The Rheb family of GTP-binding proteins

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Received 22 February 2004; accepted 17 March 2004
Available online 1 June 2004

Abstract

Rheb proteins represent a novel and unique family of the Ras superfamily GTP-binding proteins that is conserved from yeast to human. Biochemical studies establish that they bind and hydrolyze GTP. Molecular modeling studies reveal a few structural differences between Rheb and Ras, which may suggest that residues involved in biochemical activities differ between the two G-proteins. The function of Rheb has been studied in a number of organisms that point to the involvement of Rheb in cell growth and cell cycle progression. In addition, studies in fungi suggest that Rheb is involved in arginine uptake. Further studies in Drosophila and mammalian cells have shown that the effects of Rheb on growth and cell cycle progression are mediated by the effect on the insulin/TOR/S6K signaling pathway. These studies have also shown that a complex consisting of the tuberous sclerosis gene products, Tsc1/Tsc2, serves as a GAP activating protein (GAP) for Rheb, implying Rheb’s role in this genetic disorder. Finally, Rheb proteins have been shown to be farnesylated and small molecule inhibitors of protein farnesyltransferase can block the ability of Rheb to activate the TOR/S6K signaling.

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Keywords: Rheb; Tsc; GTPase; TOR/S6K signaling; Arginine uptake; Farnesylation

1. Introduction

The Ras superfamily G-proteins regulates a variety of biochemical reactions inside the cell [1]. The Ras family proteins are mainly involved in cell proliferation and differentiation, while the Rho family proteins regulate cell morphology and motility. The Rab and Arf family members affect protein transport and exocytosis.

The Rheb family GTP-binding proteins define a novel and unique family within the Ras superfamily G-proteins [2–4]. Recently, Rheb has received significant attention, partly because Rheb proteins play critical roles in regulating growth and cell cycle, and that this effect is due to its role in the insulin/TOR/S6K signaling pathway [5–7]. Rheb acts downstream of Akt and activates S6K phosphorylation dependent on TOR. Another major interest in Rheb concerns its involvement in tuberous sclerosis complex (TSC), a genetic disorder associated with seizures and mental retardation as well as with the appearance of benign tumors called hamartomas in many different parts of the body including the brain, kidney, lung and skin [8,9]. It was found that the Tsc1/Tsc2 complex functions as a GAP for Rheb, suggesting that overactivation of Rheb is a major cause for this disease [5–7]. Finally, there is significant interest in the farnesylation of Rheb protein. This posttranslational modification is critical for the function of Rheb. Small-molecule inhibitors called farnesyltransferase inhibitors (FTIs), currently evaluated as anti-cancer drugs in clinical trials [10–13], block the function of Rheb.

Recent reviews discussing Rheb have mainly dealt with the link between Rheb and the TOR/S6K signaling pathway as well as tuberous sclerosis [5–7]. However, a review focusing on the Rheb proteins has not been published. In this review, we have provided a comprehensive review on this interesting and important class of GTP-binding proteins.

2. Rheb expression and Rheb homologues

*Rheb* was initially identified as a gene whose expression is increased in rat brain by the treatment involved in the long-term potentiation scheme; rapid and transient induction of *Rheb* mRNA was detected in hippocampal granule cells by seizures and by NMDA-dependent synaptic activity [14].
Rheb expression was also induced in quiescent fibroblast cells after the addition of serum [14]. In addition, Rheb expression was induced in PC12 cells in culture after the addition of EGF or FGF [14]. UV radiation also increases expression of Rheb in human fibroblasts [15].

In humans, a gene corresponding to the original rat Rheb gene is located on chromosome 7 (7q36) [16, 17]. We have identified the second Rheb gene in mammalian cells [18]. This gene is located on chromosome 12 (12q13.12, also called RhebL1) [18]. To distinguish this gene from the original gene, we call this gene Rheb2 [18]. Finally, a pseudogene is found on chromosome 10 (10q11). Rheb1 and Rheb2 proteins share 51% amino acid identity. The Rheb1 gene is expressed as a transcript of approximately 1.2 kb in all adult human tissues with highest levels found in skeletal and cardiac muscle [16]. Rheb2 is also expressed ubiquitously (unpublished).

