ineffective in preventing seizure development under this protocol (Drion et al., 2016). Rapamycin posttreatment was ineffective in models of amygdala electrostimulation (Sliwa et al., 2012). Directly comparing the aforementioned studies is difficult because they used different seizure induction protocols, rapamycin doses, application regimens, animal species, and ages. However, the studies have some commonalities: (*i*) rapamycin pretreatment did not prevent status epilepticus, and (*ii*) rapamycin posttreatment was effective only when continuously applied. Similar to the models of genetic disorders described above, the discontinuation of rapamycin treatment resulted in the reappearance of seizures and aberrant sprouting (Buckmaster et al., 2009; Drion et al., 2016). These

differences matter only when mTOR is hyperactivated in humans with TLE. Two studies have been published to date in this regard. mTORC1 activation in the sclerotic hippocampus was confirmed by these studies, but it occurred primarily in astrocytes and more rarely in neuronal cells of the dentate gyrus (Sha et al., 2012; Sosunov et al., 2012). Thus, mTOR also appears to be activated in TLE patients and animal models of TLE, although the degree to which mTOR contributes to TLE remains to be determined.

MOLECULAR BASIS OF MTOR-RELATED NEUROPATHOLOGIES

Protein translation

Because of the important role of mTOR in protein synthesis, dysregulated translation has been considered a major molecular cause of several of the diseases discussed above. Mental retardation and autism that are associated with TSC, FXS, and Down syndrome were believed to stem from an increase in either global or local translation. Indeed, increased global translation was reported in neurons in *Fmr1*$^{-/-}$ mice, and this particular phenotype was corrected by *S6K1* knockout, similar to morphological, electrophysiological, and behavioral deficits (Bhattacharya et al., 2012). In Ts1Cje mice, a mouse model of Down syndrome, a substantial increase in synaptic translation that was caused by the excessive release of BDNF was found to be mTOR-dependent (Troca-Marín et al., 2011). FMRP and BDNF control the local synthesis of important neuronal plasticity-related proteins, suggesting that the excessive translation of synaptic proteins in mental retardation syndromes leads to the dysregulation of neuronal responses. However, some recent findings suggest selective rather than global upregulation of protein synthesis as a source of the problems with neuronal communication in mental retardation and autism. For example, Gkogkas et al. (2013) developed new animal models of ASD by knocking out *4e-bp2* or overexpressing *eif4E*. They found high selectivity in translation upregulation. Among the tested proteins, only the translation of neuroligin 1 was upregulated, and *Nlgn1* knockdown was sufficient to correct the autistic phenotype. Another protein, PARK7, was recently found to be upregulated at synapses of Tsc1-lacking neurons *in vivo*. PARK7 is involved in various cellular processes, including transcriptional regulation, mRNA binding, and the stress response (Niere et al., 2016). Although the function of PARK7 at the synapse remains unknown, its greater abundance is potentially common to TSC, ASD, and Alzheimer's disease (Niere et al., 2016). However, the links between translation and mental retardation and autism appear to be more complex. Although some behavioral phenotypes are almost indistinguishable between *Fmr1*$^{-/y}$, *4e-bp2*$^{-/-}$, and *Tsc2*$^{+/-}$ mice, the latter ones exhibit decreases in global protein synthesis, regardless of whether mTORC1 activity is increased (Auerbach et al., 2011). Among the proteins that were decreased in *Tsc2*$^{+/-}$ animals was Arc, a key synaptic plasticity protein that is involved in regulating AMPAR trafficking (Bramham et al., 2010; Auerbach et al., 2011). Global translational profiling in neurons with Tsc2 knockdown also revealed that neurons that lacked Tsc2 exhibited downregulation of the translation of some synaptogenesis genes (e.g., *Snap91* and *Cbln1*; Nie et al., 2015). The translation of 65 mRNAs, several of which are known to encode parts of the stress response (e.g., Atf3 and Ucp2), was upregulated. Rapamycin corrected the association of only 28 of these mRNAs with ribosomes (Nie et al., 2015). Thus, dysregulation of the translation of a particular group of proteins, rather than global upregulation of protein synthesis, appears to be linked to mental retardation and ASD symptoms.

In addition to neurodevelopmental diseases, aberrant mTOR-driven translation was postulated to contribute to neurodegenerative disorders. Early work by the Pei group revealed a correlation between increased activation of the mTOR pathway and mTOR-related translational machinery and the accumulation of Tau protein (An et al., 2003; Li et al., 2005). In Huntington's disease, the mTOR-dependent translation of mutated huntingtin may partially contribute to the increase in mutated protein aggregation, at least in fibroblasts (King et al., 2008). In models of Parkinson's disease, the mTORC1-dependent upregulation of RTP801 translation leads to dopaminergic neuron death (Malagelada et al., 2006, 2010). RTP801 is an inhibitor of prosurvival mTORC2-Akt signaling, and mTORC1 inhibition by rapamycin is beneficial for cell survival (Malagelada et al., 2010). *Drosophila* models of Parkinson's disease also indicate the dysregulation of mTOR-dependent translation through 4E-BP1 hyperphosphorylation (Tain et al., 2009). A recent study of mTOR hyperactivation in Leigh syndrome provided an additional mechanism by which increases in global translation challenge neurons with dysfunctional mitochondria (e.g., in Parkinson's disease). Zheng et al. (2016) showed that a decrease in the oxidative production of ATP resulted in the accumulation of amino acids, subsequent mTOR hyperactivation, and increased translation, which increases the consumption of the remaining ATP, thus making neurons more vulnerable to toxic challenges (e.g., by glutamate).

Finally, in a model of opioid-induced tolerance (i.e., repeated morphine injections), protein synthesis and eiF4A–eiF4E binding increased and were reversed by rapamycin (Xu et al., 2014). Both rapamycin treatment and mTOR knockdown in the spinal cord prevented the development and maintenance of opioid tolerance and related hyperalgesia (Xu et al., 2014). These observations led to the hypothesis of a potential role for aberrant increases in mTOR-dependent translation in chronic pain and hyperalgesia, in which mTOR is activated by NMDA and opioid receptors, respectively (Lutz et al., 2015).

