

Characteristics Determination of Rheb Gene and Protein in Raini Cashmere Goat

Research Article

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ABSTRACT

The aim of the present study was to determine characteristics of Rheb gene and protein in Raini Cashmere goat. Comparative analyses of the nucleotide sequences were performed. Open reading frames (ORFs), theoretical molecular weights of deduced polypeptides, the protein isoelectric point, protein characteristics and three-dimensional structures was predicted using online standard softwares. The full cDNA nucleotide sequence shares 99%, 99%, 99% and 94% identity with Inner Mongolia Cashmere goat, cattle, horse and human, respectively. The deduced Rheb protein of Raini Cashmere goat consist of 184 amino acid residues and its predicted molecular weight was 20478 g/mol for the unmodified protein and the estimated isoelectric point (pI) was 5.59. Results showed that Rheb has a RAS domain starting at the amino acid 4 and ending at the amino acid 170. In conclusion, our data shows the Rheb cDNA is 555 bp in length, including a complete ORF corresponding to a polypeptide of 184 amino acids. Our results in Raini Cashmere goat showed that there are 1 N-glycosylation sites, 3 protein kinase C phosphorylation sites, 2 casein kinase II phosphorylation sites, 4 microbodies C-terminal targeting signals, 2 ATP/GTP binding sites motif A (P-loop) and a prenyl group binding site for predicted Psites of Rheb. Raini Cashmere goat Rheb protein also had an interaction with other predicted proteins. Hence, can suggest that Rheb has probably role in goat cells and must detect in future investigations.

KEY WORDS base sequence, goat, interaction, isoelectric point.

INTRODUCTION

Goat production is one of the key elements contributing to the economy of farmers living in the arid and semi-arid regions including most areas of Iran (Mohammad Abadi *et al.* 2009). Raini goat is one of the most important Iranian native goats that spread in the southeast of Iran where these animals are kept for both meat and cashmere production. It has long white, black, or yellow hair and the mean weight of the fleece produced is 400 g ranging from 130 to 1,100 g per head (Moghbeli *et al.* 2013). Mean body weight at 12 months for male and female is 18.7 ± 0.04 and 15.5 ± 0.04 respectively (Hasani *et al.* 2010).

However, these economic characters are quantitative in nature, being under the control of several genes plus the environment (Askari *et al.* 2011). Rheb (Ras homolog enriched in brain) gene belongs to Ras family that encodes a carboxyl terminal CAAX box indicating that the protein may undergo post-translational farnesylation. Ras homolog enriched in brain (Rheb) is a key regulator of the mammalian target of rapamycin (mTOR) complex 1 (mTORC1) signaling pathway (Dunlop *et al.* 2009). Rheb is a member of the Ras superfamily GTPases and shares the highest homology with Ras and Rap and originally identified as immediate early gene (IEG) in 1994, encoding 184 amino acids with a deduced molecular mass of 20497

Da in hippocampus (Yamagata *et al.* 1994) The Rheb gene is highly conserved in eukaryotes from yeast to mammals (Patel *et al.* 2003). Genetic studies of fly and fission yeast indicate that Rheb plays an important role in the stimulation of cell growth and regulation of G0/G1 cell cycle progression (Yang *et al.* 2001).

The growth arrest phenotype caused by Rheb mutation in *Schizosaccharomyces pombe* can be complemented by human Rheb (Yang *et al.* 2001), suggesting the conservation of Rheb function from yeast to human. The precise physiological functions of Rheb were unknown in high eukaryotes until recently. Both genetic studies in *Drosophila melanogaster* and biochemical studies in mammalian cells have shown that Rheb is involved in signal transduction pathways that regulate cell growth (Tee *et al.* 2003). Homozygous inactivation of Rheb is lethal, while mosaic analyses of Rheb mutant cells in *Drosophila* show that the inactivation of Rheb decreases cell size. In contrast, overexpression of Rheb increases cell size (Yang *et al.* 2001). Genetic epistatic analysis demonstrates that Rheb functions between TSC1-TSC2 and TOR.

The evidences show that Rheb regulates mTOR through direct binding of Rheb-GTP to mTOR to promote activation of mTOR (Long *et al.* 2005). Other study demonstrated that FKBP38 can bind to mTOR to inhibit its activity and Rheb-GTP interacts directly with FKBP38 to prevent FKBP38 associating with mTOR (Ma *et al.* 2008).

