Ragulator—a multifaceted regulator of lysosomal signaling and trafficking

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The lysosomal Ragulator complex regulates cell metabolism and growth by coordinating the activities of metabolic signaling pathways with nutrient availability. In this issue, Filipek et al. (2017. J. Cell Biol. https://doi.org/10.1083/jcb.201703061) and Pu et al. (2017. J. Cell Biol. https://doi.org/10.1083/jcb.201703094) introduce a role for Ragulator in growth factor- and nutrient-regulated lysosomal trafficking.

Lysosomes are emerging as central regulators of a wide range of cellular functions. Besides their established role in macromolecule degradation and recycling, they control numerous other processes such as cell adhesion, cell motility, tumor invasion, cell death, metabolic signaling, cytosolic and extracellular pH, plasma membrane repair, and bone resorption. To accomplish these versatile functions, lysosomes must localize to appropriate subcellular sites. Accordingly, they can be divided into at least two spatially distinct pools, a juxtanuclear pool in the vicinity of the microtubule organizing center, which is responsible for most of the degradative “housekeeping” functions of lysosomes, and a smaller peripheral pool close to the plasma membrane. Despite our increasing knowledge of the proteins involved in the bidirectional movement of lysosomes, it has remained largely unknown how cells alter lysosomal positioning in response to changes in their environment. In this issue, Filipek et al. and Pu et al. elucidate this regulation by showing that growth factor signaling and nutrient availability enhance the outward movement of lysosomes through the weakening of the lysosomal interaction between BORC and Ragulator, thereby enhancing the recruitment of Arl8/Skip to lysosomes and the subsequent coupling of lysosomes to kinesin motors (Fig. 1).

The lysosomal Ragulator complex, which now emerges as the regulator of lysosomal positioning, is best known as an essential activation platform for metabolic signaling. It is composed of five late endosomal/lysosomal adaptor and MAPK and mammalian target of rapamycin (mTOR) activator/regulator (LAMTOR) subunits. As the long name implies, this scaffold complex regulates both MAPK and mTOR complex 1 (mTORC1). The recently solved crystal structure reveals that Ragulator is formed by tightly packed LAMTOR2/3 and LAMTOR4/5 heterodimers wrapped and held together by LAMTOR1, which anchors the complex to the lysosomal membrane by its N-terminal myristoyl and palmitoyl groups (de Araujo et al., 2017; Fig. 1). The LAMTOR2/3 heterodimer associates with RagA/B and RagC/D GTPases and serves as a guanine nucleotide exchange factor for RagA/B, whose GTP-bound forms recruit mTORC1 to the complex (Bar-Peled et al., 2012). LAMTOR2/3 also interacts with MAPK kinase 1/MEK1, thereby facilitating the activation of MAPK3/ERK1 (Wunderlich et al., 2001). Furthermore, Ragulator interacts with Axin, a scaffold protein for liver kinase B1–mediated activation of AMP-activated protein kinase (AMPK) complex on the lysosomal surface (Zhang et al., 2014). It is presently unknown which LAMTOR subunit interacts with Axin, but the ability of Axin to inhibit the guanine nucleotide exchange factor activity of LAMTOR2/3 toward RagA/B suggests that it may also bind to LAMTOR2/3 (Zhang et al., 2014). Finally, Ragulator interacts with vacuolar H⁺-ATPase and neutral amino acid transporter SLC38A9, which serve as amino acid sensors for the switching between anabolic mTORC1 and catabolic AMPK activities (Zhang et al., 2014; Wang et al., 2015; Fig. 1).

Filipek et al. (2017) and Pu et al. (2015, 2017) identified the interaction between BORC and Ragulator by tandem affinity purification with Ragulator subunits and BLOS2 (a shared subunit of BLOC1 and BORC) as baits, respectively. Using similar analyses with other BLOC1 and BORC subunits, both...
groups verified the interaction to be specific for the BORC complex, and a subsequent yeast two-hybrid (Y2H) screen for pairwise interactions between all BORC and Ragulator subunits by Pu et al. (2017) identified a strong interaction between Lyspersin and LAMTOR2. By analyzing the interactions of truncated and mutated versions of Lyspersin, both groups came to similar conclusions regarding the areas of Lyspersin that interact with LAMTOR2 and the BORC complex. Specifically, mutations in the conserved Lyspersin residues L221 (Pu et al., 2017) and K224 (Filipek et al., 2017) abolished the association with Ragulator, but not with another BORC component (Myrlysin), whereas the conserved Lyspersin residues L349/L352 (Pu et al., 2017) and K316/K357 (Filipek et al., 2017) were required for the association with Myrlysin. Pu et al. (2017) further verified these interactions using Y2H analyses. Thus, Lyspersin associates with Ragulator and BORC via two distinct but adjacent domains in its structured C-terminal part, suggesting that it can form a physical link between these two complexes.

