Molecular Neurobiology

ISSN 0893–7648/06/34(3): 205–219/$30.00
ISSN (Online) 1559–1182

The Growing Role of mTOR in Neuronal Development and Plasticity

Jacek Jaworski[1] and Morgan Sheng[2,*]

[1]International Institute of Molecular and Cell Biology, Warsaw, Poland and [2]The Picower Institute for Learning and Memory, RIKEN-MIT Neuroscience Research Center, and Howard Hughes Medical Institute, Massachusetts Institute of Technology, Cambridge, MA

Abstract

Neuronal development and synaptic plasticity are highly regulated processes in which protein kinases play a key role. Recently, increasing attention has been paid to a serine/threonine protein kinase called mammalian target of rapamycin (mTOR) that has well-known functions in cell proliferation and growth. In neuronal cells, mTOR is implicated in multiple processes, including transcription, ubiquitin-dependent proteolysis, and microtubule and actin dynamics, all of which are crucial for neuronal development and long-term modification of synaptic strength. The aim of this article is to present our current understanding of mTOR functions in axon guidance, dendritic tree development, formation of dendritic spines, and in several forms of long-term synaptic plasticity. We also aim to present explanation for the mTOR effects on neurons at the level of mTOR-regulated genes and proteins.

Index Entries: mTOR; rapamycin; neuronal development; synaptic plasticity.

Introduction

Neuronal development and synaptic transmission are plastic processes that are influenced by changing extracellular and intracellular conditions. Protein kinases are crucial for regulation of these phenomena and several of them—for example, mitogen-activated protein kinases (MAPKs) *(1,2)*, phosphoinositide-3′ kinase (PI3K) *(3–6)* and calcium/calmodulin-dependent kinases (CaMKs) *(7–11)*—have well-established roles in both neuronal development and synaptic plasticity. Recently, there has been growing interest of neuroscientists in a serine/threonine protein kinase called mammalian target of rapamycin (mTOR) that is known for its role in cell proliferation and growth in non-neural

Received June 15, 2006; Accepted August 4, 2006.
*Author to whom correspondence and reprint requests should be addressed. E-mail: msheng@mit.edu.

cells. Although neurons are postmitotic (nonproliferative), the size of the neuronal cell soma is controlled by mTOR *(12)*.

Regarding molecular mechanism, mTOR is believed to act primarily by phosphorylating eIF-4E binding protein (4E-BP) and p70 ribosomal S6 protein kinase (p70S6K), which are important regulators of protein translation *(13)*. However, "chemical genomics" performed on yeast identified 400 mutants whose phenotype (measured as rate of survival) was changed in the presence of rapamycin, a specific inhibitor of mTOR *(14,15)*. Gene expression profiling of *Drosophila melanogaster* cells treated with rapamycin further confirmed a large number of genes with expression that is down- or upregulated by mTOR inhibition *(16)*. Analysis of these rapamycin-dependent mutants has suggested that mTOR might be involved in additional cellular functions such as transcription, ubiquitin-dependent proteolysis, and microtubule stability *(14–16)*.

Because of its widespread role in cell physiology, the involvement of mTOR in the regulation of neuronal development and synaptic plasticity has been underinvestigated and poorly understood. However, several recent findings have pointed to mTOR as one of the crucial regulators of different stages of neuronal development as well as some forms of long-term modification of synapses. This article reviews the current advances in our understanding of mTOR function in axon guidance, dendrite development, dendritic spine morphogenesis, and several forms of long-term synaptic plasticity. It also discusses the known and potential molecular pathways by which mTOR exerts its effect on neurons. However, because of very diverse activities of mTOR, our knowledge about molecular targets of mTOR in neurons is far from complete.

mTOR and Signal Transduction

mTOR kinase is a major regulator of cellular metabolism involved in growth of non-neural cells. In neurons, however, it appears to play a broader role. Several excellent reviews published recently have discussed the signal transduction pathways that involve mTOR activity, the cellular processes regulated by mTOR, as well as proteins that interact with mTOR *(17–20)*. Therefore, this article provides only a brief summary of this knowledge that is indispensable for understanding mTOR actions in the nervous system (please refer to Fig. 1 for a diagram of cell signaling pathways leading to mTOR activation).

mTOR activity in most cells can be induced by three types of signals that report the metabolic state of the cell: trophic factors and hormones, availability of amino acids, and the level of adenosine monophosphate (AMP). In neurons, neurotransmitters such as glutamate can also lead to activation of mTOR via both ionotropic and metabotropic receptors *(21–23)*. The canonical pathway of mTOR activation leads from trophic factor receptor tyrosine kinase through the induction of PI3K and Akt kinases to the phosphorylation and inhibition of tuberous sclerosis complex proteins: hamartin (TSC1) and tuberin (TSC2). The TSC1/TSC2 complex acts as a GTPase-activating protein for Ras homolog enriched in brain (Rheb); elevated GTP-bound Rheb then stimulates mTOR *(24–26)*. However, it is also known that mitogens can activate mTOR independently of Akt through phospholipase D *(27)* as well as through the extracellular-signal regulated kinase (ERK)/MAPK pathway *(28,29)*. Conversely, it is not clear how availability of amino acids controls mTOR activity. It is known that increased availability of amino acids (especially leucine) activates mTOR, but this activation does not require PI3K/Akt activity *(20)*. Although trophic factors and amino acids stimulate mTOR, high levels of AMP (indicative of energy deficits in the cell) decrease mTOR activity because of activation of TSC2 by AMP-dependent protein kinase (AMPK) *(30)*.

Active mTOR interacts with one of its partners, Raptor or Rictor. Rapamycin inhibits the activity of the mTOR/Raptor complex (TORC1 complex), but it does not prevent activity of the

