MoniTORing neuronal excitability at the synapse

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Mammalian target of rapamycin (mTOR) is a key player at the synapse regulating local translation and long-lasting synaptic plasticity. Now, a new study by Sosanya et al. (2013. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb .201212089) investigates the molecular mechanism of how mTOR suppresses local protein synthesis of a key potassium channel at activated synapses.

Ever since its discovery (Sabatini et al., 1994), the mammalian target of the FK506 binding protein (FKBP12)–rapamycin complex (mammalian target of rapamycin [mTOR]) has been shown to be a critical regulator of growth in many species. It is a ubiquitously expressed, highly conserved serine/threonine kinase with many important functions in the cell. In the brain, mTOR has recently been shown to contribute to synaptic plasticity, which is thought to form the molecular basis of learning and memory (Hoeffer and Klann, 2010). At the synapse, mTOR couples the *N*-methyl-D-aspartate glutamate receptor (NMDAR) to the local translation machinery, thereby inducing long-lasting synaptic changes. The NMDAR is one specific subtype of glutamate receptors that—once coactivated by two ligands—activates critical intracellular signaling cascades through calcium influx. Two important downstream targets at the synapse are eIF4E-binding proteins and S6 kinase, both of which are critical regulators of translation, thereby providing direct links between the NMDAR, mTOR, and the nearby translation machinery. Furthermore, there is a good indication that mTOR also regulates specific RNA-binding proteins (RBPs) that are components of neuronal RNA granules, e.g., FMRP (Narayanan et al., 2008), involved in dendritic localization of a specific set of transcripts. This would allow regulation of specific subsets of mRNAs at the synapse. Another line of research suggests that mTOR regulates new protein synthesis of dendritically localized transcripts, e.g., *PSD95*, *Kv1.1*, and *CaMKIIα* (*calcium/calmodulin-dependent protein kinase α*; Lee et al., 2005; Gong et al., 2006; Raab-Graham et al., 2006). Finally, there is growing evidence indicating that dysregulation of mTOR may cause human diseases, including cancer, diabetes, and neurodevelopmental, neurodegenerative, or neuropsychiatric disorders, e.g., autism and fragile X syndrome (Hoeffer and Klann, 2010).

In previous work, Raab-Graham et al. (2006) found axonal potassium channels to be another important neuronal target of mTOR regulation. Potassium channels are evolutionarily highly conserved and constitute a very complex class of voltage-gated ion channels playing a key role in controlling neuronal excitability (Hille, 1975). The Kv1 class is 1 of 12 known channel subfamilies, representing the mammalian homologues of the Shaker channel originally described in *Drosophila melanogaster* (Jan et al., 1977). Kv1.1-deficient mice die early of spontaneous seizures. In the central nervous system (CNS), the Kv1.1 member is almost exclusively localized in axons; however, it is also found in the somatodendritic region of some CNS neurons (see Raab-Graham et al. [2006] for details).

Previously, Raab-Graham et al. (2006) reported that rapamycin increased dendritic, but not axonal, expression of Kv1.1 in rat hippocampus. Rapamycin, an immunosuppressant that binds to FKBP12, inhibits the mTOR pathway by directly binding the mTOR complex 1 (Hoeffer and Klann, 2010). When they investigated the underlying mechanism of this increased expression, they found that Kv1.1 mRNA is dendritically localized, and—upon treatment with rapamycin or phosphoinositide-3 kinase inhibitors—newly synthesized Kv1.1 protein could be detected using a clever local translation assay based on Kaede, a photoconvertible fluorescent protein (Chudakov et al., 2010).

Historically, there have been a series of experimental approaches to study local protein synthesis in axonal growth cones and/or in neuronal dendrites (Besse and Ephrussi, 2008), including Kaede or Dendra2 (Chudakov et al., 2010). Local application of UV light in dendrites converts all existing Kaede proteins to red, therefore enabling the visualization of new protein synthesis in green. This allowed for the first time to distinguish local protein synthesis in growth cones (Leung et al., 2006) and in mature dendrites (Raab-Graham et al., 2006) of cultured neurons. A similar approach has been used by Wang et al. (2009) using the monomeric Dendra2 in *Aplysia* sensory–motor synapses.

In their original study, Raab-Graham et al. (2006) generated a Sindbis virus containing Kaede as well as the coding region and the 3′-UTR of Kv1.1. They showed that inhibition of the NMDAR increased dendritic Kv1.1 expression, suggesting that synaptic activation may cause local suppression of dendritic Kv1 channels by reducing their local synthesis at the activated synapse (Fig. 1). This may sound counterintuitive at first glance, but the authors proposed an interesting hypothesis: what if inhibition of local Kv1.1 protein synthesis constitutes a positive feedback mechanism rendering the dendrite more excitable?

