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TFEBulous control of traffic by mTOR

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Cell growth is accompanied by the synthesis of macromolecules and biogenesis of organelles. The protein kinase mTOR (mechanistic or mammalian target of rapamycin) controls these processes by sensing availability of growth signals. The targeting of macromolecules and trafficking of cargo-containing vesicles into appropriate cellular compartments are also important processes that are highly controlled during growth versus stress conditions. In this issue of *The EMBO Journal*, Peña-Llopis *et al* demonstrate that mTOR complex 1 (mTORC1) could regulate endocytosis by controlling the expression of endosomal proteins such as the vacuolar (V)-ATPases. mTORC1 performs this novel function by modulating the phosphorylation and activity of the transcription factor EB (TFEB), which is required for expression of genes involved in autophagosome and lysosome biogenesis. This study, along with a related study in *Science* by Settembre *et al*, reveals how growth signals mediated by mTOR and other protein kinases such as mitogen-activated protein kinase (MAPK) can converge on TFEB to direct endosome biogenesis and trafficking.

In the presence of abundant nutrients and growth signals, mTOR controls growth by promoting synthesis of macromolecules and inhibiting autophagy, a process that allows recycling of intracellular material. mTOR is part of the two structurally and functionally distinct protein complexes, called mTORC1 and mTORC2. Cellular responses to growth cues have been linked to mTORC1, which consists of mTOR and its evolutionarily conserved partners, raptor and mLST8. mTORC1 is active in the presence of nutrients such as amino acids. It can be inhibited by rapamycin, a natural compound that is used as an immunosuppressant and anti-cancer drug. mTORC1 assembles at the surface of late endosomes/lysosomes where it is recruited by Rag GTPases (Sancak *et al*, 2010). Translocation to this compartment allows mTORC1 activation via its interactions with Rheb, presumably also located in endomembranes. The integrity of the late endosome has been shown to be critical for the ability of mTORC1 to respond to amino acids (Flinn *et al*, 2010). However, the downstream events upon mTORC1 activation in the late endosome/lysosome compartments are poorly understood.

In the recent studies of Peña-Llopis *et al*, a high-throughput screen that takes advantage of two regulators of mTORC1, namely the tuberous sclerosis complex 2 (TSC2) tumour suppressor protein and rapamycin, was employed to identify new mTORC1 targets. Genes that become upregulated in TSC2-deficient cells and undergo downregulation upon rapamycin inhibition were analysed in TSC2-null murine embryonic fibroblasts (MEFs). The screen strikingly revealed that the expression of several vacuolar (V)-ATPases is mTORC1 dependent (Figure 1). V-ATPases are large multimeric complexes that act as ATP-driven proton pumps. They generally function in the acidification of intracellular organelles such as lysosomes and endosomes during membrane fusion and trafficking. The activity of V-ATPases is regulated both at the level of subunit assembly, which has been demonstrated in yeast to be glucose-sensitive, and at the level of expression on membrane surfaces (Forgac, 2007). When mTORC1 activity is high as in TSC-deficient cells, there is increased expression of V-ATPases at both the mRNA and protein levels (Peña-Llopis *et al*, 2011). Rapamycin treatment downregulates V-ATPase expression in these cells. Thus, it is plausible that by localizing to the endosomes and lysosomes, mTORC1 is strategically positioned to control its downstream targets that have a role in the biogenesis and/or trafficking of these organelles.

What mediates the mTORC1-dependent increase in V-ATPase expression? V-ATPases are among the genes that could be transcriptionally regulated by TFEB during lysosomal biogenesis (Sardiello *et al*, 2009). TFEB is a bHLH

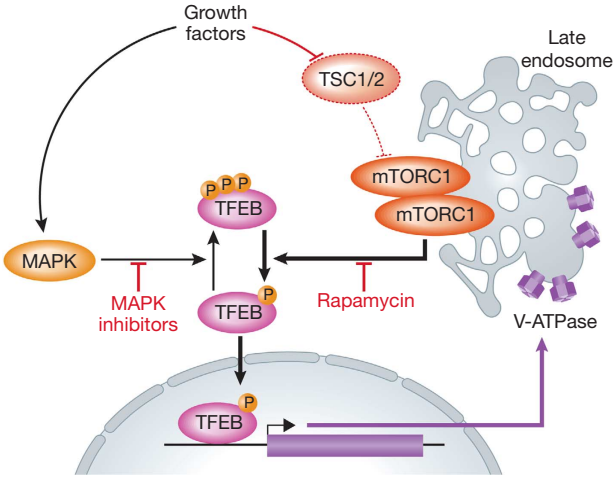

Figure 1 Model of TFEB regulation by mTORC1 and MAPK. When mTORC1 activity is high, such as in TSC-deficient cells, TFEB undergoes dephosphorylation at specific sites and localizes to the nucleus where it can regulate the expression of its target genes such as V-ATPases. Starvation or MAPK inhibition can also promote nuclear shuttling and TFEB dephosphorylation at distinct sites.