Homologues of the Rheb gene have been identified in a number of organisms. Three fungi, Saccharomyces cerevisiae, Schizosaccharomyces pombe, and Aspergillus fumigatus contain a single Rheb gene termed rhb1 (S. cerevisiae and S. pombe) and rhhA (A. fumigatus) [2, 19–22]. A single Rheb gene was found in zebrafish (Danio rerio), fruit fly (Drosophila melanogaster), slime mold (Dictyostelium discoideum), and sea squirt (Ciona intestinalis) [2]. Drosophila Rheb protein shares 63% identity and 80% similarity with the human Rheb1 protein. Likewise, 51% identity and 71% similarity are detected between S. pombe and Drosophila Rheb proteins. Human Rheb can functionally replace S. pombe Rheb, suggesting that they have conserved function [22].

3. Rheb is a guanine nucleotide-binding protein and is a GTPase

All Rheb proteins contain G1–G5 boxes, short stretches of sequences involved in the recognition of guanine ring and phosphates of guanine nucleotides (Fig. 1) [23]. We have identified three key structural features that define the Rheb family [2]. First, an arginine residue corresponding to the glycine at the 12th position of Ras is conserved in all the Rheb homologues. Second, they have very similar effector domain sequences in which 10 of the 17 residues are identical, and 4 of the remaining residues are similar. Third, they all terminate in the CAAX motif (C is cysteine, A is an aliphatic amino acid, and X is the C-terminal amino acid that is usually methionine, alanine, serine, glutamine, or cysteine) that is required for farnesylation. In fact, we as well as others have shown that Rheb is farnesylated in S. cerevisiae, S. pombe, and mammalian cells [2,20,24].

Biochemical activity to bind guanine nucleotides has been reported for rat, S. pombe, and Drosophila Rheb [14,18,25]. Detailed biochemical analysis was carried out with S. pombe Rheb (SpRheb) by Tabancay et al. [25]. Purified S. pombe Rheb protein was shown to bind GDP with a ratio approaching one GTP molecule to a molecule of Rheb. The binding is specific to guanine nucleotides, as excess amount of cold GDP or GTP but not CTP, UTP, or ATP competed with the binding of radioactive GTP. Rheb has intrinsic GTPase activity as detected by the appearance of GDP from radioactive GTP using thin layer chromatography [25]. It has been reported that Rheb GTPase activity has rates comparable to that of Ras [14]. In our hands, however, the GTPase activity appears to be lower than that of Ras (unpublished). Finally, GDP bound to Rheb can be shown to dissociate when excess GTP is added [25]. These data suggest that Rheb shuttles between a GDP-bound form and a GTP-bound form (Fig. 2A).

4. Dominant negative Rheb mutants

Introducing mutations that are known to convert Ras to constitutively active or dominant negative mutants do not confer similar properties in Rheb [19,24,25]. Introducing a Q61L mutation in Rheb does not generate a constitutively active Rheb. In fact, we still detected considerable intrinsic GTPase activity when we introduced this mutation into SpRheb (unpublished). Similarly, introducing S20N mutation into Rheb did not generate a dominant negative mutant.

To identify dominant negative mutants of Rheb, we have screened random mutant Rheb library generated by PCR mutagenesis [25]. The screen we developed was based on the finding that the inhibition of Rheb signaling leads to growth arrest and induction of the fnx1 gene [19,25]. Thus, a strain carrying a reporter construct having lacZ gene under the control of the fnx1 promoter was made. The random
mutant library of SpRheb was expressed in this strain and small blue colonies were screened on a plate containing X-gal. Multiple rounds of screens covering 25,000 transformants led to the identification of D60V mutation. To further improve the potency of dominant negative effect, we substituted D60 to all possible amino acids. This identified D60K and D60I mutations. Expression of these mutants in *S. pombe* led to the accumulation of G0/G1 phase cells [25].

To gain insight into the mechanism of the dominant negative effect, these mutant proteins were purified and their biochemical properties examined [25]. All dominant negative mutants, D60K, D60I, and D60V, lost the ability to bind GTP. D60I and D60V mutants bound GDP, while D60K mutant did not bind GDP. Therefore, it appears that the D60K mutant exists as a nucleotide free form, while D60V and D60I mutants exist as a GDP bound form. These biochemical properties are very similar to those observed with known dominant negative mutants of the Ras superfamily G-proteins [26]. It has been reported that dominant negative mutants of Ras and Rac bind tightly to their GDP/GTP exchange factors (GEFs) and sequester GEF away from its G-protein [27,28]. GEF binds preferentially to a GDP-bound form or a nucleotide free form that mimics an intermediate form during the exchange reaction [26]. From this analogy, it is likely that the mechanism of action of dominant negative effects for the Rheb mutants is a tight binding of GEF. This raises the possibility that a GEF exists for Rheb.