However, comprehensive biochemical characterization of the Rheb/FKBP38 interaction using three different *in vitro* assays has not detected an interaction between Rheb and FKBP38. Therefore, the mechanism of the interaction between Rheb and FKBP38 is still under debate and needs to be further characterized. Although the Rheb gene has been identified in mice (NM_053075) and humans (NM_005614), its physiological function has not been fully investigated and remains to be clearly defined. In farm animals, (Zheng *et al.* 2011) studied Rheb cDNA in Inner Mongolia Cashmere goat and showed that it has 555 bp in length, including a complete ORF corresponding to a polypeptide of 184 amino acids. Rheb gene was expressed in all the tested tissues; the highest level of mRNA accumulation is detected in brain tissue.

Furthermore, it has demonstrated that molecular studies are important in farm animals breeding (Javanmard *et al.* 2008; Mohammadabadi *et al.* 2010; Mohammadi *et al.* 2009; Ruzina *et al.* 2010).

So far, no study concerning the characterization of Rheb gene in Iranian farm animals, especially in Raini Cashmere goat has been published. Hence the aim of the present study was to determine for the first time characteristics of Rheb gene and protein in Raini Cashmere goat and compare with other species.

MATERIALS AND METHODS

Tissues including brain, heart, lung, pancreatic, spleen, kidney, liver and testis were collected from the Raini Cashmir goat after slaughter (3 repeats from any tissue). Extracted RNA were immediately stored at -80 °C. Quality and quantity of RNA were evaluated and cDNA was synthesized and PCR was performed. PCR Products were electrophoresed on 1.5% agarose gel and were evaluated different levels of expression in studied different tissues using SPSS (2011) software at (P<0.01) (Tohidi nezhad *et al.* 2015). The PCR products of Rheb gene from Raini Cashmere goat were sequenced. Comparative analyses of the nucleotide sequences were performed online at National Center for Biotechnology Information (NCBI). Predictions of theoretical molecular weights of deduced polypeptides were made by the protein property calculator (Kyte and Doolittle, 1982). Input data for this software was peptide sequences and outputs were predicted molecular weight, extinction coefficient and approximate volume. The protein isoelectric point was predicted by the calculation of protein isoelectric point (Kozłowski, 2016), in which was added amino acids sequence and was received estimated isoelectric point (pI). The domain of Raini Cashmere goat Rheb protein predicted with SMART software (Schultz *et al.* 1998; Letunic *et al.* 2015) and the switch I region and the switch II region were predicted with NCBI CDD program (Marchler-Bauer *et al.* 2011).

Input data for these softwares was protein sequence and outputs were basic amino composition, total number of negatively and positively charged residues, its domain starting and ending at what amino acid and indicate switch regions. Prediction of protein characteristics and three-dimensional structures provided by Molecular Bioinformatics Center of National Chiao Tung University (Chen *et al.* 2006; Chen *et al.* 2009) in which was added the query sequence in FASTA format and was received three-dimensional structure of Rheb for Raini Cashmere goat and its sites. The STRING program used for representing predicted protein interactions (Szklarczyk *et al.* 2015). Input data for this software was protein name and organism and outputs were interaction with other predicted proteins and description of predicted functional partners.

RESULTS AND DISCUSSION

The deduced Rheb protein of Raini Cashmere goat consist of 184 amino acid residues and its predicted molecular weight was 20478 g/mol for the unmodified protein and the estimated isoelectric point (pI) was 5.59. Extinction coefficient and approximate volumes were 16050 cm⁻¹m⁻¹ and 24778 A³ respectively.