Autophagy. Several studies have tested whether mTOR-dependent autophagy contributes to neurodegeneration. The benefits of rapamycin treatment in the context of autophagy induction and aggregate clearance were described in several studies that used models of Alzheimer's disease and

Alzheimer-like pathology (Caccamo et al., 2010, 2014; Spilman et al., 2010; Bove et al., 2011; Siman et al., 2015). In mouse and *Drosophila* models of Huntington's disease, mTOR inhibition induced autophagy and ameliorated the toxicity associated with huntingtin aggregates (Ravikumar et al., 2004). These aggregates were also cleared in a neuronal cell model after treatment with catalytic mTORC1 and mTORC2 inhibitors (Roscic et al., 2011). Protein aggregate clearance via the mTORC1 pathway was shown to occur in cases of other polyglutamine and polyalanine aggregate-prone proteins, including ataxin-1, ataxin-3, and tau (Berger et al., 2006). The clearance of α-synuclein aggregates upon mTORC1 inhibition was also found in animal and cellular models of Parkinson's disease (Crews et al., 2010). Interestingly, the enhancement of autophagy by mTOR inhibition was also beneficial in these Parkinson's disease models, in which α-synuclein aggregates were not a major and immediate cause of pathology (Bove et al., 2011). One of the potential targets is mitochondria, which are often damaged in Parkinson's disease because of oxidative stress. Increases in mitophagy prevented mitochondria-related proapoptotic signaling (e.g., cytochrome C release; Bove et al., 2011).

The dysregulation of mTOR-dependent autophagy also appears to participate in mTORopathies that are related to neuroplasticity. Increases in Ulk1 phosphorylation and decreases in autophagy were reported in *Tsc1*$^{fl/fl}$*;CamKIIα-Cre* and *Pten*$^{fl/fl}$*;CamKIIα-Cre* KO mice (McMahon et al., 2012). Mice that lack Atg7 (*Atg7*$^{fl/fl}$*;CamKIIα-Cre*), a key protein for autophagy initiation, also develop spontaneous seizures. One hypothesis is that the inhibition of autophagy through mTOR upregulation might contribute to epileptogenesis (McMahon et al., 2012). This hypothesis, however, awaits further testing to demonstrate a causal link and targets of autophagy during this process. Tang et al. (2014) recently suggested a contribution of autophagy inhibition to ASD and TSC. They observed diminished dendritic spine pruning in the brains of ASD patients. Further analyses showed that these deficits were accompanied by fewer autophagic vesicles and enhanced mTOR activity in neurons. These observations were supported by experiments in a TSC mouse model (Tang et al., 2014).

Autophagy may also be altered in brain tumors that accompany TSC. An analysis of TSC patient brain biopsies clearly showed a substantial increase in Ulk1 phosphorylation and the accumulation of p62 (i.e., an indication of autophagic flux blockade) in cortical tubers and SEGAs (McMahon et al., 2012; Yasin et al., 2013; Di Nardo et al., 2014). The extent to which autophagy inhibition contributes to this tumor formation remains to be established (Switon et al., 2016).

Transcription and other cellular processes. Much attention has been paid to the role of protein translation and autophagy in mTORopathies, but the contributions of other processes that are regulated by mTOR to neuropathology are comparatively less understood. One of these may be transcription. There is evidence of profound mTOR-dependent transcriptome changes in mTORopathies, the most comprehensive studies of which have been on TSC. For example, transcriptomes of *Tsc2*$^{-/-}$, *Tsc2*$^{-/+}$, and *Tsc2*$^{+/+}$ murine neuroepithelial cells were compared. Significant fivefold differences in the expression of 67 genes were found between wildtype and *Tsc2*-null cells. Analyses of these genes revealed a reduction of the expression of neuronal markers and the upregulation of genes that encode the cytoskeleton and adhesion and cell signaling proteins (Onda et al., 2002). This study also analyzed differences in the expression of 59 genes that are relevant to neuronal development, tuber formation, and epileptogenesis between giant cells and wildtype neurons. Twenty-seven of these genes were differentially expressed. Increased expression was observed for transcripts that encode subunits of neurotransmitter receptors, whereas decreases were observed for those that encode several trophic factor receptors (e.g., TrkB, PDGFRα, and PDGFRβ). Tyburczy et al. (2010) compared SEGAs from patients with control tissue. Similar to the study by the Kwiatkowski group, the expression of genes that are involved in neuronal development was downregulated, and genes that are linked to tumorigenesis were upregulated. Comparisons of untreated SEGA-derived lines with rapamycin-treated lines showed that the expression profiles of some genes could be reversed by rapamycin (e.g., *ANXA1*, *GPNMB*, *LTF*, *RND3*, *S100A11*, *SFRP4*, and *NPTX1*). The analysis of transcript abundance in TSC models was not restricted to only undifferentiated or tumor cells. The Sahin group showed that Tsc2 knockdown in neurons that were cultured *in vitro* resulted in strong upregulation of several transcripts that are involved in unfolded protein and oxidative cellular responses, and such increases were prevented by rapamycin treatment (Di Nardo et al., 2009; Nie et al., 2015). Similar changes were reported in the brains of P30 mice with *Tsc1* KO that was induced on E18 (Goto et al., 2011). Recent studies suggested that the stress-induced upregulation of Atf3 and its target genes (e.g., gelsolin) may contribute to dendritic spine changes that are observed in TSC (Nie et al., 2015). Notably, however, these examples do not necessarily prove that mTOR directly participates in transcription regulation in TSC. Increases in mTOR activity may increase cellular stress through an imbalance in protein production and degradation, which in turn triggers a transcriptional response. Furthermore, Bateup et al. (2013) found that the majority of transcriptional changes that are observed in "TSC model neurons" derive from a chronic increase in network activity, which is secondary to *Tsc1* loss. Thus, more work is needed to distinguish the primary *vs.* secondary effects of mTOR on transcriptional responses in TSC.

mTOR-dependent changes in the transcriptome were also reported in models of Parkinson's disease, Alzheimer's disease, epilepsy, glioblastoma, and retinitis pigmentosa (Rieker et al., 2011; Caccamo et al., 2014; Wu et al., 2014; Blazejczyk et al., 2016).

