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A Wacky Bridge to mTORC1 Dimerization

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The activity of the mTORC1 protein complex depends on multiple metabolic inputs that regulate dimerization, recruitment to the lysosome, and activation. In this issue of *Developmental Cell*, David-Morrison et al. (2016) show that the *Drosophila* protein Wacky and its mammalian counterpart WAC act as adaptors in the process of mTORC1 dimerization.

The mechanistic target of rapamycin (mTOR) is a conserved protein kinase present in two distinct complexes, mTORC1 and mTORC2, that play a central role in integrating extracellular signals to modulate intracellular functions. Active mTORC1 increases translational capacity while repressing macro-autophagy (hereafter called autophagy), a cellular degradation process that contributes to maintaining homeostasis and controlling macromolecule quality (Feng et al., 2014). mTORC1 also promotes glycolysis, the pentose phosphate pathway, de novo lipogenesis, and nucleotide synthesis—anabolic pathways that provide building blocks to sustain cellular growth.

Given mTOR's role in cellular homeostasis and its implication in human disease, a plethora of studies have been undertaken to decipher its regulation (Laplante and Sabatini, 2012). These studies have shown that mTORC1 activation (Dibble and Manning, 2013) directly depends on the GTPase Rheb (Ras-homolog enriched in brain), whose activity is repressed by the tumor suppressor complex (TSC) (Figure 1). TSC is regulated by REDD1 (regulated in development and DNA damage responses 1) and AMPK (AMP-activated protein kinase), which respond to oxygen and ATP levels, respectively. In the presence of amino acids, a dimer of Rag GTPases (RagA/B bound to RagC/D) recruits mTORC1 to the lysosomal membrane in close proximity to Rheb. This recruitment involves a membrane-associated vacuolar H^+-ATPase (v-ATPase) and the Ragulator, whose conformational change converts the RagA/B-RagC/D dimer into its active form. More recently, mTORC1 has also been shown to respond to tricarboxylic acid (TCA) cycle activity (Kim et al., 2013). Energetic stress associated with lower TCA cycle production of ATP provokes dissociation of the Tel2-Tti1-Tti2 (TTT)-RUVBL1/2 complex that in turn impinges on mTORC1 homodimer formation, a process that is necessary for efficient signaling (Yip et al., 2010). Thus, multiple metabolic inputs act on successive steps in the mTORC1 pathway.

The discovery of the mTOR counterpart in *Drosophila* (dTOR) (Oldham et al., 2000; Zhang et al., 2000) has enabled use of powerful genetic tools to decipher mTORC regulation. In this issue of *Developmental Cell*, using an unbiased screening strategy to identify factors involved in neurodegeneration in *Drosophila*, David-Morrison et al. (2016) found that Wacky, and ultimately its mammalian counterpart WAC, are required for metabolism-dependent mTORC1 dimerization. The authors found that in flies, loss of *wacky* function in the retina led to photoreceptor degeneration and increased autophagy. This observation contrasts with a previous study, which identified WAC as a gene product absolutely required for starvation-induced autophagy in mammalian cells (McKnight et al., 2012). To further investigate the role of Wacky in regulating autophagy, the authors analyzed mutant cells in the fat body, an insect organ with hepatic and adipose functions that responds strongly to nutritional status. They observed that, at the mid-third larval stage when larvae are normally actively feeding, *wacky* mutant cells did not undergo starvation-induced autophagy following nutrient deprivation—consistent with the requirement of WAC in starvation-induced autophagy in mammals. In contrast, when analyzed at earlier or later developmental stages, *wacky* mutant cells exhibited an increase in basal autophagy. Consistently, the authors also observed an increase in basal autophagic markers in WAC-knockdown mammalian cells. In summary, in both *Drosophila* and mammalian cells, the lack of Wacky/WAC increases basal autophagy but also impedes starvation-induced autophagy.

Proteomic analysis for Wacky partners led to the identification of dTOR, the RUVBL1/2 *Drosophila* counterparts (Pontin/Reptin), and Vha100-2, a component of the v-ATPase. Consistent with these findings, phenotypic analysis of *wacky* mutant was strongly reminiscent of the *dTOR* loss-of-function phenotype, suggesting that Wacky may regulate dTOR function. To study the mechanism by which Wacky/WAC may affect mTOR activity, the authors turned to mammalian cells. Co-immunoprecipitations using cell extracts revealed that WAC knockdown affected neither the assembly of the TTT and RUVBL1/2 complexes nor their interaction with mTORC1. It did, however, significantly reduce the association of these complexes with each other. Further investigation led to a model in which WAC acts as an adaptor linking the mTORC1-loaded TTT and RUVBL1/2 complexes, thereby facilitating mTORC1 dimerization (Figure 1). These results thus fill a gap in our understanding of the multiple steps in the mTORC1 regulatory pathway.

