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Deacetylated αβ-tubulin acts as a positive regulator of Rheb GTPase through increasing its GTP-loading

Mi Nam Lee [a], Ara Koh [a], Dohyun Park [a], Jin-Hyeok Jang [b], Dongoh Kwak [a], Hyeona Jeon [a], Jaeyoon Kim [a], Eun-Jeong Choi [a], Heeyoon Jeong [a], Pann-Ghill Suh [d], Sung Ho Ryu [a,b,c,*]

[a] Division of Molecular and Life Sciences, Pohang University of Science and Technology (POSTECH), Pohang 790-784, Republic of Korea
[b] School of Interdisciplinary Bioscience and Bioengineering, Pohang University of Science and Technology (POSTECH), Pohang 790-784, Republic of Korea
[c] Division of Integrative Biosciences and Biotechnology, Pohang University of Science and Technology (POSTECH), Pohang 790-784, Republic of Korea
[d] School of Nano-Biotechnology and Chemical Engineering, Ulsan National Institute of Science and Technology, Ulsan 689-798, Republic of Korea

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ABSTRACT

Ras homolog enriched in brain (Rheb) regulates diverse cellular functions by modulating its nucleotide-bound status. Although Rheb contains a high basal GTP level, the regulatory mechanism of Rheb is not well understood. In this study, we propose soluble αβ-tubulin acts as a constitutively active Rheb activator, which may explain the reason why Rheb has a high basal GTP levels. We found that soluble αβ-tubulin is a direct Rheb-binding protein and that its deacetylated form has a high binding affinity for Rheb. Modulation of both soluble and acetylated αβ-tubulin levels affects the level of GTP-bound Rheb. This occurs in the mitotic phase in which the level of acetylated αβ-tubulin is increased but that of GTP-bound Rheb is decreased. Constitutively active Rheb-overexpressing cells showed an abnormal mitotic progression, suggesting the deacetylated αβ-tubulin-mediated regulation of Rheb status may be important for proper mitotic progression. Taken together, we propose that deacetylated soluble αβ-tubulin is a novel type of positive regulator of Rheb and may play a role as a temporal regulator for Rheb during the cell cycle.

1. Introduction

Ras homolog enriched in the brain (Rheb) belongs to the Ras superfamily, sharing high homology with human Rap2, yeast RAS1, and human H-Ras [33,61], which plays an essential role in cell growth and proliferation in many organisms [39,49]. Rheb is well known as an upstream key activator for the mammalian target of rapamycin (mTOR) complex 1 (mTORC1), which has a central role in cell growth by regulating protein synthesis, through modulating mTOR kinase activity [3,31,66]. Other mTORC1-independent roles of Rheb have been continuously suggested. Rheb acts as a molecular switch in several cellular processes, such as misfolded protein metabolism, cellular apoptosis, vesicle formation and myogenesis [14,27,32,48,65], although their molecular targets have not been identified yet.

Like other small GTPases, Rheb cycles between the active GTP-bound and inactive GDP-bound forms, and its cellular function is mediated by regulating its GTP-/GDP-bound status [2]. For Rheb, tuberous sclerosis complex (TSC), particularly the TSC1–TSC2 complex, is the best characterized GTPase-activating protein (GAP). By binding to Rheb and stimulating its GTPase activity via the GAP domain of TSC2, the TSC1–TSC2 complex reduces the level of GTP-bound Rheb and thus inhibits Rheb-mediated mTORC1 pathway [29,53]. Several environmental cues, such as growth factors, energy status, and cellular stresses, converge on the TSC1–TSC2 complex to regulate its GAP activity toward Rheb [20]. For example, insulin induces phosphorylation of TSC2 and inhibits its GAP functions, whereas energy depletion induces AMPK-mediated phosphorylation of TSC2, which stimulates its GAP activity toward Rheb [3,23]. Although many reports have shown that diverse signals determine the nucleotide-bound status of Rheb by regulating GAP activity of TSC2, we have little information regarding how GTP-bound Rheb is generated. Guanine nucleotide exchange factors (GEFs) are generally accepted to induce the GTP-bound form of small GTPases. GEFs interact directly with small GTPases and stimulate the release of GDP from small GTPases and thereby allow its replacement by GTP [54,56]. Related to this, TCTP (translationally controlled tumor protein) was suggested as a Rheb GEF; however, recent reports have debated the role of TCTP in both the mTORC1 activity and its GEF activity toward Rheb in vitro [4,19,44,57], and raised the question as to whether physiologically relevant GEF exists for Rheb. Rheb has a high basal GTP level, while most Ras GTPases exist in the GDP-bound form in the resting state [22]. Due to this unique property of Rheb, the nucleotide-bound status of Rheb has been proposed to be regulated in an unconventional way. However, it remains unidentified yet.

In this report, we suggest the possibility that soluble αβ-tubulin functions as an unconventional Rheb regulator by affecting GTP-bound status

* Corresponding author at: Division of Molecular and Life Sciences, Pohang University of Science and Technology (POSTECH), Pohang, Kyungbook 790-784, Republic of Korea. Tel.: +82 54 279 2292; fax: +82 54 279 0645.
E-mail address: sungho@postech.ac.kr (S.H. Ryu).

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of Rheb. We found that the level of GTP-bound Rheb is determined by deacetylated soluble αβ-tubulin concentrations. Moreover, considering changes in both acetylation of αβ-tubulin and Rheb–tubulin binding throughout the cell cycle, our finding provides a novel Rheb regulation mechanism and shows the importance of Rheb regulation according to posttranslational modification of αβ-tubulin for cell cycle progression.

2. Materials and methods

2.1. Reagents and antibodies

All chemicals were obtained from Sigma Chemical Co. (St. Louis, MO) unless stated otherwise. Anti-GST and anti-HA 12CA5 was