According to Rieker et al. (2011), the downregulation of mTOR in mouse models of Parkinson's disease leads to the inhibition of TIF-1A and YY-1 transcription factors and subsequent nucleolar disruption and a lower stress response, respectively. The amyloid precursor protein mouse model of Alzheimer's disease exhibits substantial changes in the transcriptome. The removal of one copy of mTOR rescued the expression of over 200 transcripts to normal levels (e.g., Ulk1, Ncam1, and dynactin 1). However, this study did not evaluate the significance of these changes in ameliorating neuropathological symptoms. With regard to epilepsy, our recent data (discussed above) show that mTORC1 inhibition changes the expression profiles of *Elmo1*, *Abra*, *Gprc5a*, and *Gadd45g* in response to KA-induced status epilepticus (Blazejczyk et al., 2016). In the case of glioblastoma, recent studies suggest that the upregulation of mTORC2 activity may contribute to the metabolic reprogramming of tumor cells. mTORC2-driven changes in the transcription of genes that are involved in glycolytic, lipid, glutamine, and reactive oxygen species metabolism are "suspected," with postulations of the involvement of c-Myc and SREBP1 as downstream mTORC2 effectors (Wu et al., 2014). Finally, the mTOR–Hif1α-dependent transcription of genes that are involved in cell metabolism (e.g., Glut1, PK-M2, G6PD, heksokinase HK2, and ME1) was hypothesized to explain the prosurvival effects of insulin and trophic factors on photoreceptors in mouse models of retinitis pigmentosa (Venkatesh et al., 2015).

Finally, when discussing the potential contributions of mTOR to neuropathology, additional processes beyond translation, autophagy, and transcription may also be dysregulated and contribute to overall disease outcome. Among these are the feedback inhibition of receptor tyrosine kinase activity via the mTOR or S6K1 phosphorylation of receptor accessory proteins, disturbances in intracellular transport and the cell surface expression of glucose transporters, and lysosomal biogenesis/autoreformation (Malik et al., 2013b). Although these processes appear to be disturbed in diseases that are characterized by changes in mTOR activity, their direct dependence on mTOR and contribution to pathology have not been thoroughly studied.

CONCLUSIONS

Research over last few years has revealed enormous complexity of the mTOR signaling network in mammalian cells. This knowledge has just begun to change neuroscientists' perspectives on the contribution of mTOR to its numerous effects during neuronal development and physiology. This substantial signaling complexity upstream and downstream of mTOR expands the potential clinical interventions that can be tailored for particular diseases or even specific symptoms of a disease. Further research that links disturbances in mTOR-controlled processes and neuropathology is needed before reaching this stage.

COMPETING INTERESTS

None of the authors have any financial or non-financial competing interests in the manuscript.

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APPENDIX A

Table A.1. Animal models of selected mTOR-related diseases

Animal model	Expected range of genetic modification	Phenotype			mTOR inhibition effects	Ref.
		Biochemical/Cellular	neuroanatomical	Physiological/behavioral		
TSC1/2						
Tsc1$^{+/-}$	Global one Tsc1 allele KO	GluN2B, GluN2C/D function↑	Not analyzed	Epilepsy (P9–18)	Not analyzed	Lozovaya et al. (2014)
Tsc1$^{+/-}$	Global one Tsc1 allele KO	No changes in spine density and dendritic branching	No cerebral lesions; No giant dysmorphic neurons	No epilepsy (adult); Impaired hippocampus depending learning; Impaired social behavior	Not analyzed	Goorden et al. (2007)
Tsc2$^{+/-}$	Global one Tsc2 allele KO	P-S6(S235/236)↑, Hip[3]	Not analyzed	No epilepsy (adult); Impaired hippocampus depending learning; Lowered threshold for L-LTP induction	Rapamycin (5 mg/kg or 1 mg/kg, daily for 5 days): P-S6↓; Learning deficits↓; L-LTP induction threshold ↑	Ehninger et al. (2008)
Tsc1$^{fl/fl}$*;CamKIIα-Cre*	Tsc1 KO in mature forebrain neurons	P-S6(240/244)↑; neuronal cell size↑	Brain size↑; astrogliosis↑	Lifespan↓; KA-induced seizure severity↑; KA-induced death↑ pathological hindlimb clasping reflex; Hypoactivity	Rapamycin (0.2 mg/kg daily starting P1): Lifespan↑; brain size↑; Hindlimb clasping reflex↓; activity↑	Ehninger et al. (2008), Bateup et al. (2013)
Tsc2$^{+/-}$	Global one Tsc2 allele KO	Global protein synthesis↓; Arc synthesis↓	Not analyzed	mGluR LTD deficits; no context discrimination	Rapamycin (acute): Restoration of mGluR-LTD in slices	Auerbach et al. (2011)
Tsc2$^{+/-}$	Global one Tsc2 allele KO	P-S6 (S235/236)↑, retinal ganglion cells	Defects in ipsilateral retinogeniculate projections	Not analyzed in vivo	Not analyzed in vivo	Nie et al. (2010)
Tsc2$^{+/-}$	Global one *Tsc2* allele KO	Not analyzed	not analyzed	Impaired social mother-pup interactions	Not analyzed	Young et al. (2010)
Tsc2$^{+/-}$*; Tsc2*$^{fl/fl}$*; CamKIIα-Cre*	Global one *Tsc2* allele KO; *Tsc2* KO in mature forebrain neurons	Dendritic spine density↑; Spine pruning, cortical pyramidal neurons↑	Not analyzed	Impaired in novel object recognition; Impaired social interactions (tested only for Tsc2$^{+/-}$)	Rapamycin (3 mg/kg/day from P21 to P28): Dendritic spine pruning↑	Tang et al. (2014)
Tsc2$^{fl/fl}$*;CamKIIα-Cre*	*Tsc2* KO in mature forebrain neurons	P-S6(240/244)↑, Hip	Not analyzed	Lifespan↓* *Extended by daily glutamine supplementation	Not analyzed	Rozas et al. (2015)

(*continued on next page*)

Table A.1 (*continued*)