The dominant negative Rheb mutants have been shown to reverse the arginine uptake defect and canavanine resistance of *S. pombe* Tsc mutants, providing a genetic link between Tsc and Rheb [29]. In addition, dominant negative mutants of human Rheb1 provided a valuable reagent to establish that Rheb is a component of the insulin/mTOR/S6K signaling pathway in mammalian cells [25].

5. Tsc1/Tsc2 complex acts as a GTPase-activating protein for Rheb

One of the recent excitements concerning Rheb is the identification of the Tsc1/Tsc2 complex as a GTPase-activating protein for Rheb [30–34]. Mutations in the TSC1 and TSC2 genes are responsible for tuberous sclerosis, a genetic disorder characterized by the appearance of benign tumors called hamartomas [35,36]. TSC1 and TSC2 gene products, called tuberin and hamartin, respectively, form a complex and function together. Tsc2 has a region of interaction with hamartin at its N-terminus. At the C-terminus, there is a domain common to Ras GTPase activating proteins [36]. This domain, called the GAP domain, suggested that Tsc2 is a GAP protein. It has been reported that Tsc2 functions as a GAP for Rap1 [37] or Rab5 [38]. Recent studies, however, established that the Tsc1/Tsc2 complex functions as a GAP for Rheb.

Tsc1/Tsc2 complex immunoprecipitated from HEK293 cells after their expression exhibits dramatic stimulation of Rheb GTPase activity [30,31]. The GAP activity appears to require both Tsc1 and Tsc2, as only a small increase of GTPase was observed with Tsc2 or Tsc1 alone [30]. The GAP activity also requires the full-length Tsc2 protein, as neither the N-terminal nor C-terminal Tsc2 co-expressed with Tsc1 exhibited GAP activity [31]. Introducing a mutation N1651S found in tuberous sclerosis patients inactivated the GAP activity [30]. Similarly, introducing R1701Q or R1703Q mutation into Tsc2 destroyed the GAP activity [31]. These results point to the idea that it is the Tsc1/Tsc2 complex that exhibits the Rheb GAP activity. Direct interaction between Rheb and Tsc2 has been reported; the C-terminal fragment of Tsc2 expressed in 293T cells was pulled down in vitro by GST-Rheb loaded with a nonhydrolyzable analogue of GTP [32]. A number of studies report that Tsc1/Tsc2 affects the ratio of GTP/GDP bound to Rheb [30–33]. Expression of Tsc1/Tsc2 in HEK293T cells led to a significant decrease of the GTP/GDP ratio bound to Rheb. Importantly, expression of the N-
terminal domain of Tsc2 had no significant effect [31]. As a complementary approach, the ratio of GTP/GDP was examined in Tsc2-null MEFs. Significantly elevated levels of GTP/GDP ratio were observed in the Tsc2-null MEFs compared to the control MEFs [33].

Of particular interest is whether the GAP domain (GRD) is sufficient to exert the GAP activity. It has been reported that the GAP domain of Drosophila Tsc2 purified after expressing in E. coli exhibits GAP activity on Rheb [34]. However, this is the only report demonstrating that the GAP domain alone exhibits GAP activity. We have not been able to observe GAP activity of the GRD from Drosophila Tsc2 (unpublished). Further work is needed to understand whether other regions of Tsc2 are also involved in the GAP activity.

It has been observed that the ratio of GTP/GDP bound to Rheb in mammalian cells is unusually high [39]. This was shown by immunoprecipitating Rheb from mammalian cell lines using anti-Rheb antibody and examining the ratio of GTP/GDP. It was found that approximately 25% of Rheb had bound GTP, a rather high percentage compared with Ras and Rap proteins [39]. Furthermore, increasing Rheb expression by transfecting Rheb led to a further increase of the GTP/GDP ratio [39]. This may suggest that the amount of GAP is limited or that the GAP activity of Tsc1/Tsc2 complex is under tight regulation.

