

Mechanistic insights into the role of mTOR signaling in neuronal differentiation

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Temporal control of neuronal differentiation is critical to produce a complete and fully functional nervous system. Loss of the precise temporal control of neuronal cell fate can lead to defects in cognitive development and to disorders such as epilepsy and autism. Mechanistic target of rapamycin (mTOR) is a large serine/threonine kinase that acts as a crucial sensor of cellular homeostasis. mTOR signaling has recently emerged as a key regulator of neurogenesis. However, the mechanism by which mTOR regulates neurogenesis is poorly understood. In contrast to other functions of the pathway, 'neurogenic mTOR pathway factors' have not previously been identified. We have very recently used *Drosophila* as a model system to identify the gene *unkempt* as the first component of the mTOR pathway regulating neuronal differentiation. Our study demonstrates that specific adaptor proteins exist that channel mTOR signaling toward the regulation of neuronal cell fate. In this Commentary we discuss the role of mTOR signaling in neurogenesis and the significance of these findings in advancing our understanding of the mechanism by which mTOR signaling controls neuronal differentiation.

Keywords: *Drosophila*, D-Pax2, eye development, headcase, mTOR, neurogenesis, neuronal differentiation, photoreceptor, *unkempt*

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differentiation of neurons. This knowledge will also lead to breakthroughs in the understanding and treatment of neurological disease.

Mechanistic target of rapamycin (mTOR) is a large serine/threonine kinase that forms 2 complexes (mTORC1 and mTORC2) that are highly conserved and regulated by upstream signals including insulin receptor (InR)/insulin like growth factor signaling, cytosolic ATP, oxygen and amino acids.¹ mTOR was originally identified in budding yeast in a genetic screen for mutations conferring resistance to the immunosuppressive macrolide rapamycin.² mTOR was subsequently shown to be a key regulator of growth control in yeast, *Drosophila* and mammals.²⁹ The potency of the mTOR pathway as a regulator of growth comes from the direct control of translation and hence protein synthesis. In recent years mTOR signaling has been shown to control a number of additional fundamental cellular processes including energy metabolism, autophagy, transcription, cytoskeletal dynamics and lipid synthesis.⁴

Using the developing *Drosophila* eye as a model system, we were the first to show that the InR/mTOR pathway is a key regulator of neuronal differentiation.⁵⁻⁷ In this context the mTOR pathway temporally regulates the differentiation of 3 of the 8 photoreceptors and the cone cells that constitute each ommatidium (facet) of the fly eye (Fig. 1). mTOR signaling has subsequently been shown to play key roles in the genesis, migration and differentiation of neural progenitor cells in the mammalian nervous system.⁸⁻¹³ Inhibition of mTOR signaling in *Disc1* mutant mice, a mutation associated with psychiatric illness in humans, ameliorated the

The mature human brain contains around 100 billion neurons that are generated, migrate and differentiate during prenatal development. Defects in neurogenesis lead to epilepsy, autism and other common neurological disorders. To elucidate the complexity of neurogenesis we need to identify key genes and molecules that regulate the generation and

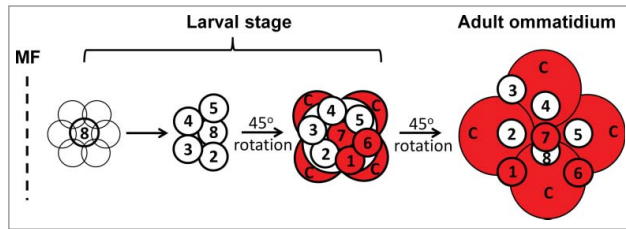


Figure 1. InR/mTOR signaling controls the timing of differentiation of photoreceptors 1,6,7 and cone cells. Differentiation of the 8 photoreceptor neurons and 4 non-neuronal cone cells (C) in the developing *Drosophila* eye begins with the differentiation of photoreceptor 8 posterior to the morphogenetic furrow (MF), followed by photoreceptors 2,5 and photoreceptors 3,4, then subsequently photoreceptors 1,6,7 and cone cells. InR/mTOR signaling, *unk* and *hdc* specifically regulates the timing of differentiation of photoreceptors 1,6,7 and cone cells (red). Anterior is to the left, dorsal is up.