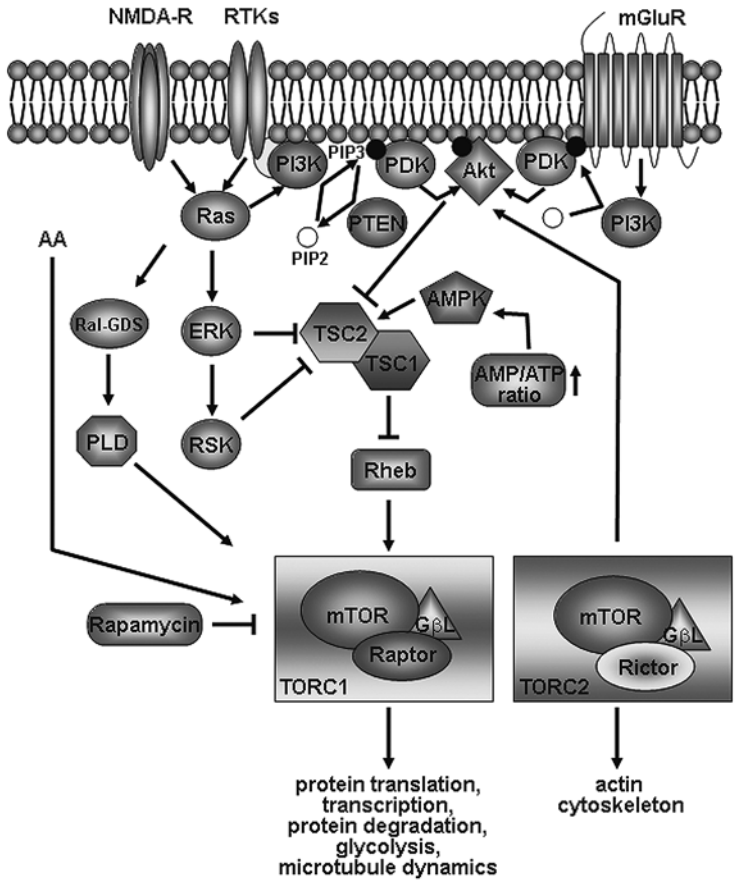

Fig. 1. Schematic diagram of signaling pathways leading to mTOR activation in mammalian cells. Stimulation of several receptors at the plasma membrane such as receptor tyrosine kinases (RTKs; e.g., TrkB), ionotropic and metabotropic glutamate receptor (NMDA-R; mGluR) through PI3K-dependent as well as PI3K-independent pathways leads to mTOR activation. PI3K is activated by Ras, leading to activation of PDK1 and Akt, which then inhibits the TSC1/2 complex. That allows GTP-bound Rheb protein to activate mTOR. Ras can also stimulate mTOR activation via ERK or phospholipase D (PLD) pathways, independently of PI3K. An increased level of amino acids (AA) induces mTOR activity in PI3K-independent manner. Increased AMP/ATP ratio, an indicator of energy deficits in the cell, activates TSC2 and inhibits mTOR. mTOR bound to Raptor protein forms the TORC1 complex that is responsible for the control of several cellular processes of which protein translation and transcription are the best described. mTOR can also form TORC2 complex with Rictor, which regulates actin dynamics and can also phosphorylate Akt. Little is known about the regulation of TORC2. Abbreviations: PI3K, phosphoinositide-3′ kinase; Ral-GDS, Ral GDP dissociation stimulator; PDK, pyruvate dehydrogenase kinase; PIP2, phosphatidylinositol bisphosphate; PIP3, phosphatidylinositol 3,4,5-trisphosphate; PTEN, phosphatase and tensin homolog deleted on chromosome ten; RSK, p90 ribosomal S6 kinase 1; TSC1, tuberous sclerosis complex protein 1, hamartin; TSC2, tuberous sclerosis complex protein 2, tuberin; Rheb, Ras homolog enriched in brain; AMPK, AMP-dependent protein kinase.

Rictor containing TORC2 complex *(31–33)*. Activation of TORC1 affects a myriad of cellular processes. The best known example is an increase in protein translation by phosphorylation of p70S6K and 4E-BP1 *(13,34)*. Phosphorylation activates p70S6K, leading to phosphorylation of ribosomal S6 protein, which is crucial for stimulating the translation rate of messenger

RNAs (mRNAs) containing a 5′ oligopyrimidine tract (TOP) *(35)*. This subgroup of mRNAs encodes primarily the proteins involved in the translation process itself, such as all ribosomal proteins and several elongation factors *(36)*. Thus, mTOR activation leads to increase in translation capacity of the cell. 4E-BP1 is an inhibitor of cap-dependent mRNA translation *(37)*. 4E-BP1 binds eIF-4E protein, which is crucial for the formation of the cap complex and prevents its recruitment to the cap complex. Phosphorylation of 4E-BP1 by mTOR results in its dissociation from eIF-4E, thereby allowing eIF-4E binding to eIF-4G, which is a crucial step for the initiation of translation.

However, p70S6K and 4E-BP1 are not the only targets of mTOR. mTOR activity, which can be inhibited by rapamycin, is important for controlling activity of proteins such as protein phosphatase PP2A *(38)*, cytoplasmic linker protein-170 *(39)*, eukaryotic elongation factor 2 kinase *(40)*, glycogen synthase *(41,42)*, hypoxia-inducible factor 1-α *(43)*, and probably many more proteins, as suggested by analysis of mammalian homologs of rapamycin-regulated genes in yeast *(14,15)*.

The recent discovery of Rictor shed light on the role of TORC2 complex. mTOR bound to Rictor regulates a different set of events than those regulated by TORC1, including those important for control of actin polymerization *(31,44)*. Recent findings have shown that chronic, but not acute, treatment with rapamycin can lead to inhibition of the TORC2 complex and Akt *(45)*. Therefore, it is important to remember that our knowledge about mTOR activity in neurons is not complete, and all of the data need to be interpreted very carefully.

mTOR Regulation of Neuronal Morphology

Axon Pathfinding and Regeneration

The navigation of axons toward their targets can occur over long distances and is regulated by multiple external cues that act as attractants (e.g., Netrin-1) or repellents (Netrin-1, Semaphorin3A, Slit2). Axonal growth cones contain mRNA and translation machinery, and growth cone responses to chemotropic cues depend at least partly on local protein synthesis *(46–50)*. Campbell and Holt *(50)* showed that similarly to general protein synthesis inhibitors such as anisomycin and cycloheximide, mTOR antagonist rapamycin was capable of preventing both repulsive turning and collapse of axon growth cones of cultured *Xenopus* retinal neurons in response to Semaphorin 3A (Sema3A). Inhibition of mTOR also blocked Sema3A-induced incorporation of ^{3}H-leucine into newly translated proteins in isolated axonal growth cones *(50)*. This observation suggests that mTOR regulates behavior of growth cone via control of protein synthesis. Similarly to Sema3A, Slit proteins also cause collapse of *Xenopus* retinal axon growth cones, but this occurs at later stages of development when retinal axons enter the tectum *(48)*. Slit-induced growth cone collapse also depends on local protein synthesis and is sensitive to rapamycin *(48)*.

In addition to repulsive turning and collapse, mTOR is involved in the directional growth cone response toward source of attractant *(50)*. Application of rapamycin to cultured retinal neurons efficiently prevented axon turning toward a source of Netrin-1. All extracellular signals discussed earlier induced phosphorylation of mTOR effectors such as 4E-BP1 locally in growth cones *(48,50)*, supporting the idea that mTOR regulates growth cone turning and collapse through regulation of local translation. Piper et al. *(48)* pointed to Sema3A- and Slit2-induced local synthesis of cofilin (an actin depolymerizing protein) *(51)* as a possible mechanism for growth cone collapse.

Besides axon growth cone guidance during development, new protein synthesis *(52,53)* and mTOR have been recently implicated in axon regeneration *(53)*. Under certain conditions, axons can efficiently regenerate after axotomy *(54,55)*, a reaction that requires formation of new growth cones *(56–58)*. Verma et al. *(53)* showed that rapamycin prevented regeneration of rat dorsal root ganglia (DRG) and retinal

axons, as well as the incorporation of ^{3}H-leucine into translated proteins induced by axotomy. Furthermore, there was a correlation between the level of phosphorylated eIF-4E protein in the cell and a higher potential for regeneration. For example, embryonic DRG neurons showed the highest level of phosphorylated eIF-4E and the highest regenerative potential, whereas adult retinal neurons had low amounts of phospho-eIF-4E and regenerated poorly. These findings suggest that in axon regeneration, the role of mTOR is also to control the level of local protein synthesis.

Dendrite Arborization

Dendrite arborization is a multistep process controlled largely by external signals *(59–62)*, including diffusible cues *(63–66)*, cell–cell interactions *(67,68)*, neuronal activity *(60,61)*, and calcium influx *(69)*. These extracellular or cell surface signals must be converted into changes in dendritic morphology. Protein kinases such as CaMKs *(7,10,11)*, MAP kinases *(70,71)*, Rho-associated coiled-coil-forming protein kinase (*72)* and Pak1 *(73)* are important regulators of dendritogenesis. Recent studies have also implicated mTOR as another protein kinase involved in the molecular mechanisms of dendritic branching. Jaworski et al. *(5)* and Kumar et al. *(6)* showed that chronic treatment with rapamycin (6 d) reduced dendrite branching in cultured hippocampal neurons. The number of primary dendrites was not changed, but the complexity of dendritic branching was perturbed by rapamycin, as shown by Sholl analysis (which measures the number of dendrites crossing circles at various radial distances from the cell soma) *(74)*. The Sholl plot reflects the "field" covered by the dendritic arbor. For example, an upward and rightward shift of the Sholl plot signifies increased complexity and outward expansion of the dendritic field. Addition of rapamycin resulted in a downward and leftward shift in the Sholl plot, suggesting a reduced area and complexity of the dendritic field *(5)*. A decrease in total number of branches and downward and leftward shift of the Sholl plot were also observed when mTOR protein expression was suppressed by small interfering RNA (siRNA) directed against mTOR *(5)*. The effect of mTOR inhibition mimicked the effects of PI3K and Akt pharmacological inhibitors on dendritic arbor development, which is consistent with mTOR acting downstream of the PI3K-Akt pathway *(5)*.