6. Molecular modeling of Rheb and comparison with Ras

Molecular modeling of Rheb highlights similarities and differences between Rheb and Ras proteins. We sought to predict the structure of Rheb by putting amino acid sequences of Rheb into the known three-dimensional structures of Ras [40] and Rap [41] using the molecular modeling program SWISS-MODEL [42]. The structure as compared to that of Ras is shown in Fig. 2B. The overall structure of Rheb is similar to that of Ras. However, there are some differences. For example, in one region (shown by a white arrow), two turns of the α-helix are detected with Rheb, while three turns are found at this position in Ras. In addition, the region close to the GTP-binding site is more tightly packed in Rheb compared to Ras. Together with the fact that Rheb has an arginine at the position corresponding to glycine-12 of Ras, these results may suggest that residues involved in GTP binding and hydrolysis differ from those of Ras protein.

7. Overview of Rheb function in a variety of organisms

Initially, it has been reported that Rheb plays a role in the Ras signaling pathway. Rheb interacts with Raf kinase [24,43] and that it acts in an antagonistic manner to Ras in the activation of the Ras/MAP kinase cascade [24,39].

<table>
<thead>
<tr>
<th>Organism</th>
<th>Gene</th>
<th>Function</th>
<th>Essential or nonessential</th>
</tr>
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<tbody>
<tr>
<td>Mammalian</td>
<td>Rheb1, Rheb2 (RhebL1)</td>
<td>Cell cycle; growth</td>
<td>Essential</td>
</tr>
<tr>
<td>D. melanogaster</td>
<td>dRheb</td>
<td>Cell cycle; growth</td>
<td>Essential</td>
</tr>
<tr>
<td>S. pombe</td>
<td>rhb1</td>
<td>Cell cycle; growth; arginine uptake</td>
<td>Essential</td>
</tr>
<tr>
<td>S. cerevisiae</td>
<td>RHB1</td>
<td>Arginine uptake; virulence</td>
<td>Nonessential</td>
</tr>
<tr>
<td>A. fumigatus</td>
<td>rhaA</td>
<td>Arginine uptake</td>
<td>Nonessential</td>
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A. fumigatus, S. cerevisiae, S. pombe, or D. melanogaster has a single Rheb gene, while mammalian cells have two Rheb genes. In A. fumigatus, S. cerevisiae, and S. pombe, Rheb functions to regulate uptake of amino acids, arginine in particular. In S. pombe, Drosophila, and mammalian cells, Rheb regulates cell growth and cell cycle progression. Rheb genes in S. cerevisiae and A. fumigatus are nonessential, while those in S. pombe and Drosophila are essential. Further work is needed to understand whether Rheb genes in mammalian cells are essential genes.

Rheb inhibited B-Raf kinase activity and prevented B-Raf-dependent activation of Elk-1 transcription factor [39]. Rheb expression also interfered with the ability of activated H-ras to transform NIH3T3 cells [24]. In Xenopus oocytes lysates, Rheb inhibited Ras activation of MAPKs [24]. Recent studies in yeast, Drosophila and mammalian cells, however, point to the role of Rheb in arginine uptake and in the insulin/TOR/S6K signaling pathway. No genetic interactions between Rheb and Ras have been detected in yeast [2,19].

Table 1 summarizes the functions of Rheb detected in a variety of organisms. In S. cerevisiae, S. pombe, and A. fumigatus, accumulating evidence points to the idea that Rheb is involved in the uptake of arginine. Studies in S. pombe, Drosophila, and mammalian cells revealed another function of Rheb to control cell growth and cell cycle progression. This function is essential for the growth of these organisms. These points will be further discussed below.