behavioral phenotypes in this model.¹⁴ Furthermore, activation of mTOR signaling partially rescued the neuronal cell death phenotype caused by loss of the apical complex protein Pals1.¹⁵ The dominant genetic disorder Tuberous Sclerosis Complex (TSC), whose features include epilepsy and autism, is caused by hyperactivation of the mTOR pathway.¹⁶ mTOR signaling has also been shown to be activated in animal models of epilepsy and in human cortical dysplasia.¹⁷⁻¹⁹

mTOR signaling clearly plays a key role in neural development and neurological disease but, in contrast to other functions of this pathway, the mechanism by which mTOR regulates neurogenesis is

unclear. Moreover, since none of the previously identified downstream effectors of mTOR have direct neurogenic roles, it cannot be assumed a priori that there is a specific mTOR pathway neurogenic mechanism. It is conceivable that the ability of mTOR signaling to regulate neurogenesis may be an indirect consequence of its ability to modulate protein synthesis, for example.

With these questions in mind we employed a genetic screen in *Drosophila* to identify putative downstream components of the mTOR pathway that regulate photoreceptor differentiation.

Through this screen we recently identified the gene *unkempt* (*unk*) as a novel

negative regulator of photoreceptor differentiation acting downstream of mTORC1 (Fig. 2).²⁰ Unk is a conserved zinc finger/RING domain protein (Fig. 3), originally identified in *Drosophila*,²¹ whose function in mammals is unknown (but see below). Unk acts together with its binding partner headcase (Hdc) to negatively regulate the differentiation of 3 of the 8 photoreceptors in the developing ommatidium, as well as non-neuronal cone cells (Fig. 1). Interestingly, neither Unk nor Hdc regulate growth and so the Unk/Hdc complex represents the point of divergence in the mTOR pathway where neuronal differentiation is uncoupled from growth control (Fig. 2).

Our work also suggests that the ability of Unk to negatively regulate cell fate comes, in part, from its interaction with the transcription factor D-Pax2. D-Pax2 is required for cone cell differentiation during *Drosophila* eye development.²² Unk physically interacts with D-Pax2 and negatively regulates D-Pax2 protein levels in vivo. Unk therefore directly connects mTOR signaling to a known transcriptional regulator of cell fate (Fig. 2). D-Pax2 regulates the differentiation of cone cells, but Unk and mTOR signaling also control the timing of differentiation of photoreceptors 1,6 and 7 (Fig. 1), so there must be additional factors that are regulated by Unk in these cells (Fig. 2).

Although the identity and phenotype of Unk as a critical regulator of neuronal differentiation acting downstream of mTORC1 is now established, several key mechanistic questions about the regulation and function of Unk remain: 1. What is the mechanism by which mTOR regulates Unk activity? 2. How does Unk control the levels of interacting proteins such as D-Pax2? Recently published studies have provided tantalizing clues to these questions. A quantitative affinity purification/mass spectrometry study of the InR/mTOR interaction proteome in *Drosophila* Kc167 cells found that Unk physically interacts with the mTORC1 components mTOR, regulatory-associated protein of mTOR (raptor), Sin1 and Lst8, as well as the mTOR substrate Thor (4E-BP).²³ Moreover, the interaction between mTOR, raptor, Lst8, Thor and Unk was increased upon activation of the InR