The authors also found that glucose/glutamine depletion, which induces a severe reduction in TCA cycle activity, results in reduced interaction between mTORC1 and components of the TTT or RUVBL1/2 complexes, suggesting that the energetic effect operates on mTORC1 loading onto these complexes before their WAC-induced association with each other. However, further experiments may be required to ascertain the timing of the interactions, since a previous study suggested that the TTT/mTORC1 interaction

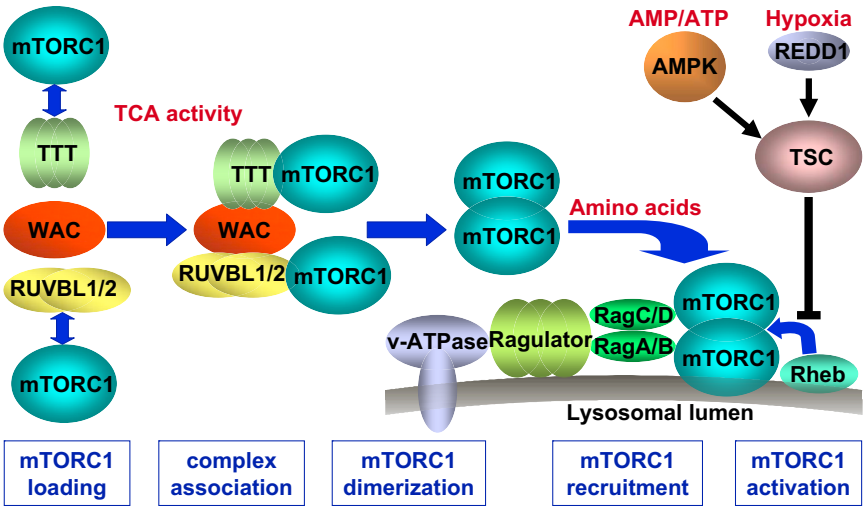

Figure 1. Several Metabolic Inputs Control the Multi-step Process of mTORC1 Activation
mTORC1 dimerization is controlled by TCA cycle activity; dimerization begins with the loading of mTORC1 onto the TTT and RUVBL1/2 complexes, followed by WAC-dependent association of the two mTORC1-containing complexes (shown by David-Morrison et al., 2016). Other metabolic inputs also affect mTORC1 activation at later steps. An increase in amino acid levels in the lysosome induces the recruitment of mTORC1, which can be activated by Rheb. TSC-mediated repression of Rheb is controlled by AMPK and REDD1, which respond to AMP/ATP levels and hypoxia. Growth factor regulation, which targets components of the TSC and mTORC1 complexes, is not shown in the figure.

also depends on RUVBL1, implying that TTT- RUVBL1/2 complex association occurs before mTORC1 loading (Kim et al., 2013).

In cancer cells, a dramatic increase in mTORC1 activity is likely due to growth factors that impinge on TSC-mediated repression (Dibble and Manning, 2013). It is intriguing that metabolic inputs operate on several distinct steps in the mTORC1 regulatory pathway (Figure 1), whereas growth factors appear to act solely on the TSC/Rheb/mTORC1 module. It is possible that these metabolically regulated steps constitute energetic checkpoints that act prior to mTORC1 growth factor stimulation. Alternatively, normal mTORC1 activity might depend predominantly on metabolic inputs, consistent with the fact that mTORC1 also operates in non-growing cells. This latter possibility is consistent with the fact that lowering mTORC1 activity in the adult eye leads to degeneration of photoreceptors, typically non-growing cells that are probably not exposed to growth factor stimulation (David-Morrison et al., 2016). It therefore appears plausible that growth factor stimulation regulates mTORC1 signaling solely under extreme growing conditions, for instance during specific developmental phases, in tumors or in culture cells raised in media containing very high levels of growth factors and nutrients, whereas in most other cells, mTORC1 activity is calibrated by metabolic inputs to maintain homeostasis.

In contrast to previous *Drosophila* screens based on growth-related phenotypes, David-Morrison et al. (2016) discovered an mTORC1 regulator in a screen for photoreceptor neurodegeneration. Interestingly, *WAC* loss-of-function mutations cause a clinical syndrome associated with intellectual disability (DeSanto et al., 2015). Since neurological disorders are often associated with accumulation of toxic aggregates due to misfolded proteins, promoting autophagy may be an appealing strategy for clearing these aggregates (Laplante and Sabatini, 2012). However, considering that WAC knockdown increases basal autophagy, the role of mTORC1 in the WAC-related syndrome will first need to be precisely evaluated.

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