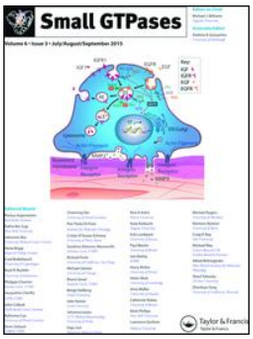

Small GTPases

ISSN: 2154-1248 (Print) 2154-1256 (Online) Journal homepage: https://www.tandfonline.com/loi/ksgt20

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To cite this article: Amanda Garrido, Marta Brandt & Nabil Djouder (2016) Transport to Rhebpress activity, Small GTPases, 7:1, 12-15, DOI: 10.1080/21541248.2015.1120846

To link to this article: https://doi.org/10.1080/21541248.2015.1120846

Published online: 21 Feb 2016.

SMALL GTPASES
2016, VOL. 7, NO. 1, 12–15
http://dx.doi.org/10.1080/21541248.2015.1120846

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COMMENTARY

Transport to Rhebpress activity

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ABSTRACT

The small GTPases from the rat sarcoma (Ras) superfamily are a heterogeneous group of proteins of about 21 kDa that act as molecular switches, modulating cell signaling pathways and controlling diverse cellular processes. They are active when bound to guanosine triphosphate (GTP) and inactive when bound to guanosine diphosphate (GDP). Ras homolog enriched in brain (Rheb) is a member of the Ras GTPase superfamily and a key activator of the mammalian/mechanistic target of rapamycin complex 1 (mTORC1). We recently determined that microspherule protein 1 (MCRS1) maintains Rheb at lysosomal surfaces in an amino acid-dependent manner. MCRS1 depletion promotes the formation of the GDP-bound form of Rheb, which is then delocalized from the lysosomal platform and transported to endocytic recycling vesicles, leading to mTORC1 inactivation. During this delocalization process, Rheb-GDP remains farnesylated and associated with cellular endomembranes. These findings provide new insights into the regulation of small GTPases, whose activity depends on both their GTP/GDP switch state and their capacity to move between different cellular membrane-bound compartments. Dynamic spatial transport between compartments makes it possible to alter the proximity of small GTPases to their activatory sites depending on the prevailing physiological and cellular conditions.

ARTICLE HISTORY
Received 15 July 2015
Revised 6 November 2015
Accepted 10 November 2015

KEYWORDS
amino acids; mTORC1; MCRS1; Ras superfamily; Rheb; small GTPases; TSC2; vesicle transport

Small GTPases from the rat sarcoma (Ras) superfamily are key regulators of fundamental cellular processes such as cell growth, cell proliferation, cell cycle progression, and autophagy. As such, they play multiple roles in maintaining cellular homeostasis[1,2] and their activity is tightly regulated. Dysfunctions in this regulation are central in the pathogenesis of various human diseases including cancer and, neurodegenerative and cardiovascular diseases.[2,3] A better understanding of their regulation could thus potentially open up new therapeutic opportunities.

The Ras superfamily is subdivided into the Ras, Rho, Rab, Arf, and Ran families, whose members are monomeric proteins of around 21 kDa with common nucleotide binding domains known as G boxes. Guanine nucleotide exchange factors (GEFs) modulate small GTPase activity by stimulating guanosine diphosphate (GDP) release and guanosine triphosphate (GTP) binding, and enhance cellular signaling processes. Conversely, GTPase activating proteins (GAPs) stimulate GTPase activity by accelerating GTP hydrolysis, favoring the GTPases' GDP-bound forms. Some members of this superfamily, including the Ras, Rho and Rab families, can also be inactivated by GDP dissociation inhibitors (GDIs).[1] GDIs reduce the GDP dissociation rate and maintain small GTPases in a soluble state. All these regulators are specific to individual families and target specific small GTPases.

Ras homolog enriched in brain (Rheb) proteins are associated with the Ras family of GTPases[1] and have been highly conserved throughout evolutionary history. They occur in species ranging from yeasts to humans, although they have not yet been detected in plants. In mammals, there are 2 genes that encode Rheb proteins, known as Rheb1 and Rheb2. Rheb2 expression is limited to certain tissues whereas Rheb1 is ubiquitously expressed and is commonly referred to simply as Rheb.