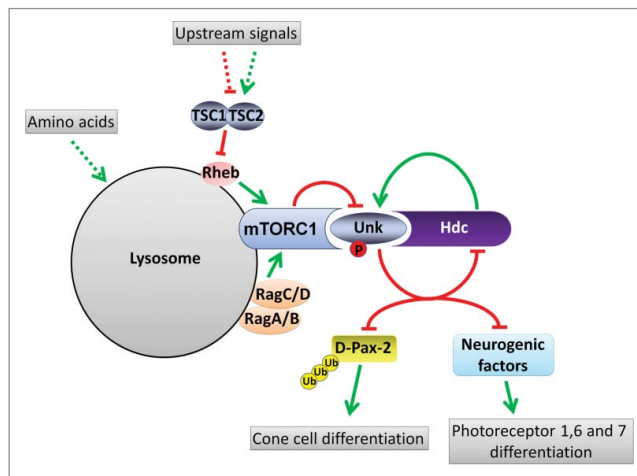


Figure 2. A model for the regulation of neuronal differentiation by mTOR signaling and Unk. Upstream signals regulate the TSC complex which acts to negatively regulate the small GTPase Rheb. When mTOR signaling is active the mTORC1 complex is recruited to the lysosome by the Rag proteins. We hypothesize that mTORC1 physically interacts with the Unk/Hdc complex and negatively regulates its activity by phosphorylating Unk. Unk/Hdc then negatively regulate D-Pax2 in cone cells and putative neurogenic factors in photoreceptors 1,6,7 to control their differentiation.

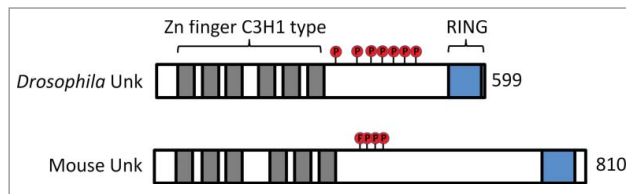


Figure 3. A schematic of the primary structure of *Drosophila* and mouse Unk proteins. See text for details.

pathway. This study strongly suggests that Unk, at least in this context, is a component of the mTORC1 complex. Unk has also been shown to be phosphorylated. A study that used pooled extracts from insulin stimulated and rapamycin treated *Drosophila* Kc167 cells identified at least 7 phosphorylated serine residues in the C-terminal region between the zinc finger and RING domains of Unk (Fig. 3).²⁴ It is tempting to speculate that these serines may be substrates for mTOR. In support of this idea several of the phospho-serine residues identified in Unk have proline or leucine at the +1 position, consistent with the unique preference for mTOR to phosphorylate residues with proline, hydrophobic and aromatic residues at the +1 position.²⁵ Moreover, mammalian Unk is also potentially phosphorylated by mTORC1 signaling (Fig. 3).^{25,26}

Loss of *unk* causes an increase in the levels of Hdc and D-Pax-2 proteins in the *Drosophila* developing eye.²⁰ Unk contains a conserved RING finger domain, characteristic of proteins involved in ubiquitin-dependent protein degradation. In accordance with this Unk has been shown to be part of a complex in human cells containing several ubiquitin conjugating enzymes.²⁷ Moreover, studies in mammalian cells have demonstrated that Unk binds to and promotes the ubiquitination of the chromatin remodelling factor BAF60b.²⁸ By analogy, we hypothesize that *Drosophila* Unk, through direct protein-protein interaction, promotes the ubiquitination and degradation of D-Pax2 and potentially other neurogenic factors (Fig. 2).

Mammalian Unk has very recently been shown to be a sequence-specific RNA binding protein.²⁹ Unk binds several hundred different mRNA species, through the recognition of a specific motif

by the zinc finger domain, and negatively regulates their translation. Unk is strongly expressed in the developing mammalian CNS and knock-down of Unk causes defects in neuronal morphology and migration. Moreover, the RNA binding function of Unk is required for the regulation of cell morphology. This study demonstrates that Unk plays a key role in regulating cell morphology and maturation during mammalian neuronal development. Whether this novel function of Unk is regulated by mTOR signaling in mammals is not known.

In conclusion, our study demonstrates that mTOR signaling is coupled to specific adaptor proteins that channel its activity toward regulating neuronal differentiation, at least in *Drosophila*. Future studies combining protein biochemistry with structure-function analyses will be required to fully address crucial mechanistic questions. These studies will further advance our understanding of the key role of mTOR signaling in neurogenesis and potentially provide novel insight into neurological disease.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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