Although dissociated cultures are a convenient preparation for neuronal morphogenesis experiments, it is important to extend these studies to a more intact preparation or to the in vivo situation. In organotypic cultures of hippocampal slice, chronic application of rapamycin or mTOR siRNAs significantly reduced the total number of basal dendrites as well as the average length of higher order apical dendrites *(5)*. Additional evidence for an involvement of mTOR in dendritic arbor development in vivo comes from Kwon et al. *(75)*, who studied knockout mice deficient in phosphatase and tensin homolog deleted on chromosome ten (PTEN), the lipid phosphatase that counteracts the function of PI3K (*see* Fig. 1). Dentate gyrus neurons of these PTEN knockout mice had more dendrites as well as increased phosphorylation of Akt and S6, which are upstream activators and downstream targets of mTOR, respectively.

In addition to the effects on dendritic arborization under normal culture conditions described earlier, inhibition of mTOR by rapamycin was able to block the dendritic branching induced in hippocampal cultures by various factors. The dendritic arbor growth stimulated by overexpression of constitutively active mutants of Ras, PI3K, and Akt was blocked when rapamycin-dependent activity of mTOR was inhibited *(5,6)*. Importantly, rapamycin also prevented the increase in the dendritic arbor complexity induced by brain-derived neurotrophic factor (BDNF) *(5)*.

What is the mechanism by which mTOR stimulates dendrite branching? Suppression of p70S6K activity by siRNA and overexpression of constitutively active form of 4E-BP1 phenocopied the effects of mTOR inhibition on

dendritic arborization *(5)*. Furthermore, overexpression of a constitutively active 4E-BP1 mutant blocked PI3K-induced dendritic arbor growth. Together, these data point to protein synthesis as a key mechanism by which mTOR contributes to dendritic tree development. At apparent odds with this conclusion, Dijkhuizen and Ghosh *(76)* showed that BDNF-induced growth of primary dendritic branches in immature cortical neurons depends on PI3K activity but was independent of protein synthesis. There are several technical differences that can explain the discrepancy; however, one intriguing explanation is that protein synthesis is important for long-term changes and stabilization of already initiated dendrites, whereas initiation of dendrites is an mTOR- and protein synthesis-independent process. Many open questions remain regarding the function and mechanism of mTOR in dendritic development. Does mTOR regulate protein synthesis generally in the neuron or locally in dendrites to influence dendrite morphogenesis? Does mTOR also control dendrite development via mechanisms unrelated to protein synthesis regulation?

Spine Morphology

In excitatory neurons, glutamatergic synapses are formed on small actin-rich protrusions of dendrites called dendritic spines. Mounting evidence suggests that synaptic plasticity is associated with changes in dendritic spine number and morphology *(77–79)*. Immature spines with potential for change are usually thin and motile. On the other hand, mature spines are larger, mushroom-shaped, and relatively stable. In coculture of the sensory and motor *Aplysia* neurons, rapamycin prevented the protein synthesis-dependent persistence of new synapses induced by serotonin application *(80)*. Kumar et al. *(6)* studied in detail the effects of pharmacological inhibition of mTOR on spine number in cultured mammalian hippocampal neurons. Chronic application of rapamycin caused a decrease in both spine and filopodia number and prevented the increase in filopodia induced by activation of MAPK and PI3K pathways. Short-term (24-h) application of rapamycin was also able to prevent BDNF-induced decrease in spine and increase in filopodia number but did not change protrusion number when used alone *(6)*. Tavazoie et al. *(81)* in cultured organotypic slices studied effects of mTOR inhibition on spine shape and showed that chronic rapamycin application led to increased spine length but not spine width or density. On the other hand, analysis of spine morphology under conditions of increased mTOR activity in slices from animals lacking TSC2 or transfected with siRNA against TSC2 showed an increase in spine width that could be reversed by rapamycin *(81)*. Although work by Tavazoie at al. *(81)* suggests that TSC2 can also regulate spine morphology via mTOR-independent mechanisms, and there are some differences between the results obtained from dissociated and organotypic hippocampal cultures regarding mTOR's role in spine number regulation, it is clear that mTOR is involved in some aspects of spine formation. This conclusion is supported by the finding of increased spine density in neurons of dentate gyrus of PTEN knockout mice *(75)*. Interestingly, changes in spine morphology resulting from TSC2 knockdown were related to altered cofilin activity but not to changes in total amount of cofilin protein *(76)*. Researchers have not determined whether the link between TSC2 and cofilin involves rapamycin- and/or mTOR-dependent mechanisms.

mTOR and Synaptic Plasticity

It is well-established that long-term memory, as well as certain forms of long-term synaptic plasticity, require *de novo* protein synthesis *(82,83)*. Therefore, mTOR was a possible candidate to play a role in the mechanisms of synaptic plasticity. Involvement of mTOR in synaptic change has now been confirmed in diverse organisms such as *Aplysia*, crayfish, and mammals.

Long-Term Facilitation and Potentiation

Long-term facilitation and long-term potentiation (LTP) are examples of longlasting enhancement of synaptic transmission, which can be achieved by various modes of synaptic stimulation, depending on the system. Casadio et al. *(80)* provided an early clue that mTOR-dependent protein synthesis might be important for the enhancement of synaptic transmission; they showed that the persistence of long-term synaptic facilitation between sensory and motor neurons of *Aplysia* in culture, induced by application of serotonin, depended on a rapamycin-sensitive component of local protein synthesis. Another example of long-term facilitation, which is achieved by prolonged high-frequency stimulation at the neuromuscular synapse of crayfish, requires local presynaptic protein synthesis and is also blocked by rapamycin *(84)*.

Rapamycin dependence of synaptic strengthening is not unique to invertebrates. In acute rodent hippocampal slices, mTOR activity is necessary for two forms of protein synthesis-dependent synaptic enhancement: late-phase LTP (L-LTP) evoked by four trains of 100-Hz stimulation, and BDNF-induced strengthening of synaptic transmission *(85)*. In this study *(85)*, evidence was also presented that components involved in mTOR-dependent protein translation, such as 4E-BP proteins and mTOR itself, were present in dendrites close to synaptic sites. In the studies cited earlier *(85)*, rapamycin was continuously present throughout the recording session. Cammalleri et al. *(22)* applied rapamycin to acute hippocampal slices before, during, or after induction of L-LTP at Schaffer collateral/commissural fiber-CA1 synapses by tetanic stimulation (3×1 s 100-Hz stimulation delivered at 10-min intervals). This approach allowed them to conclude that only inhibition of mTOR during the induction phase was able to prevent establishment of L-LTP. The same group demonstrated further that another form of protein synthesis-dependent synaptic enhancement– (conversion of short-term potentiation induced at the synapse into LTP by brief application of ACPD [*trans*–{±}-1-amino-1,3-cyclopentanedicarboxylic acid], an agonist of group I metabotropic glutamate receptors) also required active mTOR *(22)*. On the other hand, induction and establishment of protein synthesis-independent LTP was not blocked by rapamycin, indicating that mTOR activity is not essential for every type of hippocampal LTP. Recently, Vickers et al. *(86)* and Cracco et al. *(87)* showed that L-LTP induction depends on mTOR involvement in local dendritic protein synthesis because induction of L-LTP in hippocampal slices under conditions of physical separation of cell body layer from dendritic layer was blocked by rapamycin.