Unlike other small GTPases from the Ras superfamily, which are mainly targeted to the plasma membrane, Rheb is mainly localized to endomembranes such as those of the lysosomes, peroxisomes and mitochondria.[4–6] This association with cellular endomembranes is enabled by farnesylation, a post-translational lipid modification of its C-terminal CAAX motif. The process of farnesylation involves the attachment of a 15-carbon farnesyl group to the cysteine thiol, cleavage of the AAX residues, and methylation of the C-terminal carboxylate moiety. The combination of the lipid motif with

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proximal lysines promotes membrane association and ensures that the small GTPase proteins are tightly anchored to lipid membranes, which modulate their activity.[7] Membrane interaction is important in signaling mediated by small GTPases and their regulation of downstream effectors.

The mammalian/mechanistic target of rapamycin (mTOR), a serine/threonine protein kinase of the phosphoinositide kinase subfamily that senses nutrients and growth factors, is directly regulated by Rheb at lysosomal surfaces.[8–13] Although the exact mechanism of mTOR activation through Rheb is not fully understood, it is well established that amino acids influence the transport of mTOR to the lysosomes, where it can interact with Rheb-GTP and regulate its catalytic activity.[8,9,12,13] In Rheb-depleted cells, mTOR complex 1 (mTORC1) can no longer be activated even in the presence of amino acids. This suggests that amino acid-mediated Rheb/mTORC1 interaction is essential for its activation.[8] Alternative mechanisms for mTOR modulation involve phosphatidic acid (PA), whose biosynthesis is controlled by Rheb.[14] PA was shown to be necessary for S6 kinase phosphorylation by mTOR. Additionally, PA competes with the FKBP12-rapamycin complex for interaction with mTOR.[15] Growth factors also regulate Rheb/mTORC1 activity, via the phosphoinositide-3 kinase (PI3K)/protein kinase B (PKB)/tuberous sclerosis complex (TSC1-TSC2) axis.[10,16-22] In the absence of growth factors, TSC2 inactivates Rheb, keeping it in its GDP-bound state. When growth factors are present, they activate PKB, which phosphorylates and inhibits TSC2. This promotes the conversion of Rheb-GDP into Rheb-GTP, which activates mTORC1. Importantly, growth factors induce the spatial delocalization of TSC2 from the lysosomes,[6,19] suggesting another level of TSC2 regulation based on dynamic transport.

While most studies have focused on the role of growth factors, we recently dissected the role of amino acids in Rheb activation. Notably, we have shown that microspherule protein 1 (MCRS1) is an essential molecular link connecting Rheb-GTP to mTORC1.[6] MCRS1 localizes to lysosomes in an amino acid-dependent manner. There, MCRS1 hinders the GAP activity of TSC2, enabling Rheb to activate mTORC1. MCRS1 depletion exposes lysosomal Rheb-GTP to TSC2 activity, inactivating Rheb and consequently abolishing mTORC1 activation. Interestingly, amino acid or MCRS1 depletion delocalizes Rheb-GDP from lysosomes to recycling endocytic vesicles without regard for Rheb's farnesylation state. This suggests that Rheb activity is subject to dynamic spatial regulation based on nutrient availability (Fig. 1).

Recent work has shown that MCRS1 is an essential RanGTP-regulated factor for bipolar spindle assembly, protecting microtubules against depolymerisation.[23] However, MCRS1 regulates mTORC1 independently of microtubule networks and its nuclear function, suggesting that cells may contain several MCRS1 pools with different functions.[6] Clearly, a general role of MCRS1 in scaffolding small GTPase proteins cannot be excluded.