The basic amino composition is given in Table 1 and the total number of negatively charged residues (Asp+Glu) and the total number of positively charged residues (Arg+Lys) were 22 and 19 respectively. Rheb has a RAS domain starting at the amino acid 4 and ending at the amino acid 170, including a switch I region from amino acid 36 to 43 and a switch II region from amino acid 62 to amino acid 80 (Figure 1). Protein characteristics and three-dimensional structures of Rheb for Raini Cashmere goat are shown in Figure 2. Our results in Raini Cashmere goat (Figure 2) showed that there are 1 N-glycosylation sites, 3 protein kinase C phosphorylation sites, 2 casein kinase II phosphorylation sites (SAKE and TAVD), 4 microbodies C-terminal targeting signals, 2 ATP/GTP binding sites motif A (P-loop) and a prenyl group binding site (CAAX box) for predicted Psites of Rheb. Raini Cashmere goat Rheb interaction with other predicted proteins and description of predicted functional partners using the STRING program is given in Figure 3. The most interaction exists functionally between Rheb and tuberous sclerosis complex 2 (TSC2), mammalian target of rapamycin (mTOR) and tuberous sclerosis complex 1 (TSC1) respectively and the least was seen between Rheb and protein kinase B1 substrate 1 (AKT1S1) and elongation initiation factor 4E (EIF4E). [Zheng et al. \(2011\)](#) for Inner Mongolia Cashmere goat also achieved that protein consist of 184 amino acid residues and its predicted molecular weight is 20358 Da for the unmodified protein and the estimated isoelectric point (pI) is 6.27. The basic amino acids comprise 10.3% Ser, 9.2% Ile, 9.2% Val, 7.6% Lys and 7.6% Leu that confirmed results in this study. Protein characteristics and three-dimensional structures of Rheb for Raini Cashmere goat are same as results of [Zheng](#)

[et al. \(2011\)](#) for Inner Mongolia Cashmere goat. Three-dimensional structures of Rheb for Raini Cashmere goat (Figure 3) is very similar to the structure of *Mus musculus* Rheb presented by [Mazhab-Jafari et al. \(2012\)](#).

[Mazhab-Jafari et al. \(2012\)](#) proposed that whereas providing an Asn thumb as a means of accelerating catalysis, TSC2GAP may also stimulate the GTPase activity of Rheb by relieving autoinhibition and aligning Rheb's catalytic machinery.

Interaction of TSC2 with Rheb switch I may disrupt the electrostatic contact between Tyr35 and the γ -phosphate, reducing the autoinhibitory effect of this residue on GTP hydrolysis, explaining the functional and thermodynamic similarities between WT Rheb in the presence of the TSC2GAP and the Rheb Y35A mutant alone ([Mazhab-Jafari et al. 2012](#)) that confirmed results in this study. TSC1 and TSC2 form a dimeric complex that has tumor suppressor activity and TSC2 is a GTPase activating protein (GAP) for Rheb. The TSC1/TSC2 complex inhibits the activation of TOR kinase through Rheb. Rheb has also been shown to induce the formation of large cytoplasmic vacuoles in a process that is dependent on the GTPase cycle of Rheb, but independent of the target of rapamycin (TOR) kinase, suggesting Rheb plays a role in endocytic trafficking that leads to cell growth and cell-cycle progression. Most Ras proteins contain a lipid modification site at the C-terminus, with a typical sequence motif CaaX, where a, indicates an aliphatic amino acid and X, indicates any amino acid. Lipid binding is essential for membrane attachment, a key feature of most Ras proteins. The 15 amino acid in Ras protein is glycine instead of arginine in goat Rheb amino acid sequence.

Table 1 Amino acid composition of Rheb gene in Raini Cashmere goat

Amino acid full name	Amino acid name with 3 letters	Amino acid name with 1 letter	Number	%
Alanine	Ala	A	11	5.9
Arginine	Arg	R	5	2.7
Asparagine	Asn	N	6	3.2
Aspartic acid	Asp	D	9	4.9
Cysteine	Cys	C	1	0.5
Glutamine	Gln	Q	10	5.4
Glutamic acid	Glu	E	13	7.1
Glycine	Gly	G	12	6.5
Histidine	His	H	3	1.6
Isoleucine	Ile	I	18	9.7
Leucine	Leu	L	13	7.1
Lysine	Lys	K	14	7.6
Methionine	Met	M	5	2.7
Phenylalanine	Phe	F	7	3.8
Proline	Pro	P	4	2.1
Serine	Ser	S	19	10.3
Threonine	Thr	T	9	4.9
Tryptophan	Trp	W	1	0.5
Tyrosine	Tyr	Y	8	4.3
Valine	Val	V	16	8.6