Animal model	Expected range of genetic modification	Phenotype			mTOR inhibition effects	Ref.
		Biochemical/Cellular	neuroanatomical	Physiological/behavioral		
Tsc1$^{+/-}$ *Tsc2*$^{+/-}$	Global one *Tsc1* or *Tsc2* allele KO	P-S6K1(T389)↑; *Tsc1, Gsk3b, Ulk1, Erk2, Eef2k* mRNA (tested only for Tsc2$^{+/-}$)↑	Not analyzed	Impaired social interaction	Rapamycin (2, 5 and 10 mg/kg once daily for 2 consecutive days): P-S6K1↓; *Tsc1, Gsk3b, Ulk1, Erk2, Eef2k* mRNA↓; Social interaction improvement	Sato et al. (2012)
Tsc1$^{-/fl}$*;Synl-Cre*	Second copy Tsc1 KO in Tsc1 heterozygous neurons starting E12.5	P-S6(S235/236)↑; Cell soma size↑; Improper axonal orientation; spine density↓	Lamination defects, Cx; Enlarged ectopic cells, Hip; Dysplastic neurons hypomyelination	Lifespan↓ Body mass↓; Clasping; Tremor	Rapamycin and RAD001 (both 6 mg/kg every 2nd day starting P7–9): Lifespan↑; Body mass↑; Clasping↓; tremor↓; Neuronal cell size ↓ (but no effects on dysplastic features); Myelination↑	Meikle et al. (2007, 2008), Di Nardo et al. (2009)
Tsc1$^{fl/fl}$*;GFAP-Cre* (*Tsc1*^{GFAP1}CKO)	*Tsc1* KO in astrocytes (not verified in radial glia)	P-S6[1], Hip, Cx ↑; Expression of Glt-1↓[2]	Glutamate levels, Hip↑; Neuronal cell death, Hip↑; Astrocyte proliferation↑; astrogliosis↑; Brain size↑; Hip size↑; Disorganization of layers; No cortical tubers	Lifespan↓; Epilepsy; impaired hippocampal LTP; Impaired hippocampus-dependent learning	Rapamycin (3 mg/kg, 5 days/week for 5 weeks starting at P14): P-S6↓; Astrogliosis↓; brain size ↓; Hip size↓; Hippocampal disorganization↓; Lifespan↑; Epilepsy↓; Rapamycin (3 mg/kg, 5 days/week for 5 weeks starting at P60 for 3 weeks; after seizure onset): Epilepsy↓; Lifespan↑	Uhlmann et al. (2002), Zeng et al. (2008)
Tsc2$^{fl/fl}$*;GFAP-Cre* *(Tsc2*^{GFAP1}*CKO)*	*Tsc2* KO in astrocytes (not verified in radial glia)	P-S6[1], Hip, Cx ↑[*]; Glt-1 expression↓[*] [*]More severe than in *Tsc1*^{GFAP1}CKO	Brain size↑; Astrocyte proliferation↑; Astrogliosis↑; Lamination defects, Hip[*]	Lifespan↓; Epilepsy	Rapamycin (3 mg/kg, 5 days/week for 5 weeks starting at P14): P-S6↓;	Zeng et al. (2011)

			*More severe than in *Tsc1*^{GFAP1}CKO		Astrocyte proliferation↓; Disorganization of hippocampus↓; Lifespan↑; Epilepsy↓	
Tsc1$^{fl/fl}$;*hGFAP-Cre* (*Tsc2*^{GFAP2}*CKO*) *Tsc1*$^{-/fl}$;*hGFAP-Cre*	*Tsc1* KO in radial glia	P-S6(S235/236), Cx↑; P-Akt(S473)↓; P-Stat(S727)↑; Neuronal cell soma size↑; Disarranged projections of Cx pyramidal neurons	Brain size↑; Cortical thickness↑; Lamination defects, Hip, Cx; Presences of ectopic neurons; Hypomyelination; Number of astrocytes↑; Lateral ventricles size↑; SVZ expansion; SEN-like lesions	Lifespan↓; Epilepsy	Rapamycin (6 mg/kg every 2 days starting P8): Epilepsy↓; P-S6↓; Cortical thickness↓; Astrogliosis↓; Size of lateral ventricles↓; SVZ expansion↓; SEN-like lesions↓	Magri et al. (2013)
Tsc2$^{fl/fl}$;*hGFAP-Cre* (*Tsc2*^{GFAP2}*CKO)*	*Tsc2* KO in radial glia	P-S6, Pax6 positive cells, neurons, astrocytes↑; Cell soma size, neurons and astrocytes↑	Brain size↑; Lamination defects, Hip, Cx; Heterotopias; Astrogliosis↑; Hypomyelination	Body weight↓	Not analyzed	Way et al. (2009)
Tsc1$^{-/fl}$;*GFAP-Cre,* *Tsc2*$^{-/fl;}$*GFAP-Cre,* *Tsc1*$^{-/fl}$;*GFAP-Cre* *x* *Tsc2*$^{-/fl}$;*GFAP-Cre*	Single or double Tsc1 and Tsc2 KO in radial glia	P-S6(S235/236; S240/244)↑*; P-Akt(S473)↓*; neuronal cell size, Cx↑* *No difference between single and double mutants	Cortical thickness↑*; Ectopic Cux-positive neurons in lower cortical layers*; Number of early born neurons in cortical layer IV↓*; Lamination defects, Hip**; Ring heterotopias, Hip***; Astrogliosis↑*; Number of mature oligodendrocytes↓*; Hypomyelination**Similar in all 3 mutants **Subtle differences between mutants ***Only in Tsc2 and double mutants	Lifespan↓*; Body mass↓ *Double mutant life span significantly shorter	Not analyzed	Mietzsch et al. (2013)
Tsc1$^{fl/fl}$;*Dlx5/6-Cre*	*Tsc1* KO in GABA- (Magri et al. (2011); Carson et al. (2012) ergic neurons	P-S6[1] ↑; Cell size, GABAergic neurons, Hip, Cx,↑	Number of CR and NPY positive GABAergic neurons, Cx↓	Body weight↓; Lifespan↓; Sensitivity to flurothyl-induced seizures↑	Not analyzed	Fu et al. (2012)

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Table A.1 (*continued*)