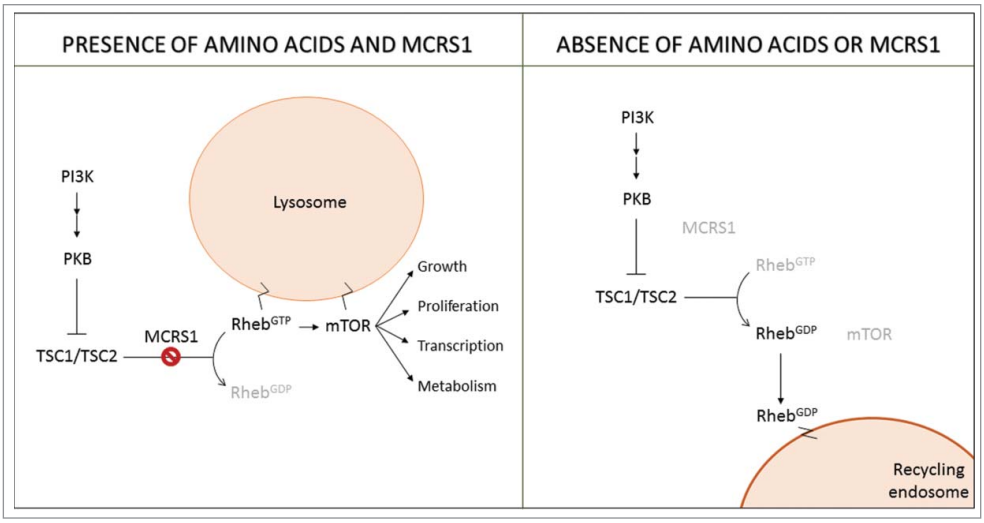

Figure 1. An amino acid-dependent mechanism essential for Rheb-GTP-mediated mTORC1 activation. In the presence of MCRS1 and amino acids, MCRS1 protects Rheb from TSC2 GAP activity; Rheb-GTP activates mTOR, leading to the phosphorylation of its downstream effectors. In the absence of amino acids or MCRS1, TSC2 inactivates Rheb, thereby delocalizing it to recycling endosomes and taking it away from the lysosomal activation platform. mTOR becomes inactivated and mTOR-dependent signaling pathways are not triggered. PI3K: phosphoinositide-3 kinase. PKB: protein kinase B. MCRS1: microspherule protein 1. TSC1: tuberous sclerosis complex 1. TSC2: tuberous sclerosis complex 2. mTOR: mechanistic/mammalian target of rapamycin.

There is an increasing amount of evidence indicating that Rheb is transported between different cellular membrane-bound compartments, including the Golgi and endoplasmic reticulum [24,25] as well as the mitochondria.[26] In mitochondria, Rheb interacts with other proteins such as BCL2/adenovirus E1B 19 kDa-interacting protein 3 (BNIP3) and Nip3-like protein X (NIX), to regulate mitophagy.[27] BNIP3 and NIX are members of the BCL-2 family of proteins and part of the BH3 domain containing protein family. They are thus pro-apoptotic mitochondrial factors with important roles in the induction of cell death and autophagy. The interactions of mitochondrial BNIP3 and NIX with Rheb apparently influence Rheb activity. This suggests that Rheb activity is regulated both by spatial transport and by the expression of specific regulatory factors at particular sites within the cell.

Ras proteins were also reported to be spatially distributed in membrane-bound compartments [28] such as the Golgi and endoplasmic reticulum,[29,30] and multiple mechanisms for their transport have been identified. After being released from the plasma membrane, Ras proteins shuttle back through mechanisms involving phosphodiesterase subunit delta (PDEδ) or chaperones. These proteins recognize specific posttranslational lipid modifications of small GTPases and carry them back, first to the endoplasmic reticulum and then to the plasma membrane.[29,31] These lipids also affect membrane affinity, so the variability in lipid modifications between Ras family members makes the kinetics of this cycle slightly different in each case. These findings provide important new insights into the regulation of small GTPase proteins from the Ras superfamily, whose activity depends on their GTP/GDP switch state but also on their capacity to shuttle between different cellular membrane-bound compartments. This dynamic transport ensures that small GTPases can be located in close proximity to their activatory sites or be distant from them, depending on cellular and physiological conditions.

In recent years farnesyltransferase inhibitors have been developed as new anti-cancer agents that prevent the farnesylation of small GTPases and thus their attachment to cellular membranes, taking them away from their activatory sites. While such inhibitors have performed well *in vitro* and in rodents,[32,33] they were less effective in clinical trials.[34,35] Potential alternative therapeutic strategies include inhibiting "scaffolders" such as MCRS1 or favoring small GTPases' vesicular circulation away from their activation platforms. These approaches could be useful in treating both cancer and other diseases involving misregulation of small GTPase activity.

Abbreviations

mTOR	mammalian/mechanistic target of rapamycin
MCRS1	microspherule protein 1
Rheb	Ras homolog enriched in brain
GEFs	guanine nucleotide exchange factors
GAPs	GTPase activating proteins.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

A.G is a recipient of Severo Ochoa PhD fellowship. M.B is a recipient of La Caixa PhD fellowship. N.D is a recipient of the Spanish Ramón y Cajal fellowship. This work was supported by the Spanish Ministry of Economy and Competitiveness (SAF2010–18518 and SAF2013–46089-R).

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