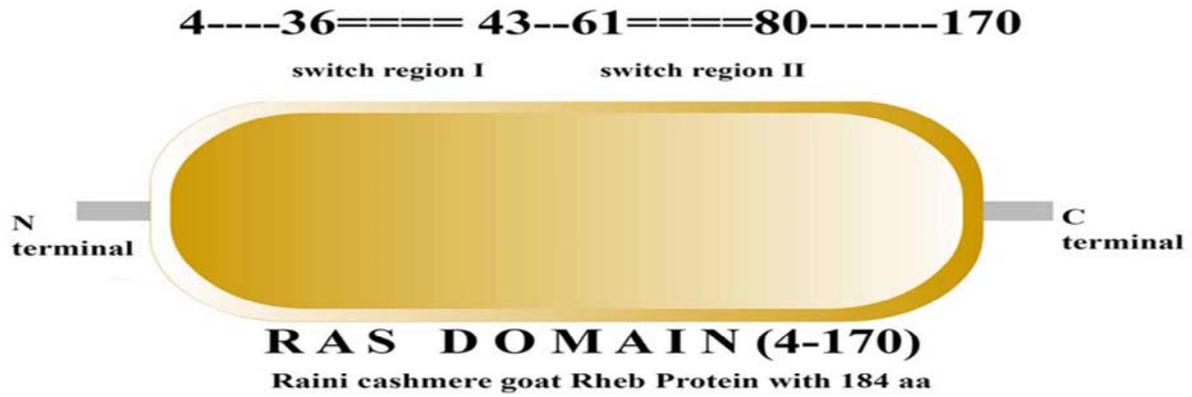


Figure 1 The predicted domain of Raini Cashmere goat Rheb protein (the RAS domain ranges from amino acid 4 to 170, including a switch region I starting from the amino acid 36 to the amino acid 43 and a switch region II from amino acid 61 to 80)