Animal model	Expected range of genetic modification	Phenotype			mTOR inhibition effects	Ref.
		Biochemical/Cellular	neuroanatomical	Physiological/behavioral		
Tsc2$^{fl/fl}$;Nex-Cre	*Tsc2* KO in early postmitotic excitatory neurons in embryonic forebrain	P-S6 (S235/236)↑; P-Akt (S473, T308)↓; No. of axons/cell↑; Dendritic branching↑	Cell dispersion, Cx, Hip; Astrogliosis↑	Body weight↓; Lifespan↓	RAD001 (10 mg/kg daily starting P1): Lifespan↑; P-S6 (S235/236)↑; P-Akt (S473, T308)↓; Astrogliosis↓	Crowell et al. (2015)
Tsc1$^{fl/fl}$;Nestin-Cre	*Tsc1* KO in neural stem cells during early development	P-S6 (S235, S240)↑; Cell size, Cx↑	Brain size↑; Cell density, Cx layers IV and V, ↓ Astrogliosis↑ Normal layer formation, Cx	Lifespan↓ (due to impaired maternal pup interaction)	Rapamycin (single dose 1 mg/kg given to pregnant mouse) : Pup survival↑; P-S6↓; Cell density↑; No change in cell size Additional rapamycin (gradually increasing doses [1 to 3 mg/kg] starting P8): lifespan↑; P-S6↓; P-Akt↑	Anderl et al. (2011)
Tsc2F36-37$^{fl/fl}$; Emx1-Cre	Selective KO of GAP domain of TSC2 in neural progenitor cells giving rise to cortical neurons	P-S6 (S240/244), Cx, ↑; P-4EBP (T37/46), Cx, ↑	Not analyzed	Lifespan↓; Body mass↓	Rapamycin (0,1 mg/kg/day postnatally): Lifespan↑; Body mass↑	Fu and Ess (2013)
Tsc1$^{fl/fl}$;Emx1-Cre *Tsc1$^{-/fl}$;Emx-Cre*	*Tsc1* KO in embryonic NCSs of wild type mice or second Tsc1 allele KO in embryonic NCSs of Tsc1 heterozygous mice	P-S6 (S235/236)↑; P-ERK↑; P-Akt (S473)↓; neuronal cell size↑	Brain size↑; Cortical thickness↑; Hydrocephaly; Displastic and ectopic neurons, Cx; Ring heterotopias; Astrogliosis↑; Hypomyelination; Expanded and disorganized hippocampal regions; Lamination defects, Cx; Disorganization of postnatal SVZ niche; Tumor-like lesions in	Lifespan↓; Lower body mass↓; Epilepsy	Rapamycin (6 mg/kg every 2 days starting P8): P-S6↓; Body mass↑; Lifespan↑; Epilepsy↓; Astrogliosis↓; Hypomyelination↓; Cortical thickness↓; Hydrocephalus↓; Rapamycin (3 mg/kg, 5 days/week for 4 weeks starting at P15): P-S6↓; Astrogliosis↓;	Magri et al. (2011), Carson et al. (2012)

			ventricles; Migration defects along RMS		Brain size↓; Epilepsy↓; Lifespan↑; Tumor like lesions↓	
Tsc1$^{fl/fl}$;*L7-Cre*	*Tsc1* knockout in Purkinje cells (PCs)	P-S6^{1}↑; Neuronal cell size↑; spine density↑; Aberrant axonal sprouting; Cleaved caspase 3↑; ER stress↑; Oxidative stress↑	Number of PC cells↓	Excitability of PC cells↓; Autistic like behaviors↑; Ataxic behaviors↑	Rapamycin (6 mg/kg,3 times a week starting P7): PC cell survival↑; Autistic like behaviors↓; Ataxic behaviors↓	Tsai et al. (2012)
Tsc2$^{fl/fl}$;*Pcp2-Cre*	*Tsc2* knockout in Purkinje cells (PC, starting P6)	P-S6(S240/244)↑; neuronal cell size↑; ER stress↑; Oxidative stress↑	Number of PC cells↓	Motor functions↓	Rapamycin (2 mg/kg, 3x week, starting P10): Neuronal cell size↓; PC cell survival↑; ER stress↓; Motor functions↑	Reith et al. (2011)
Tsc2$^{fl/fl}$;*Olig2-Cre*	Tsc2 KO in oligodendrocite precursors	P-S6(240/244)↑; P-NDRG1↓; MBP, CNP↓	Hypomyelination; axon density↓; Oligodendrocite number↓; Astrogliosis, Cx and white matter↑	No significant deficits in motor, anxiety and social behavior	Rapamycin (0.1 mg/kg, P3–17; P30–60): Myelination↑	Carson et al. (2015)
Tsc1$^{fl/fl}$;*Gbx2-CreERT*	Tamoxifen-induced *Tsc1* KO in thalamic neurons (E12 and 18)	P-S6(240/244)↑; Neuronal cell size↑; Membrane input resistance (E12 KO)↓; input capacitance (E12 KO)↑	Altered thalamocortical projections	Abnormal neural activity (local field potentials in cortex); Overgrooming; Epilepsy (E12 KO)	Not analyzed	Normand et al. (2013)
Tsc1$^{fl/fl}$;*Lhx2-Cre*	*Tsc1* KO in eye committed neural progenitor cells	P-S6 (S235/236; S240/244) in eye cells↑	Eye size↑, Retinal folding; Loss of ora serrata integrity; Heterotopias and hamartomas; Astrogliosis↑; Changes in optic nerve morphology; Aberrant retinogeniculate topography	Not analyzed	Not analyzed	Jones et al. (2015)

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Table A.1 (*continued*)

Animal model	Expected range of genetic modification	Phenotype			mTOR inhibition effects	Ref.
		Biochemical/Cellular	neuroanatomical	Physiological/behavioral		
Tsc2$^{c-del3/fl;}$*Syn1-Cre* (Tsc2cc) *Tsc2*$^{-/c-del3;}$*Syn1-Cre* (Tsc2kc)	Graded reduction of Tsc2 activity in neurons due to introduction of either two hypomorphic Tsc2 alleles (Tsc2cc) or one null, one hypomorphic (Tsc2kc)	P-S6(S235/236) ↑; neuronal cell size↑; *Proportional to the extent of Tsc2 loss	Number of ectopic neurons in the hippocampus↑	Lifespan↓*; Locomotor activity↑; anxiety↓*; Impaired social interaction *Proportional to the extent of Tsc2 loss	Not analyzed	Yuan et al. (2012)
Tsc1$^{fl/fl}$*xNestin-rtTAxTRE-Cre* Dox given at E8, E10, E13, E16–17	doxycycline induced mosaic *Tsc1* KO in different brain areas; E8 small proportion of cells thalamus, brain stem, spinal cord; E10 widespread recombination in all brain areas, E16 restricted KO in cortex, hippocampus and cerebellum	*E13 dox:* P-S6 (S235,S 240), neurons, glia, ↑, mTORC2 activity, neurons, glia↓, Dysmorphic neurons with increased cell size; Nucleus size↑; complexity of basal dendrites, Cx,↑; Aberrant orientation of apical dendrite; ER stress↑; Inflammatory response↑; Vacuolated giant cells with immature lysosomes and mitochondrial expansion	*E8 dox:* Degeneration of white matter in a spinal cord *E13 dox:* Brain size↑; Astrogliosis↑; Hypomyelination; Vacuolated giant cells, Cx	*All dox:* Lifespan↓; *E13 dox:* Hyperactivity; Epilepsy	Rapamycin (starting P8): Lifespan↓; Epilepsy of E13 dox mice↓; mTORC1 activity↓; astrogliosis↓; ER stress and inflammation↓; Partial correction of improper apical dendrite polarity	Goto et al. (2011)
Tsc1$^{fl/fl}$*;Nestin-CreERT2*	Tamoxifen induced *Tsc1* KO in neural stem cells (P7 or P30)	Giant cells with increased P-S6[1] within tumor mass; NeuN expression in DCX positive neuroblasts	Brain size↑; Hydrocephalus; Nodular protrusions on lateral ventricle walls (SEN-like); Small tumors near intraventricular foramen (SEGA-like);	Not analyzed	Not analyzed	Zhou et al. (2011)