In all the aforementioned experiments, inhibition of mTOR did not change basal synaptic properties. Accordingly, application of rapamycin for 4 to 6 h to dissociated cultures of cortical neurons did not change the number of AMPA receptor subunit 2 (GluR2) clusters on dendrites *(88)*. On the other hand, a potential increase in mTOR activity induced by knockout of TSC1 led to increased AMPA currents, as shown by both increased miniature evoked postsynaptic potential (mEPSC) amplitude and increased AMPA to *N*-methyl-D-aspartate (NMDA) ratio of EPSC *(81)*.

Phosphorylation of p70S6K is increased at threonine-289 (the phosphorylation site for mTOR) when hippocampal cultures are stimulated with NMDA or elevated KCl to mimic strong synaptic activation leading to calcium influx *(22)*. By immunostaining, phosphorylated p70S6K has a punctate appearance along the dendrite with occasional presence in dendritic spines. The immunocytochemical pattern for phosphorylated p70S6K resembled the punctate staining observed for hotspots of dendritic translation *(22,89)*. These results are consistent with the idea that mTOR is activated by synaptic stimulation, leading to enhanced dendritic protein translation via p70S6K.

Long-Term Depression and Depotentiation

Similarly to LTP, long-term depression (LTD) can be protein synthesis- and mTOR-dependent. A form of hippocampal CA1 LTD can be induced by activation of metabotropic glutamate

receptors (mGluR-LTD) and requires translation of pre-existing mRNAs *(90)*. Recently, Hou and Klann *(23)* found that rapamycin could block mGluR-LTD evoked by DHPG ([*RS*]-3,5,dihydroxyphenylglycine), an agonist of metabotropic glutamate receptors. Moreover, the induction of mGluR-LTD led to increased phosphorylation of mTOR, a hallmark of mTOR activation, both in the whole slice lysates and in synaptoneurosome preparations. DHPG also induced phosphorylation of 4E-BP proteins in hippocampal slices in a PI3K-, eIF-4E- and rapamycin-dependent fashion *(91)*. Moreover, mGluR-LTD in the hippocampus was enhanced and insensitive to rapamycin in mouse with knockout of 4E-BP2-the predominant isoform of 4E-BP in the hippocampus *(91,92)*.

Zho et al. *(93)* developed a protocol in which CA1 synapses with already established LTP (induced by 100-Hz tetanic stimulation) were efficiently depotentiated by application of DHPG. This depotentiation was blocked by rapamycin *(93)*. Similar results were obtained with inhibitors of general protein synthesis but not with actinomycin D, an RNA transcription inhibitor. Therefore, both mGluR-LTD- and mGluR-dependent depotentiation depend on mTOR activity.

LTP and LTD are considered electrophysiological substrates of learning and memory processes in the brain *(94)*. This suggests that mTOR might be involved in the mechanisms of memory formation. Indeed, Tischmeyer et al. *(95)* showed that injection of rapamycin into the auditory cortex of Mongolian gerbils shortly after linearly frequency-modulated tones discrimination training could prevent consolidation of cerebral cortex-dependent long-term memory.

Molecular Mechanisms Underlying mTOR Regulation of Development and Plasticity

Although evidence is mounting for the importance of mTOR in neuronal development and synaptic plasticity, we know relatively little about the signaling pathways in neurons mediated by mTOR. There is relatively good documentation that activity of mTOR and its best-characterized effectors p70S6K and 4E-BP1 can be regulated in neurons by various extracellular stimuli such as trophic factors and hormones (BDNF, insulin) and by neurotransmitters (glutamate), usually in a PI3K-dependent manner *(21,22,96,97)*. The activity of TSC1 can be downregulated by BDNF in dendrites *(97)*. Jaworski et al. *(5)* showed that mTOR is important for induction of dendritic branching by Ras mutants specific for activation of PI3K, Raf-ERK, or PLD. These findings imply that activation of Ras signaling (e.g., through receptor tyrosine kinases, G protein-coupled receptors, ionotropic receptors) can stimulate mTOR through PI3K and PI3K-independent pathways.

There is growing agreement that the effects on neuronal development and synaptic plasticity are related, at least partly, to mTOR's function in regulation of the protein synthesis. Several recent papers showed that axon guidance molecules, BDNF, insulin, and glutamate could stimulate local protein synthesis through activation of mTOR *(21,50,96,97)*. However, we still have very little knowledge of the identity of the proteins crucial for axon guidance, dendritic branching, or synaptic plasticity, which are actually translated in mTOR-dependent fashion. In case of axon guidance, an attractive candidate known as cofilin has been discovered.

Large genetic and microarray screens for *Drosophila* genes involved in axon and synapse development *(98)* and mTOR-regulated genes involved in control of cell growth and division *(16)* identified another regulator of axon development whose transcription is downregulated by mTOR. This gene encodes for a transcription factor, an ortholog of human *ASH2L,* and rapamycin induced its expression *(16)*. Mutation in this gene resulted in a "path finding" phenotype *(98)*.

Recent work by Schratt et al. *(99,100)* and Takei et al. *(97)* has revealed a set of potential rapamycin-regulated genes that are good candidates for key regulators of spinogenesis and synaptic plasticity. Analysis of RNAs that asso-

ciated with polysomes in response to BDNF in a rapamycin-dependent manner identified 79 and 48 genes in young (4 d in vitro) and older (14 d in vitro) cortical neuron cultures, respectively *(99)*. Several of them (e.g., CamKIIα, NMDA receptor subunit NR1, Homer2, Pyk2, LIMK-1, e-NOS) are already implicated in synapse development and plasticity. Indeed, expression of LIMK-1, which is regulated by rapamycin *(99)* and microRNA134 *(100)*, is involved in spine development. LIMK-1 is a negative regulator of cofilin and therefore inhibits actin depolymerization *(101)*. Its increased expression and activity promotes stabilization of actin cytoskeleton and maturation of dendritic spine *(102,103)*.

Other candidate proteins mediating mTOR effects on dendrites and synapses include Arc, a neuronal protein whose translation in synaptoneurosomes is induced by BDNF in a rapamycin-sensitive manner (*92*). Knockdown of Arc by means of antisense technology leads to impaired LTP and to deficits in spatial memory *(104)*. Arc can also influence synapse development via internalization of glutamate receptors *(105)*. Translation of PSD-95, an important postsynaptic scaffold protein of excitatory synapses, has been shown to require PI3K and mTOR *(96,106)*. Overexpression of PSD-95 promotes the maturation of dendritic spines and strongly potentiates AMPAR-mediated EPSCs *(107)*. Deficiency of PSD-95 in vivo prevents the maturation of orientation preference in the visual cortex *(108)* and eliminates behavioral sensitization induced by chronic cocaine administration *(109)*. The aforementioned examples are by no means a complete list of candidate synaptic plasticity proteins whose expression levels might be regulated by mTOR.