Having established the interaction between BORC and Ragulator, Filipek et al. (2017) and Pu et al. (2017) examined its functional consequences using Lyspersin and LAMTOR1-depleted cells. Lysosomes in cells depleted for LAMTOR1 (and lacking the entire Ragulator complex) were dispersed to the cellular periphery and had increased Arl8-BORC association, whereas lysosomes depleted for Lyspersin alone or together with LAMTOR1 clustered in the perinuclear area and failed to recruit Arl8 (Filipek et al., 2017; Pu et al., 2017). C-terminal Lyspersin fragments retaining binding to BORC fully rescued the perinuclear lysosomal phenotype of Lyspersin knockout cells, whereas C-terminal fragments binding only Ragulator and N-terminal fragments binding neither BORC nor Ragulator failed to do so (Filipek et al., 2017; Pu et al., 2017). Collectively, this comprehensive set of data indicates that Ragulator negatively regulates the BORC-Arl8 association and the Arl8-mediated outward movement of lysosomes via the LAMTOR2–Lyspersin interaction, and that the C-terminal part of Lyspersin is necessary and sufficient for BORC to recruit Arl8 to lysosomes and subsequent lysosomal dispersion to the cellular periphery.

The association of both BORC and mTORC1-activating Rag GTPases with LAMTOR2 raised the question whether BORC regulates the mTORC1-activating association between Rag GTPases and Ragulator to influence lysosomal positioning, or vice versa. These questions were addressed by Pu et
al. (2017) by examining the mTORC1 activity and lysosomal positioning after the depletion of various BORC subunits and mTORC1 inhibition, respectively. Notably, BORC silencing and subsequent perinuclear clustering of lysosomes did not affect mTORC1 activity, and neither genetic nor pharmacological mTORC1 inhibitors altered lysosomal positioning (Pu et al., 2017). Based on these results, Pu et al. (2017) concluded that BORC does not interfere with mTORC1 activation and that lysosomal positioning is independent of mTORC1 activity. However, this does not exclude the possibility that BORC and Rag GTPases compete for the same or overlapping binding site in LAMTOR2 as was suggested previously by Schweitzer et al. (2015) based on the ability of overexpressed Lyspersin to disrupt the Rag GTPase–Ragulator interaction and inhibit mTORC1 activity. In line with this, silencing the relatively little expressed Lyspersin by Pu et al. (2017) may have increased the binding of Rag GTPase–mTORC1 without having a detectable increase in already high mTORC1 activity. Additionally, inhibiting mTORC1 signaling may have failed to affect lysosomal positioning because Rag GTPases could compete with Lyspersin expression in cell protrusions and invasion of breast cancer cells (Rafn et al., 2012). These events are mediated by complex signaling events, including the activation of the MAPK–ERK pathway (Rafn et al., 2012). It remains, however, to be tested whether the MAPK–ERK pathway or other EGF/Erbb2-induced signaling pathways directly control the hydrophobicity of LAMTOR2 and thereby the interaction between LAMTOR2 and Lyspersin. If this is the case, it would open attractive therapeutic possibilities to control lysosomal trafficking by targeting MAPK–ERK or other kinases responsible for LAMTOR2 phosphorylation.

Overall, it is becoming evident that Ragulator plays a variety of cellular functions in regulating lysosomal biogenesis, activity, and, as illustrated in these two papers, positioning. Identification of Ragulator (via Lamtor2) as the negative regulator of anterograde directed movement and demonstration that this regulation is controlled by both amino acids and the EGF, highlights the varied levels of cellular regulation that converge on the Ragulator complex. How these proteins spatially interact with the Ragulator and how such interactions are regulated will be important in understanding how a Rag GTPase–LAMTOR2 interaction influences a Lyspersin–LAMTOR2 interaction, and will better enable us to manipulate the regulatory pathways to target lysosomal positioning for therapies ranging from the aforementioned invasive cancer to neurodegenerative disorders and infectious diseases.

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