8. Rheb is involved in arginine uptake in fungi

Studies in yeast suggested that Rheb is involved in arginine uptake. S. cerevisiae cells with a Rheb disruption was viable but exhibited hypersensitivity to canavanine, a toxic analogue of arginine [2]. It was then shown that the rhei disruptants exhibit increased uptake of arginine and lysine [2]. The effects of the rhei disruption on arginine uptake appears to be due to the effect on Can1 permease, as the rhei disruption effect is not observed when Can1 is deficient [2]. How then does Rheb affect arginine uptake? Although further work is needed, this may involve the Btn2 protein that has recently been shown to interact with Rheb [44]. Interestingly, btn2 mutants exhibit hypersensitivity to canavanine [44].
Rheb also affects arginine uptake in *S. pombe*. Mutants of *S. pombe* defective in protein farnesyltransferase, *cpp1*, exhibit hypersensitivity to canavanine and increased uptake of arginine [20]. To demonstrate that these phenotypes are due to the defect in the farnesylation of Rheb, a mutant form of Rheb that can bypass farnesylation was expressed in the *cpp1* mutant. The farnesylation bypass mutant was generated by changing the C-terminal amino acid to leucine so that the protein can be geranylgeranylated instead of farnesylated. Expression of this mutant suppressed canavanine hypersensitivity of the *cpp1* mutant [20].

Involvement of Rheb in arginine uptake in *S. pombe* was also gleaned from the studies on tsc mutants. *S. pombe* has *Tsc1* and *Tsc2* genes [45]. Like mammalian Tsc proteins, Tsc1 and Tsc2 form a complex [45]. One striking phenotype of the *Tsc*-deficient mutants is that they exhibit increased resistance to canavanine and decreased uptake of arginine [29]. Expression of dominant negative Rheb in the *tsc* mutants suppressed these phenotypes; the canavanine resistance was suppressed and arginine uptake was increased [29]. *Tsc* mutants also exhibit decreased uptake of leucine and adenine [45]. Interestingly, these mutants were also shown to exhibit mislocalization of an amino acid permease [45].

Effects of Rheb in arginine uptake were also observed in *A. fumigatus* [46]. This was shown by the increased uptake of arginine when the *rhbA* gene was disrupted [46]. The *rhbA* gene was able to complement the canavanine hypersensitivity of *S. cerevisiae rhl1* mutant [21]. Interestingly, the *rhbA* mutant exhibited reduced virulence in a model of invasive pulmonary aspergillosis [46].

9. Rheb plays critical roles in the regulation of cell growth and cell cycle progression in *S. pombe*, *Drosophila*, and mammalian cells

*Rheb* is an essential gene in *S. pombe* [19,22]. Conditional inhibition of Rheb expression was achieved by placing the gene under the control of the *nmt* promoter that is repressible by the addition of thiamine. This led to an accumulation of cells in the G0/G1 phase and growth inhibition [19,22]. The accumulated cells are small and resemble those observed after nitrogen starvation [19,22]. In fact, *fnx1* and *mel2*, genes that are induced by nitrogen starvation, were induced by shutting down Rheb expression [19].

Similar involvement of Rheb in cell growth and cell cycle progression was observed by inhibiting Rheb expression in a *Drosophila* tissue culture cell line S2 [18]. When Rheb expression was inhibited by the use of siRNA, we observed dramatic accumulation of G0/G1 phase cells [18]. Forward scatter analysis showed that the cells accumulated had small size [18]. On the other hand, overexpression of Rheb in S2 cells led to a significant increase of S-phase cells and a slight increase in cell size [18]. Thus, Rheb functions at the boundary of G1 and S phases.

*Drosophila Rheb* mutants were identified independently by three groups [18,47,48]. In our case, P-element insertion mutant collections were screened to identify genes whose overexpression in the hindgut causes a morphological alteration of this organ [18]. This led to the identification of an allele designated AV4 that has a P-element inserted in the 5’ upstream region of the *Drosophila Rheb* gene [18]. Use of the GMR-GAL4 driver enabled overexpression of Rheb in developing imaginal disc, which resulted in a dramatic increase in the size of head and eyes [18]. Overexpression of Rheb also promoted growth in the wing and in the salivary gland [18]. In the case of the wing, the Rheb overexpression led to the increase of wing cell size, as it was possible to count the number of wing cells by counting the number of hairs [18]. Fly clones having a deficiency in Rheb expression in the head and eye have been generated [18]. These clones had small heads and eyes. *Rheb* is an essential gene and homozygous *Rheb* mutants stop growing after reaching the first instar during larval development [18,47,48]. Presumably, maternal supply of Rheb is enough to drive development through embryogenesis.