Analysis of the TSC2-null murine neuroepithelial progenitor cells that are characterized by increased activation of the mTOR pathway showed elevated expression of several mRNAs encoding important plasticity-related proteins, suggesting that mTOR can also control synaptic function at the transcriptional level *(110)*. Among identified mRNAs were several subunits of glutamate receptors, including GluR2 and NR2B. Passafaro et al. *(111)* showed that overexpression of GluR2 subunit itself is sufficient to induce spine growth and maturation. On the other hand, NR2B subunit appears to play important role in LTD because NR2B-selective antagonists abolish LTD *(112)* and leads to increased presence of GluR1 AMPA receptor subunits at the cell surface, whereas its overexpression results in removal of GluR1 from the cell surface *(113)*. There are two more candidates (orthologs of human *ASH2L* and *SP-1-like protein*) that might be involved in executing mTOR effects on synapse formation in *Drosophila (16)*. Both of them encode for transcription factors and their expression was upregulated in the presence of rapamycin (*16*).

There are still no known proteins translated in a mTOR-dependent fashion that are proven to regulate dendritic branching. However, several candidate proteins that are known to be locally translated into dendrites and are likely involved in dendrite development include CaMKIIα, MAP2, FMRP, BDNF, and AMPA receptor subunits.

All of the aforementioned examples account for only the rapamycin-dependent functions of mTOR. However, it is now believed that the mTOR-Rictor complex can also regulate actin polymerization independently of protein translation *(31)*. Given the importance of actin cytoskeleton for neuronal morphogenesis, the mTOR-Rictor pathway could also contribute to axon growth *(114)*, dendritic branching *(115)*, and spine formation *(116)*.

Conclusion

This article presented recently acquired knowledge showing that the role of mTOR in neurons goes much beyond simple control of cell growth. However, we still need to learn more about the complexity of the signaling cascades that involve mTOR, as well as about their functional significance in neuronal physiology. In the postgenomic era, one can hope that the powerful methods for analysis of whole transcriptomes

and proteomes will soon allow a more complete picture of mTOR regulation and function. Once we define mTOR signaling pathways in neurons, we will need to learn when, where, and which of mTOR-regulated proteins are activated during particular phases of neuronal development and in different types of synaptic plasticity. This information will also yield a better understanding of the molecular events that underlie some aspects of neurodegenerative disorders *(117,118)* as well as mTOR-related human multi-organ diseases that affect brain function, such as tuberous sclerosis *(119–122)* and neurofibromatosis *(122,123)*. It can also help extend our knowledge about neurodevelopmental disorders that are linked to disturbance of local protein synthesis, such as Fragile X syndrome *(124)*.

Acknowledgments

M. Sheng is Investigator of the Howard Hughes Medical Institute. Work of J. Jawoski has been financed by Polish Ministry of Science and Higher Education (research grant—2P04A01530). We thank B. Kaminska-Kaczmarek and C.C. Hoogenraad for comments on this manuscript.

References

1. Wu, G. Y., Deisseroth, K., and Tsien, R. W. (2001) Spaced stimuli stabilize MAPK pathway activation and its effects on dendritic morphology. *Nat. Neurosci.* **4**, 151–158.
2. Adams, J. P., and Sweatt, J. D. (2002) Molecular psychology: roles for the ERK MAP kinase cascade in memory. *Annu. Rev. Pharmacol. Toxicol.* **42**, 135–163.
3. Atwal, J. K., Massie, B., Miller, F. D., and Kaplan, D. R. (2000) The TrkB–Shc site signals neuronal survival and local axon growth via MEK and P13-kinase. *Neuron* **27**, 265–277.
4. Sanna, P. P., Cammalleri, M., Berton, F., et al. (2002) Phosphatidylinositol 3-kinase is required for the expression but not for the induction or the maintenance of long-term potentiation in the hippocampal CA1 region. *J. Neurosci.* **22**, 3359–3365.
5. Jaworski, J., Spangler, S., Seeburg, D. P., Hoogenraad, C. C., and Sheng, M. (2005) Control of dendritic arborization by the phosphoinositide-3′-kinase-Akt-mammalian target of rapamycin pathway. *J. Neurosci.* **25**, 11,300–11,312.
6. Kumar, V., Zhang, M. X., Swank, M. W., Kunz, J., and Wu, G. Y. (2005) Regulation of dendritic morphogenesis by Ras–PI3K–Akt–mTOR and Ras–MAPK signaling pathways. *J. Neurosci.* **25**, 11,288–11,299.
7. Fink, C. C., Bayer, K. U., Myers, J. W., Ferrell, J. E., Jr., Schulman, H., and Meyer, T. (2003) Selective regulation of neurite extension and synapse formation by the beta but not the alpha isoform of CaMKII. *Neuron* **39**, 283–297.
8. Jourdain, P., Fukunaga, K., and Muller, D. (2003) Calcium/calmodulin-dependent protein kinase II contributes to activity-dependent filopodia growth and spine formation. *J. Neurosci.* **23**, 10,645–10,649.
9. Colbran, R. J., and Brown, A. M. (2004) Calcium/calmodulin-dependent protein kinase II and synaptic plasticity. *Curr. Opin. Neurobiol.* **14**, 318–327.
10. Redmond, L., Kashani, A. H., and Ghosh, A. (2002) Calcium regulation of dendritic growth via CaM kinase IV and CREB-mediated transcription. *Neuron* **34**, 999–1010.
11. Wu, G. Y., and Cline, H. T. (1998) Stabilization of dendritic arbor structure in vivo by CaMKII. *Science* **279**, 222–226.
12. Kwon, C. H., Zhu, X., Zhang, J., and Baker, S. J. (2003) mTor is required for hypertrophy of Pten-deficient neuronal soma in vivo. *Proc. Natl. Acad. Sci. USA* **100**, 12,923–12,928.
13. Burnett, P. E., Barrow, R. K., Cohen, N. A., Snyder, S. H., and Sabatini, D. M. (1998) RAFT1 phosphorylation of the translational regulators p70 S6 kinase and 4E–BP1. *Proc. Natl. Acad. Sci. USA* **95**, 1432–1437.
14. Xie, M. W., Jin, F., Hwang, H., et al. (2005) Insights into TOR function and rapamycin response: chemical genomic profiling by using a high-density cell array method. *Proc. Natl. Acad. Sci. USA* **102**, 7215–7220.
15. Chan, T. F., Carvalho, J., Riles, L., and Zheng, X. F. (2000) A chemical genomics approach toward understanding the global functions of the target of rapamycin protein (TOR). *Proc. Natl. Acad. Sci. USA* **97**, 13,227–13,232.

