Suppressing mTORC1 on the lysosome

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The mechanistic target of rapamycin, mTOR, is a protein kinase that integrates environmental and nutritional inputs into regulation of cell growth and metabolism. Key outputs of mTOR signalling occur from the lysosome membrane in the form of the multi-subunit mTOR complex 1 (mTORC1), which phosphorylates multiple targets. While class I phosphoinositide kinase (PI3K-I) is a well-known activator of mTORC1, a recent paper (Marat *et al*, 2017) shows that a class II PI3K with a different substrate specificity, PI3K-C2 β , serves to inhibit mTORC1 on lysosomes under conditions of growth factor deprivation.

See also: **AL Marat *et al*** (June 2017)

Control of metabolism by mTORC1 is crucial for normal physiology, and dysregulation of mTORC1 is associated with diseases, including cancer and diabetes (Saxton & Sabatini, 2017). Upregulation of anabolic processes through activation of mTORC1 is promoted by amino acids and growth factors whereas low energy status and cellular stress serve as inhibitory inputs and trigger catabolic processes such as autophagy (Saxton & Sabatini, 2017). Even though spectacular progress in understanding mTORC1 signalling has been made during the past two decades, it still remains incompletely understood how signalling from growth factor receptors at the cell surface can control mTORC1 on the lysosome membrane.

A central mediator of mTORC1 activation is PI3K-I, which is recruited by activated growth factor receptors to convert phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P₂] into PtdIns(3,4,5)P₃ at the plasma

membrane (Dibble & Cantley, 2015). This lipid in turn activates the protein kinases PDK1 and AKT1, and the latter causes (by an indirect mechanism) activation of a small GTPase, Rheb, which activates mTORC1 on the lysosome membrane (Saxton & Sabatini, 2017). AKT1 can also be activated by a dephosphorylation product of PtdIns(3,4,5)P₃, PtdIns(3,4)P₂ (Franke *et al*, 1997). Surprisingly, however, Marat *et al* observed that under conditions of serum (growth factor) deprivation, lysosomal PtdIns(3,4)P₂, generated by PI3K-II PI3K-C2 β -mediated phosphorylation of PtdIns4P, instead causes mTORC1 inhibition (Marat *et al*, 2017). This suggests that PtdIns(3,4)P₂ produces different signalling outputs depending on its subcellular localisation. Another implication of these findings is that the low mTORC1 activity observed under serum deprivation is not only caused by the absence of activating inputs but also by the presence of inhibitory factors.

PI3K-C2 β is not permanently associated with lysosomes but rather translocates there from the cytosol under conditions of serum deprivation (Marat *et al*, 2017). This appears to occur mainly via direct binding to the mTORC1 scaffolding subunit Raptor. How does lysosomal PtdIns(3,4)P₂, produced by PI3K-C2 β , inhibit mTORC1? A PtdIns(3,4)P₂-binding repressor of mTORC1 activity known as 14-3-3 γ was found to associate with Raptor in a PI3K-C2 β - and PtdIns(3,4)P₂-dependent manner. Another clue comes from the finding that PI3K-C2 β promotes perinuclear clustering of lysosomes (Marat *et al*, 2017), a condition that by itself limits mTORC1 activation presumably by spatially segregating mTORC1 from growth factor receptors (Korolchuk *et al*, 2011). Thus, PtdIns(3,4)P₂ exerts a dual

mechanism for mTORC1 inhibition on the lysosome.

The emerging picture is that mTORC1 signalling is controlled positively and negatively by spatially restricted 3-phosphoinositide pools (Fig 1). Not only do PtdIns(3,4,5)P₃ and PtdIns(3,4)P₂ at the plasma membrane function as positive inputs and lysosomal PtdIns(3,4)P₂ as a negative input, but PtdIns3P has previously been shown to promote both amino acid- and insulin-stimulated mTORC1 activation (Nobukuni *et al*, 2005), and PtdIns(3,5)P₂ also elevates insulin-stimulated mTORC1 activation by a direct interaction with Raptor (Bridges *et al*, 2012). The precisely tuned generation of phosphoinositides in specific cellular membranes makes them particularly well suited for reversible recruitment and regulation of cytosolic protein complexes such as mTORC1. A recent study has also revealed the involvement of another lipid in mTORC1 signalling, namely lysosomal cholesterol, which controls the membrane recruitment and activation of mTORC1 by a mechanism similar to that of amino acids (Castellano *et al*, 2017).

The new findings on PI3K-C2 β provide important insight into negative regulation of mTORC1. During prolonged amino acid deprivation, the entire mTORC1 complex dissociates from lysosome membranes and becomes inactive (Sancak *et al*, 2010). However, when amino acids are present, mTORC1 can reside on lysosomes and still be largely inactive, provided that the cells are deprived of growth factors (Korolchuk *et al*, 2011; Marat *et al*, 2017). The newly identified mechanism explains how mTORC1 activity is repressed under these conditions. It will now be interesting to learn what causes PI3K-C2 β to associate with

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Figure 1. Regulation of mTORC1 by phosphoinositides.

Green arrows show mTORC1 stimulation by phosphoinositides, with dotted arrows indicating indirect regulation. mTORC1 inhibition via PI3K-C2β-generated PtdIns(3,4)P₂ is indicated in red. Phosphoinositide kinases are shown in light blue.

Raptor under serum deprivation and how PI3K-C2β controls the positioning of lysosomes. It is also interesting that an mTOR complex with a distinct subunit composition and distinct functions, mTORC2, is not regulated by PI3K-C2β (Marat *et al.*, 2017). Whereas mTORC1 is activated by multiple mechanisms, mTORC2 is mainly activated by growth factors (Saxton & Sabatini, 2017), and it remains to be seen whether negative

regulatory factors for this complex exist as well.

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