On the Rab again—the PATh to mTORC1 activation

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Rab proteins are crucial players in membrane transport pathways and several Rabs have been implicated in the regulation of autophagy. However, it is not clear whether Rabs control autophagy indirectly through the transport of factors influencing upstream signalling, such as amino acid transporters, or if they are implicated in the generation of the autophagosome. Autophagy is initiated by the generation of membrane cisternae in the phagophore, which ultimately encloses a portion of the cytoplasm targeted for degradation. In yeast, the phagophore is located at the vacuole—equivalent to the mammalian lysosome. In mammalian cells, several organelles have been suggested to provide lipids to the phagophore, such as the endoplasmic reticulum, mitochondria and endosomes. Whether the phagophore membrane elongates by direct lipid insertion or whether it receives lipids by vesicular transport remains an area of intensive investigation (see [1] for review). So far, the quest for a specific Rab or Rab effector that would regulate membrane delivery to the phagophore has remained inconclusive.

In this issue of *EMBO reports*, Matsui and Fukuda [2] report the results of a two-step screen to find Rab proteins involved in the regulation of autophagy. They identified Rab12 as the most potent regulator of autophagy. Rather than directly regulating a membrane transport step that might add membrane to autophagosomes, Rab12 affects autophagy indirectly by stimulating the constitutive degradation of the proton-assisted amino acid transporter 4 (PAT4). This negatively regulates the mammalian target of rapamycin complex 1 (mTORC1) and stimulates autophagy (Fig 1).

The authors performed a small interfering RNA (siRNA) screen against 58 Rabs. Only

six candidates (Rab1A, 1B, 11A, 12, 18 and 40B) changed the levels of two autophagic marker proteins tested, LC3-II and p62. Among these six candidates, Rab1 [3] and Rab11 [4] had previously been implicated in autophagosome formation, thus validating the screen. The authors further deciphered the role of Rab12. They show that autophagosome formation and maturation remain intact despite the knockdown of Rab12, and that autophagy can still be triggered by starvation. However, autophagic activity is always lower on Rab12 knockdown than in control cells. The authors concluded that Rab12 might exert its effect upstream from autophagosome formation.

How does Rab12 regulate autophagy?

Rab12 is mainly localized to recycling endosomes. It participates in a poorly characterized membrane transport pathway from recycling endosomes to lysosomes [5]. Until now, the sole cargo known to be following the Rab12 route is the transferrin receptor.

The mTOR kinase is a central regulator of cell growth and metabolism and negatively regulates autophagy. It functions on both the single cell level, and on the level of whole organisms. Two functionally distinct protein complexes, mTORC1 and mTORC2, regulate temporal and spatial aspects of cell growth, respectively. The two complexes differ in their sensitivity to rapamycin (TORC2 being resistant) and in their localization—mTORC1 resides on endolysosomal membranes under nutrient-replete conditions, whilst mTORC2 mainly localizes to the plasma membrane. mTORC1 hyperactivation is linked to cell proliferation and cancer [6].

Intracellular amino acids activate mTORC1. Matsui and Fukuda found that depletion of Rab12 by siRNA leads to increased amino acid levels and altered

Fig 1 | Proton-assisted amino acid transporter 4 (PAT4) cycles from the plasma membrane to recycling endosomes. A portion of PAT4 is constitutively targeted for degradation in a Rab12-dependent fashion. On Rab12 depletion, the plasma membrane pool of PAT4 increases, allowing for enhanced amino acid uptake and mTORC1 signalling. A sensing and signalling role for PAT4, which is similar to those of the V-ATPase at the lysosome, cannot be excluded. ER, endoplasmic reticulum; mTORC1, mammalian target of rapamycin complex 1.

transport of the amino acid transporter PAT4, which is normally constitutively targeted for degradation. Consequently, in

Rab12-depleted cells PAT4 accumulates at the plasma membrane, augmenting amino acid uptake. Accordingly, overexpression of PAT4 hyperactivates mTORC1 in cells with normal Rab12 levels. Expressing a constitutively active form of Rab12 rescues this effect.

Intracellular amino acid levels are controlled by amino acid transporters, the expression and localization of which is regulated in response to the available nutrients. Several studies place amino acid transporters upstream from TORC1 (see [7] for review). SLC7A5/SLC3A2, a bidirectional leucine–glutamine exchanger on the plasma membrane, is required for leucine activation of mTORC1. In *Drosophila* and mammalian cells, proton-assisted amino acid transporters on both the plasma membrane (PAT4) and the lysosome (PAT1) regulate mTORC1 activity. PAT1 and PAT4 are required for proliferation of breast cancer cells. Interestingly, transport activity of PAT4 might not be crucial for the signalling to mTORC1. Given that PAT4 is a low-capacity, high-affinity transporter, it is probably saturated over a wide range of amino acid concentrations. PAT4 might act not only as transporter, but also as a transceptor—it might act as a receptor and sensor for amino acids. At its localization, the plasma membrane, it would be well placed to sense rapid changes in nutrient availability. The endosomal pool of PAT4 might represent a reservoir facilitating rapid remobilization of PAT4 to the plasma membrane. Elucidating the amino acid specificity of PAT4 and other amino acid transporters would further help to understand how exactly mTORC1 activity is controlled by amino acids.

Lysosomes were proposed as platforms for nutrient sensing and TOR signalling. With mTORC1 localizing on lysosomes, and lysosomes being major storage organelles for amino acids, lysosomal content could be sensed in a vacuolar H⁺-ATPase-dependent manner towards Rag-GTPases and mTORC1 [8]. Indeed, leucine, glutamine and arginine, the main amino acids in yeast vacuoles—and probably lysosomes—have the biggest effect on mTORC1 activation.

In yeast and mammalian cells, an aminoacyl-transfer RNA (tRNA) synthetase communicates leucine levels to TORC1 [9]. Whilst these enzymes generally reside in cytosolic multi-protein complexes, a portion is also localized to endolysosomal membranes at which mTORC1-directed amino acid signalling could be initiated. In addition to amino acids, α -ketoglutarate, a key intermediate in cellular metabolism that can be generated by glutaminolysis, also activates mTORC1 [10]. It has been proposed that mTORC1 could integrate inputs from different sources depending on the cell type and the specific nutrients available [10]. In mammalian cells, leucine sensing through aminoacyl-tRNA synthetase occurs through RagD, whilst activation by glutaminolysis occurs through RagB. In this context, it will be interesting to determine the role of the different Rag GTPases in integrating different upstream signals.

Few membrane transport events are known to have an influence on mTORC1 activity and, except for yeast (control of Gap1p transport by TORC1; [11]), there is little data on how TORC1 impinges on membrane transport. One could speculate that

Rab12 is part of a feedback loop responding to decreased amino acid levels. If Rab12 activity or abundance were controlled by mTORC1, inactivation of mTORC1 by decreasing levels of amino acids could result in an inactivation of Rab12, thereby diminishing the degradation of PAT4 and enhancing amino acid uptake. Finally, modulating the transport and activity of PATs, and thereby mTORC1 activity, might be a new route for therapy of cancer and other diseases in which this pathway is deregulated.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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