16. Guertin, D. A., Guntur, K. V., Bell, G. W., Thoreen, C. C., and Sabatini, D. M. (2006) Functional genomics identifies TOR-regulated genes that control growth and division. *Curr. Biol.* **16**, 958–970.
17. Jacinto, E., and Hall, M. N. (2003) Tor signalling in bugs, brain and brawn. *Nat. Rev. Mol. Cell Biol.* **4**, 117–126.
18. Sarbassov dos, D., Ali, S. M., and Sabatini, D. M. (2005) Growing roles for the mTOR pathway. *Curr. Opin. Cell Biol.* **17**, 596–603.
19. Schmelzle, T., and Hall, M. N. (2000). TOR, a central controller of cell growth. *Cell* **103**, 253–262.
20. Harris, T. E., and Lawrence, J. C., Jr. (2003) TOR signaling. *Sci. STKE* **2003**, re15.
21. Lenz, G., and Avruch, J. (2005) Glutamatergic regulation of the p70S6 kinase in primary mouse neurons. *J. Biol. Chem.* **280**, 38121–38124.
22. Cammalleri, M., Lutjens, R., Berton, F., et al. (2003) Time-restricted role for dendritic activation of the mTOR–p70S6K pathway in the induction of late-phase long-term potentiation in the CA1. *Proc. Natl. Acad. Sci. USA* **100**, 14,368–14,373.
23. Hou, L., and Klann, E. (2004) Activation of the phosphoinositide 3-kinase-Akt-mammalian target of rapamycin signaling pathway is required for metabotropic glutamate receptor-dependent long-term depression. *J. Neurosci.* **24**, 6352–6361.
24. Garami, A., Zwartkruis, F. J., Nobukuni, T., et al. (2003) Insulin activation of Rheb, a mediator of mTOR/S6K/4E–BP signaling, is inhibited by TSC1 and 2. *Mol. Cell* **11**, 1457–1466.
25. Tee, A. R., Manning, B. D., Roux, P. P., Cantley, L. C., and Blenis, J. (2003) Tuberous sclerosis complex gene products, Tuberin and Hamartin, control mTOR signaling by acting as a GTPase-activating protein complex toward Rheb. *Curr. Biol.* **13**, 1259–1268.
26. Manning, B. D., Tee, A. R., Logsdon, M. N., Blenis, J., and Cantley, L. C. (2002) Identification of the tuberous sclerosis complex-2 tumor suppressor gene product tuberin as a target of the phosphoinositide 3-kinase/akt pathway. *Mol. Cell* **10**, 151–162.
27. Fang, Y., Vilella–Bach, M., Bachmann, R., Flanigan, A., and Chen, J. (2001) Phosphatidic acid-mediated mitogenic activation of mTOR signaling. *Science* **294**, 1942–1945.
28. Tee, A. R., Anjum, R., and Blenis, J. (2003) Inactivation of the tuberous sclerosis complex-1 and -2 gene products occurs by phosphoinositide 3-kinase/Akt-dependent and -independent phosphorylation of tuberin. *J. Biol. Chem.* **278**, 37,288–37,296.
29. Ma, L., Chen, Z., Erdjument–Bromage, H., Tempst, P., and Pandolfi, P. P. (2005) Phosphorylation and functional inactivation of TSC2 by Erk implications for tuberous sclerosis and cancer pathogenesis. *Cell* **121**, 179–193.
30. Kimura, N., Tokunaga, C., Dalal, S., et al. (2003) A possible linkage between AMP–activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR) signalling pathway. *Genes Cells* **8**, 65–79.
31. Sarbassov, D. D., Ali, S. M., Kim, D. H., et al. (2004) Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton. *Curr. Biol* **14**, 1296–1302.
32. Hara, K., Maruki, Y., Long, X., et al. (2002) Raptor, a binding partner of target of rapamycin (TOR), mediates TOR action. *Cell* **110**, 177–189.
33. Kim, D. H., Sarbassov, D. D., Ali, S. et al. (2002) mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. *Cell* **110**, 163–175.
34. Hara, K., Yonezawa, K., Kozlowski, M. T., et al. (1997) Regulation of eIF–4E BP1 phosphorylation by mTOR. *J. Biol. Chem.* **272**, 26,457–26,463.
35. Jefferies, H. B., Fumagalli, S., Dennis, P. B., Reinhard, C., Pearson, R. B., and Thomas, G. (1997) Rapamycin suppresses 5′TOP mRNA translation through inhibition of p70s6k. *EMBO J.* **16**, 3693–3704.
36. Meyuhas, O. (2000) Synthesis of the translational apparatus is regulated at the translational level. *Eur. J. Biochem.* **267**, 6321–6330.
37. Beretta, L., Gingras, A. C., Svitkin, Y. V., Hall, M. N., and Sonenberg, N. (1996) Rapamycin blocks the phosphorylation of 4E–BP1 and inhibits cap-dependent initiation of translation. *EMBO J.* **15**, 658–664.
38. Peterson, R. T., Desai, B. N., Hardwick, J. S., and Schreiber, S. L. (1999) Protein phosphatase 2A interacts with the 70-kDa S6 kinase and is activated by inhibition of FKBP12-rapamycinassociated protein. *Proc. Natl. Acad. Sci. USA* **96**, 4438–4442.
39. Choi, J. H., Bertram, P. G., Drenan, R., Carvalho, J., Zhou, H. H., and Zheng, X. F. (2002) The FKBP12-rapamycin-associated protein (FRAP) is a CLIP-170 kinase. *EMBO Rep.* **3**, 988–994.
40. Redpath, N. T., Foulstone, E. J., and Proud, C. G. (1996) Regulation of translation elongation

factor-2 by insulin via a rapamycin-sensitive signalling pathway. *EMBO J.* **15**, 2291–2297.
41. Azpiazu, I., Saltiel, A. R., DePaoli-Roach, A. A., and Lawrence, J. C. (1996) Regulation of both glycogen synthase and PHAS-I by insulin in rat skeletal muscle involves mitogen-activated protein kinase-independent and rapamycin-sensitive pathways. *J. Biol. Chem.* **271**, 5033–5039.
42. Shepherd, P. R., Nave, B. T., and Siddle, K. (1995) Insulin stimulation of glycogen synthesis and glycogen synthase activity is blocked by wortmannin and rapamycin in 3T3–L1 adipocytes: evidence for the involvement of phosphoinositide 3-kinase and p70 ribosomal protein-S6 kinase. *Biochem. J.* **305 (Pt 1)**, 25–28.
43. Hudson, C. C., Liu, M., Chiang, G. G., et al. (2002) Regulation of hypoxia–inducible factor 1alpha expression and function by the mammalian target of rapamycin. *Mol. Cell Biol.* **22**, 7004–7014.
44. Jacinto, E., Loewith, R., Schmidt, A., et al. (2004) Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. *Nat. Cell Biol.* **6**, 1122–1128.
45. Sarbassov dos, D., Ali, S. M., Sengupta, S., et al. (2006) Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. *Mol. Cell* **22**, 159–168.
46. Bassell, G. J., Zhang, H., Byrd, A. L., et al. (1998) Sorting of beta-actin mRNA and protein to neurites and growth cones in culture. *J. Neurosci.* **18**, 251–265.
47. Martin, K. C. (2004) Local protein synthesis during axon guidance and synaptic plasticity. *Curr. Opin. Neurobiol.* **14**, 305–310.
48. Piper, M., Anderson, R., Dwivedy, A., et al. (2006) Signaling mechanisms underlying Slit2-induced collapse of *Xenopus* retinal growth cones. *Neuron* **49**, 215–228.
49. Piper, M., and Holt, C. (2004) RNA translation in axons. *Annu. Rev. Cell Dev. Biol.* **20**, 505–523.
50. Campbell, D. S., and Holt, C. E. (2001) Chemotropic responses of retinal growth cones mediated by rapid local protein synthesis and degradation. *Neuron* **32**, 1013–1026.
51. Abe, H., Obinata, T., Minamide, L. S., and Bamburg, J. R. (1996) Xenopus laevis actin-depolymerizing factor/cofilin: a phosphorylation-regulated protein essential for development. *J. Cell Biol.* **132**, 871–885.
52. Willis, D. E., and Twiss, J. L. (2006) The evolving roles of axonally synthesized proteins in regeneration. *Curr. Opin. Neurobiol.* **16**, 111–118.
53. Verma, P., Chierzi, S., Codd, A. M., et al. (2005) Axonal protein synthesis and degradation are necessary for efficient growth cone regeneration. *J. Neurosci.* **25**, 331–342.
54. Selzer, M. E. (2003) Promotion of axonal regeneration in the injured CNS. *Lancet Neurol.* **2**, 157–166.
55. Chuckowree, J. A., Dickson, T. C., and Vickers, J. C. (2004) Intrinsic regenerative ability of mature CNS neurons. *Neuroscientist* **10**, 280–285.
56. Wessells, N. K., Johnson, S. R., and Nuttall, R. P. (1978) Axon initiation and growth cone regeneration in cultured motor neurons. *Exp. Cell Res.* **117**, 335–345.
57. Blackmore, M., and Letourneau, P. C. (2006) Changes within maturing neurons limit axonal regeneration in the developing spinal cord. *J. Neurobiol.* **66**, 348–360.
58. Sahly, I., Khoutorsky, A., Erez, H., Prager-Khoutorsky, M., and Spira, M. E. (2006) Online confocal imaging of the events leading to structural dedifferentiation of an axonal segment into a growth cone after axotomy. *J. Comp. Neurol.* **494**, 705–720.
59. Jan, Y. N., and Jan, L. Y. (2003) The control of dendrite development. *Neuron* **40**, 229–242.
60. McAllister, A. K. (2000) Cellular and molecular mechanisms of dendrite growth. *Cereb. Cortex* **10**, 963–973.
61. Wong, R. O., and Ghosh, A. (2002) Activity-dependent regulation of dendritic growth and patterning. *Nat. Rev. Neurosci.* **3**, 803–812.
62. Miller, F. D., and Kaplan, D. R. (2003) Signaling mechanisms underlying dendrite formation. *Curr. Opin. Neurobiol.* **13**, 391–398.
63. Wirth, M. J., Brun, A., Grabert, J., Patz, S., and Wahle, P. (2003) Accelerated dendritic development of rat cortical pyramidal cells and interneurons after biolistic transfection with BDNF and NT4/5. *Development* **130**, 5827–5838.
64. Whitford, K. L., Marillat, V., Stein, E., Goodman, C. S., Tessier-Lavigne, M., Chedotal, A., and Ghosh, A. (2002) Regulation of cortical dendrite development by Slit-Robo interactions. *Neuron* **33**, 47–61.
65. Polleux, F., Morrow, T., and Ghosh, A. (2000) Semaphorin 3A is a chemoattractant for cortical apical dendrites. *Nature* **404**, 567–573.
66. Horch, H. W., and Katz, L. C. (2002) BDNF release from single cells elicits local dendritic growth in nearby neurons. *Nat. Neurosci.* **5**, 1177–1184.

