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SPOTLIGHT

Establishing spatial control over TORC1 signaling

Oliver Schmidt[1] and Mariana E.G. de Araujo[1]

Target-of-rapamycin complex 1 resides on lysosomes/vacuoles and additionally on signaling endosomes. Gao et al. (2022. *J. Cell Biol.* https://doi.org/10.1083/jcb.202109084) set out to define the molecular identity of signaling endosomes, along with players required for the formation and maintenance of this endosomal subpopulation.

The target-of-rapamycin complex 1 protein kinase (TORC1 in budding yeast, mTORC1 in mammals) is an evolutionary conserved master regulator of cell growth that integrates signals from multiple pathways to activate anabolic processes (e.g., protein and lipid synthesis) and inhibit catabolic processes (e.g., lysosome biogenesis, autophagy; 1). The major pool of mTORC1/TORC1 resides on lysosomes or the analogous organelle in yeast, the vacuole. Recently, in a series of publications, the laboratories of de Virgilio and Ungermann have shown in budding yeast that part of TORC1 also resides on an endosomal organelle, called the signaling endosome (SE). The endosomal and the vacuolar pool are simultaneously activated by amino acids sensing, but their preferential downstream effectors are different: vacuolar TORC1 predominantly regulates protein synthesis via phosphorylation of Sch9 (2), and endo-/lysosomal lipid composition through Fab1 (3). In contrast, endosomal TORC1 predominantly phosphorylated Atg13 and Vps27 to inhibit macro- and microautophagy, respectively (2). In a collaboration the two laboratories have now defined the molecular identity of SEs in comparison to early and late endosomal markers and investigated how this endosomal subpopulation is formed (4).

Ivy1 is an inverted BAR domain protein and the suggested yeast ortholog of human missing-in-mitosis (MIM). Ivy1 is an effector of the Rab7-like GTPase Ypt7 and found on late endosomes and the vacuolar surface, with a preference for membrane regions of negative curvature (5). It has previously been implicated in TORC1 signaling (5, 6), suggesting it could be a marker for SEs. Gao et al. (4) now demonstrated that most Ivy1-positive puncta colocalized with Ypt7 and were devoid of Vps4, the driving force of the endosomal sorting complexes required for transport (ESCRT) machinery, suggesting they have a late endosomal nature but are distinct from multivesicular bodies. Approximately half of the TORC1-positive punctate structures colocalized with Ivy1, and—reciprocally—30% of Ivy1-positive puncta colocalized with TORC1 and with the EGO (exit from G0) complex, the yeast equivalent of the human late endosomal/lysosomal adaptor and MAPK and mTOR activator (LAMTOR)/Ragulator complex. Hence, a considerable fraction of SEs contains Ivy1, although the role of Ivy1 in endosomal TORC1 signaling remains unclear. Notably, TORC1-positive puncta colocalized to a similar extent with late endosomal Ypt7 and with the early endosomal Rab5 GTPase (Vps21) or its effector, the class C core vacuole/endosome tethering (CORVET) complex. Therefore, TORC1 likely also resides on early endosomes and/or endosomal maturation intermediates.

The formation and maintenance of Ivy1-positive SEs required the function of the homotypic fusion and protein sorting (HOPS) tethering complex, as well as functional ESCRT and retromer machineries. Specific HOPS inactivation increased the number of Ivy1-positive puncta and depleted them of early endosomal markers, indicative of continuous endosomal maturation but defective fusion with the vacuole (a major HOPS function). Intriguingly, HOPS inactivation markedly shifted TORC1 activity from vacuolar to endosomal targets. Yet, it remains unclear from where endosomal TORC1 activity now originated, because under these conditions Ivy1-positive puncta no longer contained EGO and TORC1.

Collectively, yeast TORC1 localizes to a heterogenous population of structures of late and early endosomal origin—50% with and 50% without Ivy1. Homo- and heterotypic fusions of late endosomal organelles are critical to maintain the population of SEs. Some of the observed structures may also represent biosynthetic carriers supplying SEs or vacuoles with TORC1 signaling components. Discrimination is difficult, as it is currently unclear which of the observed structures actively participate in endosomal TORC1 signaling. New tools to localize active TORC1 within cells and a detailed ultrastructural analysis may more accurately determine the nature of SEs in the future.

In mammalian cells, the situation appears to be even more challenging: mTORC1, Rheb, and Raptor have been found on the endo-lysosomal system, but also at the ER, the Golgi apparatus, peroxisomes, stress granules, the nucleus, and mitochondria. Whereas the function within the nucleus, controlling gene expression, is well

[1]Institute of Cell Biology, Biocenter Innsbruck, Medical University of Innsbruck, Innsbruck, Austria.

Correspondence to Oliver Schmidt: oliver.schmidt@i-med.ac.at; Mariana Eca Guimaraes de Araujo: mariana.araujo@i-med.ac.at.

established, characterizing unambiguous physiological roles for some of the other mTORC1 pools has proven considerably harder. This is partially due to the motile nature of lysosomes, where the canonical pool of mTORC1 resides, and their intrinsic capability to establish membrane contacts or fuse with other organelles. As an example, growth factors promote lysosomal mTORC1 activation and simultaneously trigger the translocation of the organelle to the cell periphery. These signaling lysosomes specifically target focal adhesions, specialized domains of the plasma membrane, in a process that is required to support mTORC1 activity (7).

What is the evidence for a functionally distinct endosomal pool of mTORC1 in mammalian cells? Previous work on mouse embryonic fibroblasts revealed that the Ragulator/LAMTOR complex was not only present on lysosomes, but that 10% of Ragulator/LAMTOR-positive organelles corresponded to early endosomes and about 30% to late endosomes/multivesicular bodies (8). Although it is undisputable that the integrity of late endosomes and endo-/lysosomal biogenesis are essential for mTORC1 activity, there is comparably little evidence for mTORC1 signaling from endosomes and a subsequent regulation of discrete targets. The generalized use of the term late endosomes/lysosomes in literature reflects the difficulties in discriminating the relative contributions of different late endocytic organelles. Interestingly, sustained endosomal mTORC1 activity in T cells from older individuals prevented the induction of the TFEB-dependent transcriptional program. The resulting inability to restore lysosome catabolic activity led to progressive organelle dysfunction and impaired T cell function (9). Although these data argue for a specific role of endosomal mTORC1 signaling, they failed to identify unique targets. Notably, the same study showed that HOPS is also required to sustain endosomal mTORC1 signaling in human cells, corroborating the results from Gao et al. There is also evidence of a reverse regulation, as phosphorylation of UVRAG by mTORC1 prevents its association with HOPS, suppressing endosomal and autophagosome maturation (10). Taken together, these results indicate that mTORC1/TORC1 signaling from late endocytic compartments and their biogenesis are tightly interconnected and jointly contribute to cell growth and metabolism.

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