			No. of granule neurons in OB (due to decreased migration)↓			
Tsc1$^{fl/fl}$;AscII-CreERTM	Tamoxifen induced *Tsc1* selectively in transit amplifying cells of SVZ	Not analyzed	No gross brain abnormalities; Hydrocephalus in some cases; SEN- and SEGA-like abnormalities	Not analyzed	Not analyzed	Zhou et al. (2011)
Tsc1$^{fl/fl}$;Cag-CreERT	Tamoxifen induced *Tsc1* KO in adult mice	P-S6 (235/236)↑	No hypomyelination; no astrogliosis	L-LTP threshold↓; excitability↑; epilepsy; Seizure induced lethality	Rapamycin (10 mg/kg post seizures): Epilepsy↓	Abs et al. (2013)
Tsc1$^{fl/fl}$;Nestin-CreERT2	Tamoxifen induced postnatal *Tsc1* KO in neural stem cells (P7)	Not analyzed	SEGA-like lesions, SVZ; lesions in RMS; mutated cell mistargeted to cortex.	Not analyzed	Not analyzed	Feliciano et al. (2012)
Tsc1$^{fl/fl}$ + AAV-Cre injection to CA1	*Tsc1* KO in infected CA1 neurons	P-S6(S240/244)↑; Intrinsic excitability↓	Not analyzed	Inhibitory transmission↓; Excitatory-inhibitory synaptic imbalance	Rapamycin (5 mg/kg, 7 days prior to experiment): Correction of excitatory-inhibitory synaptic imbalance	Bateup et al. (2013)
Tsc1$^{fl/-}$ + Cre electroporation (Epo)	Second *Tsc1* allele KO in electroporated neural stem cells (E15–16)	P-S6(240/244)↑; Neuronal soma size↑; Multinucleated neurons	*E16 Epo, P15:* Heterotopic nodules with enlarged neurons, Cx; *E15 Epo, P15:* Tuber like lesions, Cx	Threshold for PTZ-induced seizures↓	Not analyzed	Feliciano et al. (2011)
Tsc1$^{fl/-}$ + Cre electroporation @ P0–1	Postnatal second *Tsc1* allele KO in SVZ neural stem cells	P-S6(240/244)↑; Increased HIF1α expression↑; Neuronal cell size↑; Nuclei size↑; Increased size and more complex morphology of cells in RMS; Hypertrophy of OB neuron dendritic arbors	Heterotopias in and outside RMS consisting of neuronal or neuroglial cells; Neuronal micronodules in OB; Mutated cell mistargeted to cortex	Not analyzed	Not analyzed	Feliciano et al. (2012, 2013)
Tsc2 shRNA *in utero* electroporation	Embryonic *Tsc2* knockdown in neural stem cells (E14)	P-S6 (S235/236)↑; Neuronal cell size↑	Lamination defects, Cx	Not analyzed	Fetal rapamycin (5 mg/kg): Neuronal cell volume↓; Lamination defects↓	Tsai et al. (2014)

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Table A.1 (*continued*)

Animal model	Expected range of genetic modification	Phenotype			mTOR inhibition effects	Ref.
		Biochemical/Cellular	neuroanatomical	Physiological/behavioral		
Tsc1$^{fl/fl}$ + AAV-Cre	*Tsc1* KO in transduced neurons (viral injection at P0)	P-S6 (S235/236)↑; Neuronal cell size↑	Brain size↑; Mild hydrocephalus; Nodules and thickening of the ventricular lining	Lifespan↓	Not analyzed	Prabhakar et al. (2013)
ΔRG mice	Global overexpression of dominant negative Tsc2	Not analyzed in brain tissue	Aggregates of granular cells at the cerebellum surface	Anxiety↑; Impaired social behavior Impaired motor learning	Not analyzed	Govindarajan et al. (2005), Ehninger and Silva (2011), Chévere-Torres et al. (2012)
Tsc2 shRNA postnatal electroporation (Wistar rats)	Postnatal Tsc2 knockdown in neural stem cells of SVZ (P0)	P-S6(235/236)↑; Neuroblast cell size, RMS↑	Not analyzed	Not analyzed	Not analyzed	Malik et al. (2015b)
PTEN						
Pten$^{+/-}$	Global one *Pten* allele KO	Not analyzed	Not analyzed	Aggression↓; Repetitive behavior↑	Not analyzed	Clipperton-Allen and Page (2014, 2015)
Pten$^{fl/fl}$*;GFAP-Cre* (NS-*Pten*)	*Pten* KO in granular neurons of DG and cerebellum	P-S6(S235/236), P-Akt(S473), Cb, Hip↑: Neuronal cell size, DG, Cb	PCs atrophy; Dysplastic neurons, Cb	Lifespan↓; Epilepsy	CCI-779 (10–50 mg/kg, 5 days a week for 4–8 weeks): P-S6↓; Neuronal cell size, DG↓; Lifespan↑; Epilepsy↓	Kwon et al. (2001, 2003)
Pten$^{fl/fl}$*;GFAP-Cre* (NS-*Pten*)	*Pten* KO reported also for cortical neurons	P-S6(S240/244),↑; Neuronal cell size, Cx↑	Brain size↑; Aberrant mossy fiber sprouting	Epilepsy	Rapamycin (10 mg/kg 5 days a week, for 2 weeks starting at week 4): P-S6↓*; Neuronal cell size↓*; Mossy fiber sprouting↓*; Epilepsy↓*; *only up to 5 weeks post rapamycin); Repeated rapamycin 2 week rapamycin treatments (weeks 4–5, 10–12, 16–18): Extension of seizure free period; Lifespan↑	Ljungberg et al. (2009), Sunnen et al. (2011)