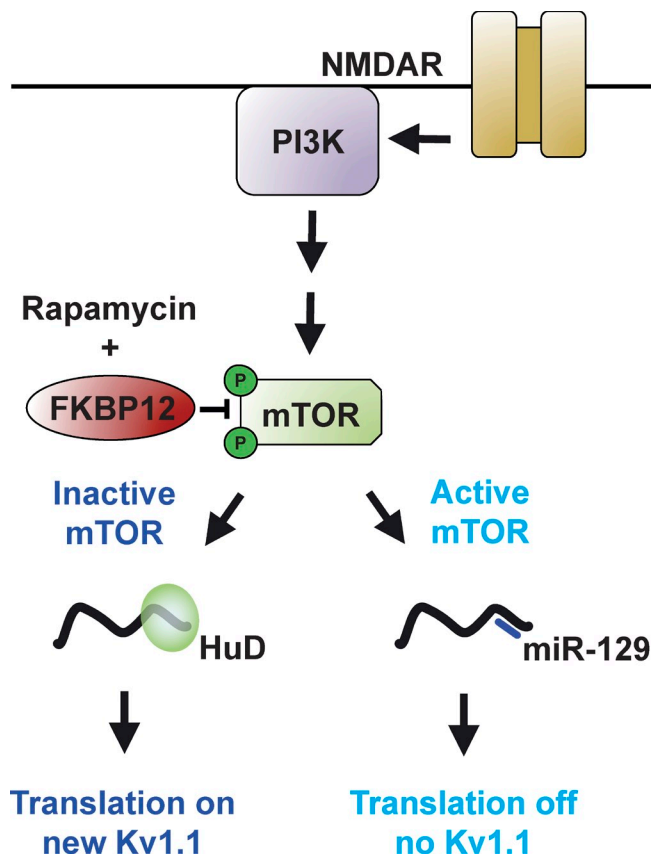Correspondence to Michael A. Kiebler: michael.kiebler@med.uni-muenchen.de

Figure 1. **mTOR-mediated translational control of the Kv1.1 potassium channel at the synapse.** This cartoon summarizes a hypothetical model based on the work by Sosanya et al. (2013) linking neuronal receptors, e.g., the NMDAR, with both intracellular mTOR signaling as well as translational control at the synapse (Hoeffer and Klann, 2010). When mTOR is active (right), miR-129 binds to Kv1.1 mRNA, repressing its translation at the synapse. When mTOR is inactivated by adding the mTOR inhibitor rapamycin or by inhibiting the NMDAR (left), HuD displaces miR-129 to relieve translational repression of Kv1.1.

A paper in this issue of *JCB* (Sosanya et al., 2013) takes a significant step forward toward a more complete understanding of how translational repression at the active synapse might be achieved. Obviously, many possibilities exist in cells: translation might be regulated by classical translational regulators, or alternatively, miRNAs might be involved in this process. As a competition assay expressing excess 3′-UTR of Kv1.1 yielded a fivefold increase in its surface expression, the authors analyzed this 3′-UTR and identified a conserved binding site for miR-129. Knockdown of miR-129 using a locked nucleic acid probe consequently relieved translational repression. Next, the authors asked the question whether overexpression of an miR-129 precursor would mimic repression of Kv1.1 by rapamycin (Raab-Graham et al., 2006). Rewardingly, it did mimic, demonstrating that it is miR-129 binding to Kv1.1 mRNA that represses its translation when mTOR is active.

The authors then went on to investigate a very important question: how does synaptic activity relieve translational repression (Fig. 1)? Recent work in the miRNA field showed that investigating only a given miRNA and its target transcript in cells might be too simplistic. At least one additional player has to be considered: RBPs, e.g., HuD, HuR, Pumilio2, GW182, and

Ago, which all regulate miRNA binding and subsequent function (Kundu et al., 2012). As Kv1.1 mRNA contains three putative HuD binding sites in its coding region, this led the authors to test whether overexpressed HuD in the presence of active mTOR would overcome miR-129–mediated repression of Kv1.1 mRNA. Consequently, HuD overexpression increased dendritic Kv1.1 expression by almost fourfold, indicating that HuD actually promotes translation.

In a final set of experiments, the authors investigated how HuD operates. Interestingly, overexpression of a high affinity HuD target, coding for the CaMKIIα, prevented the relief of translational repression by HuD. These findings led the authors to propose that HuD might switch from high affinity targets, e.g., CaMKIIα (under conditions in which mTOR is active), to low affinity targets, e.g., Kv1.1 mRNA, at the inactive synapse. This is possible as mTOR inhibition appears to cause degradation of high affinity targets, thereby making HuD available to now bind to Kv1.1 mRNA.

The resulting working model based on the work from Sosanya et al. (2013) is depicted in Fig. 1. A first and important lesson is that translational repression is not generally relieved at the activated synapse but that this may occur in a transcript-specific manner. In the current study, the authors report miR-129–dependent repression of Kv1.1 mRNA. However, other groups have previously reported that active mTOR stimulates dendritic protein synthesis of *PSD95* and *CaMKIIα* (Lee et al., 2005; Gong et al., 2006). A second important aspect of this work is the key role of HuD displacing miRNA-129 to relieve translational repression of Kv1.1 mRNA, which is in line with similar roles of HuR, Pumilio2, GW182, or Ago. A third and novel finding is that mTOR inhibition appears to cause degradation of high affinity targets; however, the precise way of how this occurs is currently unknown. We anticipate that the near future will shed significantly new light on the underlying molecular mechanisms of how a given synapse can be functionally and structurally remodeled in an experience-dependent manner.

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