

Deacetylated $\alpha\beta$ -tubulin acts as a positive regulator of Rheb GTPase through increasing its GTP-loading

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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 $\alpha\beta$ -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 $\alpha\beta$ -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 $\alpha\beta$ -tubulin levels affects the level of GTP-bound Rheb. This occurs in the mitotic phase in which the level of acetylated $\alpha\beta$ -tubulin is increased but that of GTP-bound Rheb is decreased. Constitutively active Rheb-overexpressing cells showed an abnormal mitotic progression, suggesting the deacetylated $\alpha\beta$ -tubulin-mediated regulation of Rheb status may be important for proper mitotic progression. Taken together, we propose that deacetylated soluble $\alpha\beta$ -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.

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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 $\alpha\beta$ -tubulin functions as an unconventional Rheb regulator by affecting GTP-bound status

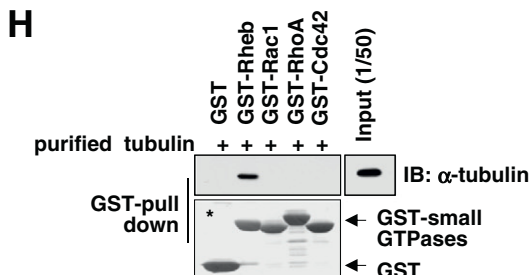
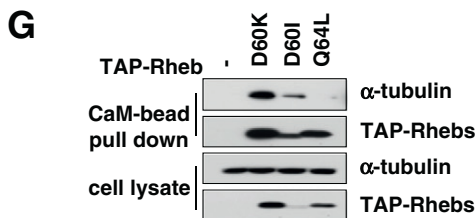
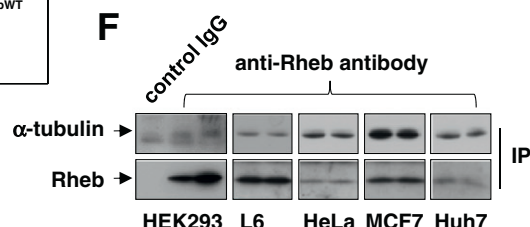
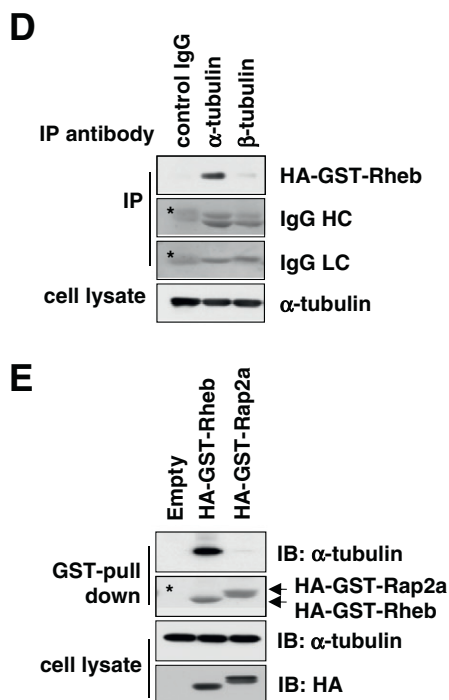
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2. Materials and methods

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



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