Pten$^{fl/fl}$;GFAP-Cre (NS-Pten)	As above	P-S6(240/244)↑; P-Akt(S473)↑	Astrogliosis↑, Microgliosis↑	lifespan↓; epilepsy	Rapamycin (10 mg/kg 5 days a week, starting at postnatal week 9): P-S6↓; P-Akt↓; GFAP↓; IBA1↓; Established epilepsy↓; Lifespan↑	Nguyen et al. (2015)
Pten$^{fl/fl}$;GFAP-Cre (NS-Pten)	As above	P-S6(240/244), S6K1, P-Akt(S473), FMRP, P-FMRP (S499), Hip,↑	Not analyzed	Trace and contextual memory impairment	Not analyzed	Lugo et al. (2013)
Pten$^{fl/fl}$;hGFAP-Cre	*Pten* KO in astrocytes and subset of neurons. KO observed also in some oligodendrocites	P-Akt(S473)↑; Neuron and astrocyte cells size↑; Increased astrocyte proliferation↑; Neuronal ribosome density↑; nucleoli size↑; increased spine density↑; Pre-synaptic terminals size↑; Postsynaptic densities extending past the pre-synaptic terminal	Brain size↑; Myelination ↑	LTP, CA3-CA1↓	Not analyzed	Fraser et al. (2004, 2008)
Pten$^{fl/fl}$;hGFAP-Cre	*Pten* KO in radial glia	P-Akt, GFAP, DCX↑; NR2B, PSD95↓; Prolonged proliferation of radial glia	Brain size↑; Gliosis; Hyperplasia; Hydrocephalus; Impaired lamination, Hip, Cb; Number of neuronal progenitors↑;	Not analyzed	Not analyzed	Wen et al. (2013)
Pten$^{fl/fl}$;DAT-Cre	*Pten* KO in dopaminergic neurons	DA neuron cell size↑	Not analyzed	Impaired social interaction	Not analyzed	Clipperton-Allen and Page (2014)
Pten$^{fl/fl}$;Nse-Cre	*Pten* KO in a subset of postmitotic	Neuron cell size, DG, Cx↑;	Brain size↑; Cell dispersion, DG	Impaired social interaction and learning;	Rapamycin (10 mg/kg daily for 5 days a week):	Kwon et al. (2006), Ogawa et al. (2007),

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Table A.1 (*continued*)

Animal model	Expected range of genetic modification	Phenotype			mTOR inhibition effects	Ref.
		Biochemical/Cellular	neuroanatomical	Physiological/behavioral		
	neurons of cortex and hippocampus; (max. Cre expression ∼ P30)	Dendritic caliber, DG,↑; Ectopic dendrites, DG; Spine density↑		Dysregulation of theta burst induced LTP and mGluR-dependent LTD, medial perforant path-to-dentate gyrus synapses of young mice; Epilepsy	Neuron cell size↓; Dendritic caliber↓; Brain size↓; Behavioral deficits↓; Effects of rapamycin on LTP and LTD not tested	Zhou et al. (2009), Takeuchi et al. (2013)
Pten$^{+/fl}$;*Emx1-Cre,* *Pten*$^{fl/fl}$;*Emx1-Cre*	*Pten* KO in neural progenitor cells of the developing forebrain	P-Akt (S473), P-GSK3 (S9)↑*, P-S6 (235/236)↑***both hetero- and homozygous Pten KO **only homozygous Pten KO	Brain mass↑*; Cerebral cortex mass↑*; Cell number↑**; *Partly mTOR dependent **mTOR independent	Not analyzed	*Rptor*$^{+/-}$ does not prevent effects of *Pten* haploinsuficiency	Chen et al. (2015)
Pten$^{fl/fl}$;*En2-Cre*	Pten KO in developing cerebellum cells	P-Akt (S473), dysplastic neurons and glia↑; Cell size of all precursor cells and their progeny↑	Cerebellum size; Lack of lamination; Dysplastic neurons and astrocytes; PCs death	Ataxia; Impaired balance; Activity↓	Not analyzed	Marino et al. (2002)
Pten$^{fl/fl}$;*L7-Cre*	*Pten* KO in PCs	P-Akt (S473), PCs↑; PCs cell size↑; dendrites and axons caliber↑	PCs death (after 17–20 weeks of age)	Not analyzed	Not analyzed	Marino et al. (2002)
Pten$^{fl/fl}$;*Gli1-CreERT2*	*Pten* KO only in a subset of postnatally generated neurons (DG and OB)	P-S6[1]↑; DG neurons size↑; Number of apical dendrites↑; Presence of basal dendrites, spine density↑	Ectopic neurons in DG; Mossy fiber sprouting	Lifespan↓; Epilepsy	Rapamycin (6 mg/kg daily/5 days a week starting P14): P-S6↓,; epilepsy↓; mossy fiber sprouting↓	Pun et al. (2012)
Pten$^{fl/fl}$;*Nestin-CreERT2*	*Pten* KO induced in neural stem cells/ progenitors (tamoxifen injection on P0–1, P11–12 or P28–30)	P-S6(S235/236)↑; Premature cell differentiation	SVZ size↑; Diameter of terminal RMS↑; Density of granule neurons, OB↓	Not analyzed	Rapamycin (as described in Kwon et al. (2003); 3 days prior to tamoxifen): SVZ expansion↓	Zhu et al. (2012)