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transcription factor that binds E-box-related DNA sequences. TFEB overexpression induces lysosomal biogenesis, autophagy and increased degradation of complex molecules (Sardiello *et al*, 2009; Settembre *et al*, 2011). Peña-Llopis *et al* (2011) and the related study by Settembre *et al* (2011) now show that TFEB can mediate the expression of its target genes by control of its phosphorylation and nuclear shuttling. In HeLa cells, TFEB localizes to the nucleus upon starvation (Settembre *et al*, 2011). Surprisingly, this nuclear accumulation of TFEB is mimicked by pharmacological inhibition of the MAPK pathway but not rapamycin treatment. Intriguingly, in TSC2-deficient cells wherein mTORC1 activity is elevated, nuclear accumulation of TFEB was also observed (Peña-Llopis *et al*, 2011). In these cells, rapamycin can prevent the mobilization of TFEB to the nucleus. Thus, it seems contradictory that TFEB would localize to the nucleus either upon starvation or when mTORC1 activity is high. Nevertheless, both studies agree that TFEB phosphorylation is altered, seemingly hypophosphorylated, upon nuclear translocation. However, whereas Settembre *et al* reported that TFEB phosphorylation at Ser142 by the MAPK, ERK2, correlates with nuclear exclusion of TFEB, expression of a Ser142Ala mutant in the studies by Peña-Llopis *et al* does not affect nuclear localization in wild-type or $TSC2^{-/-}$ MEFs. Instead, they found that TFEB phosphorylation is regulated in a complex manner. A serine-rich region between amino acids 462–469 in TFEB appears to be critical for the mTORC1-dependent nuclear localization. Previously, at least 10 phosphosites have been identified for TFEB. Furthermore, whereas general nutrient and growth factor deprivation increases nuclear TFEB (Settembre *et al*, 2011), withdrawal of specific nutrients differentially affects TFEB phosphorylation. Thus, TFEB is likely dynamically regulated under different conditions by multiple kinases.

These two recent reports raise an interesting question: if TFEB is a master controller of transcriptional changes that occur in response to autophagy and endocytic processes, then how can mTORC1 negatively regulate autophagy and yet positively regulate TFEB activity and endocytosis? Despite the fact that rapamycin can stimulate autophagy (Zoncu *et al*, 2011), the finding that mTORC1 inhibition by rapamycin is not sufficient to promote TFEB nuclear translocation suggests that rapamycin does not fully mimic starvation signals that regulate TFEB. Thus, rapamycin treatment could be adequate to trigger acute but not sustained autophagic responses that require transcriptional changes. But why would enhanced mTORC1 activity, as occurs in TSC-deficient cells, promote TFEB nuclear translocation? Loss of TSC proteins has also been reported to promote endoplasmic reticulum (ER) stress and the unfolded protein response (Ozcan *et al*, 2008). Interestingly, ER stress also upregulates autophagy (Ravikumar *et al*, 2010). Thus, TFEB activity could be enhanced by conditions that promote autophagy. However, it is not clear if autophagy is augmented in TSC2-deficient cells. Speculatively, the enhanced membrane trafficking occurring during endocytosis in these cells could instead trigger increased TFEB activity. It is also worth mentioning that the distinct experimental systems used could explain some discrepancies in the findings between the two studies. Future investigation should provide more insights on how TFEB could be subject to numerous inputs to coordinately control endosome biogenesis and trafficking. Lastly, deregulated TFEB has been linked to renal tumours (Peña-Llopis *et al*, 2011). The findings described here provide further rationale for targeting mTOR and MAPK pathways, if not TFEB itself, for the treatment of this type of tumours and perhaps other cancers as well.

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Conflict of interest

The author declares that she has no conflict of interest.

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