10. Rheb is a component of the insulin/TOR/S6K signaling

Characterization of *Drosophila Rheb* mutants provided evidence that Rheb is a component of the insulin/Tor/S6K signaling pathway. We have found that heterozygous *Rheb* mutants exhibit hypersensitivity to rapamycin, an inhibitor of Tor; the time for eclosion was delayed more in the *Rheb* mutant compared to the wild type by the addition of rapamycin [18]. Epistasis analysis showed that Rheb is a component of this signaling pathway [47,48]. Co-expression of Rheb suppressed *PTEN*-mediated growth inhibition in the adult eye [47,48]. Similarly, Rheb expression suppressed growth inhibition observed when Tsc1–Tsc2 was overexpressed [47,48]. The lethality of *Tsc1*-null flies was partially rescued by Rheb heterozygosity [34]. Rheb overexpression effect was suppressed by a mutation in TOR [47,48]. In addition, Rheb overexpression effect was partially attenuated by a mutation in S6K [47,48]. Reduced activity of S6K was observed in larvae extracts of the Rheb mutants, while Rheb expression resulted in an increase in S6K activity [48]. Effects of Rheb in the activation of S6K were also demonstrated using *Drosophila* tissue culture cells [47,48].

Involvement of Rheb in the insulin/TOR/S6K signaling pathway was further investigated in mammalian cells. Transient transfection of Rheb1 or Rheb2 in HEK293 cells results in the increase of the phosphorylation of ribosomal S6 kinase (S6K) [25,30–33]. Increase of the phosphorylation of S6 as well as 4E-BP1 was observed [30,31,33]. In contrast, Rheb did not stimulate Akt phosphorylation [30,33]. The increase of S6K activity by the transient
expression of Rheb was inhibited by the addition of rapamycin but not by wortmannin, an inhibitor of PI3K [25,30–35]. The use of our dominant negative Rheb mutants provided convincing evidence that Rheb is a component of the insulin/TOR/S6K signaling pathway [25]. COS7 cells were starved for serum and nutrients, and then stimulated by the addition of serum. This led to the activation of S6K as detected by its phosphorylation. However, expression of dominant negative Rheb mutant, Rheb1D60K, blocked serum-induced activation of S6K [25]. Similarly, amino-acid-induced activation of S6K was blocked by the expression of dominant negative Rheb [25].

The overall picture of the involvement of Rheb in the insulin/mTOR/S6K signaling pathway is shown in Fig. 3. During serum activation of this signaling pathway, insulin or IGF1 is received by their respective receptors at the cell surface. This results in the activation of PI3K, which then phosphorylates Akt. The activated Akt phosphorylates Tsc2 and inhibits the ability of Tsc1/Tsc2 to negatively regulate mTOR. The inhibition of Tsc1/Tsc2 leads to the activation of Rheb, which then causes activation of mTOR. At least two downstream events, activation of p70S6K and phosphorylation of S6, as well as phosphorylation of 4E-BP1 contribute to the increase in protein synthesis and progression of cell cycle [49]. Nutrients such as amino acids also stimulate this signaling pathway, and this is believed to be mediated by Tsc1/Tsc2 [50]. It is conceivable that a GEF protein also plays a role in mediating serum and nutrient signals to Rheb. Tsc2 also plays critical roles in mediating cellular energy response to control cell growth and this is catalyzed by the AMP-activated protein kinase (AMPK) [51].

11. Significance of Rheb in genetic disorders and cancers

Tuberous sclerosis (TSC) is a genetic disorder with a prevalence of 1 in 6000 to 10,000 births [8,9]. Most TSC patients develop hamartomas, typically benign tumors, in the brain, skin, kidneys, and heart [8,9]. Neurological disorders such as mental retardation, autism, and seizures are also common. Positional cloning led to the identification of two genes responsible for this disorder. TSC1 is located on chromosome 9 (9q34) and encodes a protein called hamartin, while Tsc2 is on chromosome 16 (16q13.3) and encodes a protein called tuberin [35,36]. Mutations in either TSC1 or TSC2 gene cause symptoms that are similar, although TSC2 mutation symptoms are more severe. The finding that Rheb serves as a target of Tsc1/Tsc2 GAP activity clearly points to the significance of Rheb in the pathogenesis of this genetic disorder. In support of this idea, constitutive activation of S6K is detected in Tsc-null MEFs [52,53].