Pten$^{fl/fl}$;Nestin-CreERT2	*Pten* KO induced in neural stem cells/ progenitors by (tamoxifen injection at 4 weeks of age)	P-S6(S235/236), P-Akt(S473), P-GSK3β (S9)↑; Cell size↑; Dendrite number and length↑; Abnormal neuron polarity; Accelerated differentiation of neuronal progenitor cells	Lamination defects, DG granular cell layer	Impaired social interaction; Infrequent epilepsy	Not analyzed	Amiri et al. (2012)
Pten$^{fl/fl}$;Nex-Cre	*Pten* KO in early postmitotic excitatory neurons in embryonic forebrain	P-S6(S240/244), P-Akt(T308, S473)↑; MAP2, NR2A, NR2B↑; neuron soma size↑;	Brain size↑	Lifespan↓; Body mass↓	Rapamycin (0.5 mg/kg subcutaneous Injections every other day, 2 weeks starting at P1): Lifespan↑; Body mass↑	Kazdoba et al. (2012)
Pten$^{fl/fl}$;Rip-Cre	*Pten* KO in hypothalamus	not analyzed	No analyzed	Postnatal growth↓; Serum insulin levels↓; Blood glucose levels↓	Not analyzed	Choi et al. (2008)
Pten$^{fl/fl}$;Dmbx1-Cre	*Pten* KO in midbrain	Not analyzed	Hydrocephalus	Lifespan↓	Not analyzed	Ohtoshi (2008)
Lv-shPTEN	*Pten* knockdown in neonatal and young adult mice DG	Neonatal and young adult: Cell soma size↑; Axons and dendrite caliber↑; Spine density↑; Input resistance↓; mEPSC freq.↑;Young adults: frequency of spontaneous EPSCs↑; mEPSC freq. and amplitude↑	Not analyzed	Not analyzed	Not analyzed	Luikart et al. (2011)
Pten$^{fl/fl}$ + RetroV-Cre	*Pten* KO only in neural stem cells of DG	P-S6(S235/236), P-Akt (S473), P-GSK3β (S9)↑; c-Fos↑; Neuron soma size↑; Basal electrophysiological	Aberrant neuronal migration, DG granular cell layer		Rapamycin (10 mg/kg, daily, 3–14 dpi, 14–24 dpi): P-S6↓; Neuron soma size↑; Migration defects prevention (but not reversal);	Williams et al. (2015), Getz et al. (2016)

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Table A.1 (*continued*)

Animal model	Expected range of genetic modification	Phenotype			mTOR inhibition effects	Ref.
		Biochemical/Cellular	neuroanatomical	Physiological/behavioral		
		properties alteration; Excitability↑; Dendritic arbors complexity↑; spine density↑			Effects on electrical properties and dendritic morphology not analyzed	
NF1						
Nf1$^{+/-}$, *Nf1*$^{fl/fl}$; *GFAP-Cre, Nf1*$^{-/fl}$*;GFAP-Cre*	*Nf1*$^{fl/fl}$*;GFAP-Cre:* *Nf1* KO in astrocytes starting E14 *Nf1*$^{-/fl}$*;GFAP-Cre:* global single *Nf1* allele KO (E0), second *Nf1* allele KO selectively in astrocytes (E14)	*Nf1*$^{-/GFAP:Cre}$*:* number of P-S6 positive cells in tumor-bearing mice↑	*Nf1*$^{fl/fl}$*;GFAP-Cre:* astrocyte proliferation↑ *Nf1*$^{-/fl}$*;GFAP-Cre:* Optic nerve gliomas	Not analyzed	*Nf1*$^{-/fl}$*;GFAP-Cre:* Rapamycin (5 or 20 mg/kg daily): P-S6↓; Tumor proliferation↓	Bajenaru et al. (2002, 2003), Dasgupta et al. (2005), Hegedus et al. (2008)
Nf1$^{fl/-}$ *Nf1*$^{fl/fl}$*;SynI-Cre*	*Nf1*$^{fl/-}$: global one *Nf1* allele KO *Nf1*$^{fl/fl}$*;SynI-Cre:* *Nf1* KO in neurons	*Nf1*$^{fl/fl}$*;SynI-Cre:* P-ERK↑	*Nf1*$^{fl/fl}$*;SynI-Cre:* Forebrain weight↓; Cerebral cortex thickness↓; Cell density↑; astrogliosis↑; NO tumor development	*Nf1*$^{fl/-}$*:* Lifespan↓ *Nf1*$^{fl/fl}$*;SynI-Cre:* Growth retardation starting P3–4	Not analyzed	Zhu et al. (2001)
Nf1$^{+/-}$*:p53*$^{+/-}$ *(NPcis mice)*	*Nf1* and *p53* null alleles in *cis*	P-S6(S235/236), tumors↑; Cyclin D1, tumors,↑	Tumor development (MPNSTs)	Lifespan↓	Rapamycin (5 mg/kg starting at the time of tumor detection, 5 days a week): P-S6↓; Cyclin D1↓; Tumor growth↓	Cichowski et al., (1999) and Johannessen et al. (2008)
FXR1						
Fmr1$^{-/-}$	Global *Fmr1* KO	P-S6 (S235/236; S240/244), P-eiF4B↑; Global protein synthesis↑; Expression of PSD95, Shank3, CamKIIα, eEF2↑	Abnormal spine morphology; Spine density↑	Hippocampal mGluR-LTD↑; Impaired motor and social behavior	Crossing *Fmr1*$^{-/-}$ with *S6K1*$^{-/-}$: Signaling abnormalities↓; Spine morphology correction; Spine density; mGluR-LTD↓;	Bhattacharaya et al. (2012)

Fmr1$^{-/-}$	Global *Fmr1* KO	P-mTOR(S2448), P-S6K1(T389), P-4E-BP(Thr37/46)↑; Raptor-mTOR interaction↑; eiF4G–eiF4E interaction↑* *no upregulation by DHPG	Not analyzed	mGluR-LTD, Hip,↑	Behavioral deficits↓ Rapamycin (20 nM): No effect on enhanced mGluR-LTD in acute slices	Sharma et al. (2014)
Fmr1$^{fl/fl}$*;Nse:Cre*	*Fmr1* KO in mature cortical and hippocampal neurons	VGLUT1, VGAT, Cx, ↓; P-S6K1(T389), P-Akt (S473), P-GSK3β (S9)↑; Dendritic arbor complexity, Hip CA3↓	Changes is spine shape; No global changes in gross morphology; No change in spine density,	No effect on tested behavior	Not analyzed	Amiri et al. (2014)

[1]Exact epitope not defined by the authors.
[2]Astrocyte specific glutamate transporter.
[3]Hip – hippocampus, Cx – cortex, Cb – cerebellum, DG – dentate gyrus, NSCs – neural stem cells.

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