67. Yu, X., and Malenka, R. C. (2003) Beta-catenin is critical for dendritic morphogenesis. *Nat. Neurosci.* **6**, 1169–1177.
68. Redmond, L., Oh, S. R., Hicks, C., Weinmaster, G., and Ghosh, A. (2000) Nuclear Notch1 signaling and the regulation of dendritic development. *Nat. Neurosci.* **3**, 30–40.
69. Lohmann, C., Myhr, K. L., and Wong, R. O. (2002) Transmitter-evoked local calcium release stabilizes developing dendrites. *Nature* **418**, 177–181.
70. Bjorkblom, B., Ostman, N., Hongisto, V., et al. (2005) Constitutively active cytoplasmic c-Jun N-terminal kinase 1 is a dominant regulator of dendritic architecture: role of microtubule-associated protein 2 as an effector. *J. Neurosci.* **25**, 6350–6361.
71. Wayman, G. A., Impey, S., Marks, D., et al. (2006) Activity-dependent dendritic arborization mediated by CaM-kinase I activation and enhanced CREB-dependent transcription of Wnt-2. *Neuron* **50**, 897–909.
72. Nakayama, A. Y., Harms, M. B., and Luo, L. (2000) Small GTPases Rac and Rho in the maintenance of dendritic spines and branches in hippocampal pyramidal neurons. *J. Neurosci.* **20**, 5329–5338.
73. Hayashi, K., Ohshima, T., and Mikoshiba, K. (2002) Pak1 is involved in dendrite initiation as a downstream effector of Rac1 in cortical neurons. *Mol. Cell Neurosci.* **20**, 579–594.
74. Sholl, D. A. (1953) Dendritic organization in the neurons of the visual and motor cortices of the cat. *J. Anat.* **87**, 387–406.
75. Kwon, C. H., Luikart, B. W., Powell, C. M., et al. (2006) Pten regulates neuronal arborization and social interaction in mice. *Neuron* **50**, 377–388.
76. Dijkhuizen, P. A., and Ghosh, A. (2005) BDNF regulates primary dendrite formation in cortical neurons via the PI3-kinase and MAP kinase signaling pathways. *J. Neurobiol.* **62**, 278–288.
77. Hering, H., and Sheng, M. (2001) Dendritic spines: structure, dynamics and regulation. *Nat. Rev. Neurosci.* **2**, 880–888.
78. Yuste, R., and Bonhoeffer, T. (2004) Genesis of dendritic spines: insights from ultrastructural and imaging studies. *Nat. Rev. Neurosci.* **5**, 24–34.
79. Tada, T., and Sheng, M. (2006) Molecular mechanisms of dendritic spine morphogenesis. *Curr. Opin. Neurobiol.* **16**, 95–101.
80. Casadio, A., Martin, K. C., Giustetto, M., et al. (1999) A transient, neuron-wide form of CREB-mediated long-term facilitation can be stabilized at specific synapses by local protein synthesis. *Cell* **99**, 221–237.
81. Tavazoie, S. F., Alvarez, V. A., Ridenour, D. A., Kwiatkowski, D. J., and Sabatini, B. L. (2005) Regulation of neuronal morphology and function by the tumor suppressors Tsc1 and Tsc2. *Nat. Neurosci.* **8**, 1727–1734.
82. Kelleher, R. J., 3rd, Govindarajan, A., and Tonegawa, S. (2004) Translational regulatory mechanisms in persistent forms of synaptic plasticity. *Neuron* **44**, 59–73.
83. Squire, L. R., and Davis, H. P. (1981) The pharmacology of memory: a neurobiological perspective. *Annu. Rev. Pharmacol. Toxicol.* **21**, 323–356.
84. Beaumont, V., Zhong, N., Fletcher, R., Froemke, R. C., and Zucker, R. S. (2001) Phosphorylation and local presynaptic protein synthesis in calcium- and calcineurin-dependent induction of crayfish long-term facilitation. *Neuron* **32**, 489–501.
85. Tang, S. J., Reis, G., Kang, H., Gingras, A. C., Sonenberg, N., and Schuman, E. M. (2002) A rapamycin-sensitive signaling pathway contributes to long-term synaptic plasticity in the hippocampus. *Proc. Natl. Acad. Sci. USA* **99**, 467–472.
86. Vickers, C. A., Dickson, K. S., and Wyllie, D. J. (2005) Induction and maintenance of late-phase long-term potentiation in isolated dendrites of rat hippocampal CA1 pyramidal neurones. *J. Physiol.* **568**, 803–813.
87. Cracco, J. B., Serrano, P., Moskowitz, S. I., Bergold, P. J., and Sacktor, T. C. (2005) Protein synthesis–dependent LTP in isolated dendrites of CA1 pyramidal cells. *Hippocampus* **15**, 551–556.
88. Wang, Y., Barbaro, M. F., and Baraban, S. C. (2006) A role for the mTOR pathway in surface expression of AMPA receptors. *Neurosci. Lett.* **401**, 35–39.
89. Job, C., and Eberwine, J. (2001) Identification of sites for exponential translation in living dendrites. *Proc. Natl. Acad. Sci. USA* **98**, 13037–13042.
90. Huber, K. M., Roder, J. C., and Bear, M. F. (2001) Chemical induction of mGluR5- and protein synthesis-dependent long-term depression in hippocampal area CA1. *J. Neurophysiol.* **86**, 321–325.
91. Banko, J. L., Hou, L., Poulin, F., Sonenberg, N., and Klann, E. (2006) Regulation of eukaryotic