Significance of Rheb may not be limited to a genetic disorder, because the insulin/Akt/mTOR signaling pathway is activated in a number of human cancers. For example, PTEN mutations have been identified in a significant percentage of prostate cancer [54]. This leads to the activation of Akt. Her2/neu is overexpressed in a significant percentage of breast cancer and that the Her2 overexpression correlates with increased pathogenicity of breast cancer [55]. One of the major downstream events of Her2/FTI (farnesytransferase Inhibitor)

Fig. 3. Rheb is a component of the insulin/mTOR/S6K signaling pathway in mammalian cells. Rheb functions upstream of mTOR but downstream of Akt. Activation of the insulin signaling by insulin or IGF1 leads to the activation of PI3K leading to the activation of Akt. Akt then phosphorylates Tsc2 and inhibits negative regulation of Rheb. This then leads to the accumulation of GTP-bound Rheb that results in the activation of mTOR. Tsc2 is also regulated by AMPK. Farnesytransferase inhibitor can block activation of the insulin/mTOR/S6K signaling by blocking farnesylation of Rheb.
neu is the activation of Akt. Akt activation is also detected in multiple myeloma [56]. It will be interesting to survey a variety of cancers to see whether Rheb activation is observed.

12. Inhibition of Rheb function by the use of farnesyltransferase inhibitors

Because of its significance in genetic disorders and possibly cancer, Rheb represents a critical target for therapy. One of the ways to inhibit Rheb function is to inhibit its membrane association by blocking its protein farnesylation.

Significance of farnesylation for the function of Rheb has been demonstrated by a variety of experiments. In S. cerevisiae, Rheb is involved in arginine uptake and canavanine sensitivity, and this function requires farnesylation, as mutations of the CAAX motif of S. cerevisiae Rheb result in the loss of Rheb function to suppress canavanine hypersensitivity of the rheb disruption mutants [2]. In S. pombe, Rheb is required for cell growth and cell cycle progression, and this function also requires farnesylation, as Rheb CAAX mutants do not suppress growth inhibition due to the inhibition of Rheb expression [22]. In mammalian cells, the ability of Rheb to activate S6K has been established. This function is dependent on the presence of the CAAX motif, as Rheb CAAX mutants cannot activate S6K [30,32].

Farnesyltransferase inhibitors have been developed as anticancer drugs and are currently evaluated in clinical trials [10–13]. A variety of inhibitors including CAAX competitive inhibitors, FPP competitive inhibitors, as well as zinc chelators have been identified [10–13,57]. Recent studies suggest that FTI is effective in blocking activation of the insulin/TOR/S6K signaling. When cells were treated with FTI, activation of S6K by Rheb was blocked [32]. FTI also blocked nutrient stimulation of S6K (unpublished). These results raise the possibility that FTI provides an effective means to inhibit the TOR/S6K signaling. The ability of FTI to inhibit the TOR/S6K signaling has previously been reported [58].

13. Concluding remarks

Rheb has emerged as a GTP-binding protein playing critical roles in cell growth and cell cycle progression in Drosophila and mammalian cells. This effect is mediated by its involvement in the insulin/TOR/S6K signaling pathway. The major question to be addressed is how Rheb activates the TOR kinase. Does Rheb directly interact with TOR? The answer awaits further characterization of proteins interacting with mTOR. Recently, TOR complexes have been identified [59]. Identification and characterization of proteins that are components of these complexes may provide hints about how Rheb influences TOR kinase.

Understanding how Rheb is regulated is critical to gaining insight into its function. While we have significant knowledge about its negative regulator, Tsc1/Tsc2, little is known about a positive regulator RhebGEF. Our work on dominant negative Rheb mutants raises the possibility that a GEF exists. Identification of this putative GEF is one of the immediate goals in the study of Rheb.

Another major question concerns how Rheb affects arginine uptake and whether this function is mediated by the TOR signaling pathway. Studies in fungi clearly imply that Rheb as a key regulator of amino acid uptake, and that this appears to be mediated by the effect of Rheb on amino acid permeases. Further genetic studies in these organisms may provide insight into whether TOR is involved in this effect.

Acknowledgements

We thank members of the Tannano laboratory for discussions. In particular, we acknowledge Chia-Ling Gau and Dr. Nitika Parmar for providing information on Rheb2. This work is supported by NIH grants CA41996 and CA32737.

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