initiation factor 4E by converging signaling pathways during metabotropic glutamate receptor-dependent long-term depression. *J. Neurosci.* **26**, 2167–2173.
92. Banko, J. L., Poulin, F., Hou, L., DeMaria, C. T., Sonenberg, N., and Klann, E. (2005) The translation repressor 4E–BP2 is critical for eIF4F complex formation, synaptic plasticity, and memory in the hippocampus. *J. Neurosci.* **25**, 9581–9590.
93. Zho, W. M., You, J. L., Huang, C. C., and Hsu, K. S. (2002) The group I metabotropic glutamate receptor agonist (S)-3,5-dihydroxyphenylglycine induces a novel form of depotentiation in the CA1 region of the hippocampus. *J. Neurosci.* **22**, 8838–8849.
94. Malenka, R. C. (1994) Synaptic plasticity in the hippocampus: LTP and LTD. *Cell* **78**, 535–538.
95. Tischmeyer, W., Schicknick, H., Kraus, M., et al. (2003) Rapamycin-sensitive signalling in long-term consolidation of auditory cortex-dependent memory. *Eur. J. Neurosci.* **18**, 942–950.
96. Lee, C. C., Huang, C. C., Wu, M. Y., and Hsu, K. S. (2005) Insulin stimulates postsynaptic density-95 protein translation via the phosphoinositide 3-kinase-Akt-mammalian target of rapamycin signaling pathway. *J. Biol. Chem.* **280**, 18543–18550.
97. Takei, N., Inamura, N., Kawamura, M., et al. (2004) Brain-derived neurotrophic factor induces mammalian target of rapamycin-dependent local activation of translation machinery and protein synthesis in neuronal dendrites. *J. Neurosci.* **24**, 9760–9769.
98. Kraut, R., Menon, K., and Zinn, K. (2001) A gain-of-function screen for genes controlling motor axon guidance and synaptogenesis in *Drosophila*. *Curr. Biol.* **11**, 417–430.
99. Schratt, G. M., Nigh, E. A., Chen, W. G., Hu, L., and Greenberg, M. E. (2004) BDNF regulates the translation of a select group of mRNAs by a mammalian target of rapamycin-phosphatidylinositol 3-kinase-dependent pathway during neuronal development. *J. Neurosci.* **24**, 7366–7377.
100. Schratt, G. M., Tuebing, F., Nigh, E. A., et al. (2006) A brain-specific microRNA regulates dendritic spine development. *Nature* **439**, 283–289.
101. Yang, N., Higuchi, O., Ohashi, K., et al. (1998) Cofilin phosphorylation by LIM-kinase 1 and its role in Rac-mediated actin reorganization. *Nature* **393**, 809–812.
102. Meng, Y., Zhang, Y., Tregoubov, V., et al. (2002) Abnormal spine morphology and enhanced LTP in LIMK-1 knockout mice. *Neuron* **35**, 121–133.
103. Zhou, Q., Homma, K. J., and Poo, M. M. (2004) Shrinkage of dendritic spines associated with long-term depression of hippocampal synapses. *Neuron* **44**, 749–757.
104. Guzowski, J. F., Lyford, G. L., Stevenson, G. D., et al. (2000) Inhibition of activity-dependent arc protein expression in the rat hippocampus impairs the maintenance of long-term potentiation and the consolidation of long-term memory. *J. Neurosci.* **20**, 3993–4001.
105. Vazquez, L. E., Chen, H. J., Sokolova, I., Knuesel, I., and Kennedy, M. B. (2004) SynGAP regulates spine formation. *J. Neurosci.* **24**, 8862–8872.
106. Akama, K. T., and McEwen, B. S. (2003) Estrogen stimulates postsynaptic density-95 rapid protein synthesis via the Akt/protein kinase B pathway. *J. Neurosci.* **23**, 2333–2339.
107. El-Husseini, A. E., Schnell, E., Chetkovich, D. M., Nicoll, R. A., and Bredt, D. S. (2000) PSD-95 involvement in maturation of excitatory synapses. *Science* **290**, 1364–1368.
108. Fagiolini, M., Katagiri, H., Miyamoto, H., et al. (2003) Separable features of visual cortical plasticity revealed by N-methyl-D-aspartate receptor 2A signaling. *Proc. Natl. Acad. Sci. USA* **100**, 2854–2859.
109. Yao, W. D., Gainetdinov, R. R., Arbuckle, M. I., et al. (2004) Identification of PSD-95 as a regulator of dopamine-mediated synaptic and behavioral plasticity. *Neuron* **41**, 625–638.
110. Onda, H., Crino, P. B., Zhang, H., et al. (2002) Tsc2 null murine neuroepithelial cells are a model for human tuber giant cells, and show activation of an mTOR pathway. *Mol. Cell Neurosci.* **21**, 561–574.
111. Passafaro, M., Nakagawa, T., Sala, C., and Sheng, M. (2003) Induction of dendritic spines by an extracellular domain of AMPA receptor subunit GluR2. *Nature* **424**, 677–681.
112. Liu, L., Wong, T. P., Pozza, M. F., et al. (2004) Role of NMDA receptor subtypes in governing the direction of hippocampal synaptic plasticity. *Science* **304**, 1021–1024.
113. Kim, M. J., Dunah, A. W., Wang, Y. T., and Sheng, M. (2005) Differential roles of NR2A- and NR2B-containing NMDA receptors in Ras–ERK signaling and AMPA receptor trafficking. *Neuron* **46**, 745–760.
114. Dent, E. W., and Gertler, F. B. (2003) Cytoskeletal dynamics and transport in growth cone

motility and axon guidance. *Neuron* **40**, 209–227.
115. Luo, L. (2002) Actin cytoskeleton regulation in neuronal morphogenesis and structural plasticity. *Annu. Rev. Cell Dev. Biol.* **18**, 601–635.
116. Matus, A. (2000) Actin-based plasticity in dendritic spines. *Science* **290**, 754–758.
117. Khurana, V., Lu, Y., Steinhilb, M. L., Oldham, S., Shulman, J. M., and Feany, M. B. (2006) TOR-mediated cell-cycle activation causes neurodegeneration in a *Drosophila* tauopathy model. *Curr. Biol.* **16**, 230–241.
118. Ravikumar, B., Vacher, C., Berger, Z., et al. (2004) Inhibition of mTOR induces autophagy and reduces toxicity of polyglutamine expansions in fly and mouse models of Huntington disease. *Nat. Genet.* **36**, 585–595.
119. Kwiatkowski, D. J. (2003) Rhebbing up mTOR: new insights on TSC1 and TSC2, and the pathogenesis of tuberous sclerosis. *Cancer Biol. Ther.* **2**, 471–476.
120. Jozwiak, J., and Jozwiak, S. (2005) Giant cells: contradiction to two-hit model of tuber formation? *Cell Mol. Neurobiol.* **25**, 795–805.
121. Kwiatkowski, D. J. (2003) Tuberous sclerosis: from tubers to mTOR. *Ann. Hum. Genet.* **67**, 87–96.
122. Inoki, K., Corradetti, M. N., and Guan, K. L. (2005) Dysregulation of the TSC-mTOR pathway in human disease. *Nat. Genet.* **37**, 19–24.
123. Johannessen, C. M., Reczek, E. E., James, M. F., Brems, H., Legius, E., and Cichowski, K. (2005) The NF1 tumor suppressor critically regulates TSC2 and mTOR. *Proc. Natl. Acad. Sci. USA* **102**, 8573–8578.
124. Antar L. N., and Bassell G. J. (2003) Sunrise at the synapse: the FMRP mRNP shaping the synaptic interface. *Neuron* **37**, 555–558.