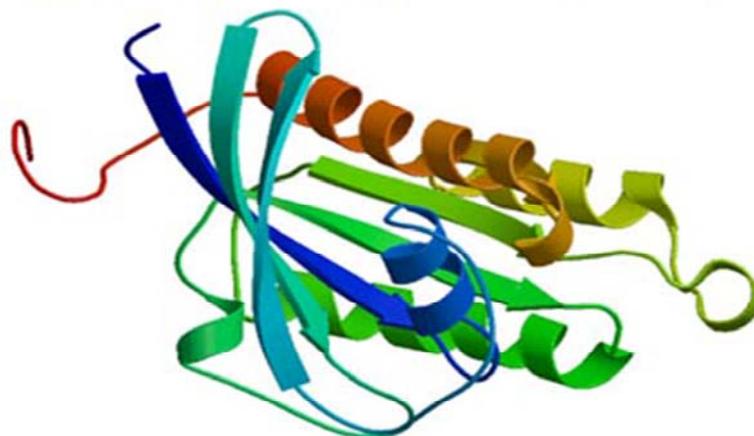
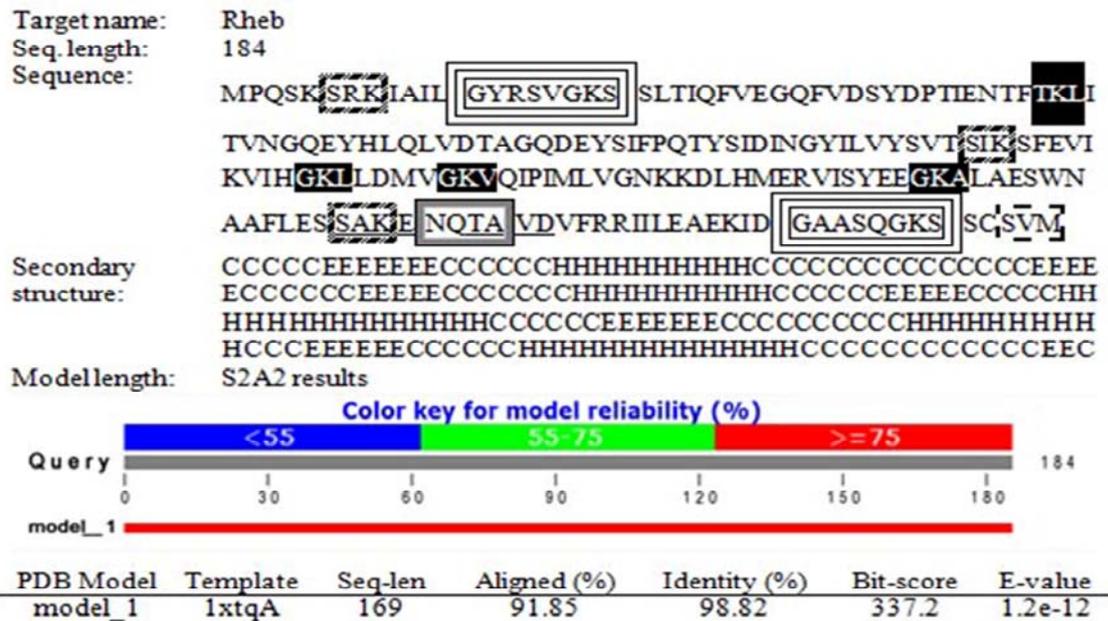
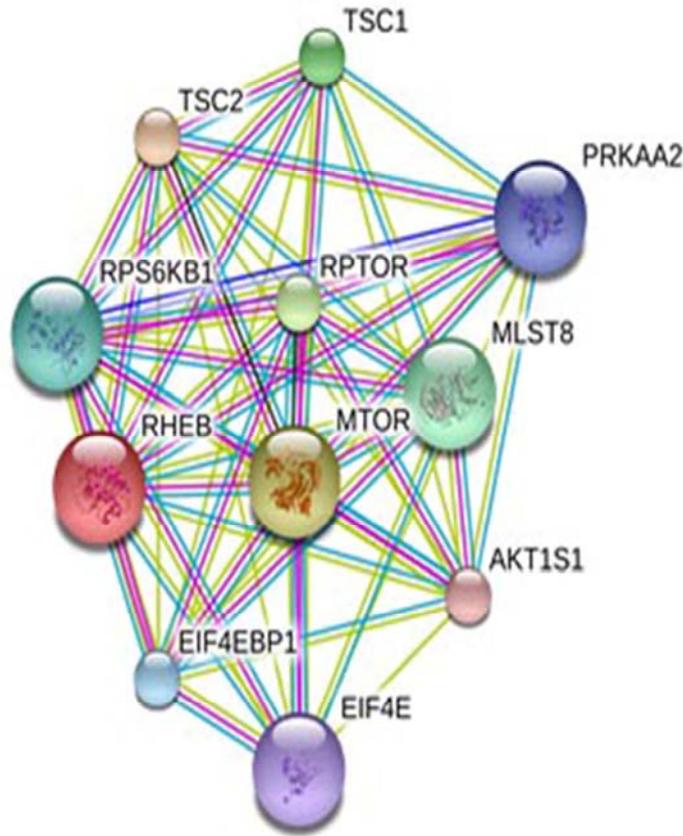


Figure 2 Protein characteristics and three-dimensional structures of Rheb for Raini Cashmere goat

NA indicates N-glycosylation sites; SK indicates protein kinase C phosphorylation sites; indicates casein kinase II phosphorylation sites; indicate microbodies C-terminal targeting signals; GS indicates ATP/GTP binding sites motif A (P-loop) and SVM indicates a prenyl group binding site (CAAX box) for predicted Psites



Input:

Rheb

| Neighborhood
| Gene Fusion
| Cooccurrence
| Experiments
| Databases
| Textmining
| [Homology]

Score

Predicted Functional Partners:

	TSC2	tuberous sclerosis 2 (1815 aa)					● ● ●	0.999
	MTOR	mechanistic target of rapamycin (serine/threonine kinase) (2550 aa)					● ● ●	0.999
	RPTOR	regulatory associated protein of MTOR, complex 1 (1335 aa)					● ● ●	0.998
	TSC1	tuberous sclerosis 1 (1167 aa)					● ● ●	0.997
	MLST8	MTOR associated protein, LST8 homolog (<i>S. cerevisiae</i>) (326 aa)					● ● ●	0.981
	RPS6KB1	ribosomal protein S6 kinase, 70kDa, polypeptide 1 (525 aa)					● ● ●	0.975
	EIF4EBP1	eukaryotic translation initiation factor 4E binding protein 1 (118 aa)					● ● ●	0.967
	PRKAA2	protein kinase, AMP-activated, alpha 2 catalytic subunit (462 aa)					● ● ●	0.964
	EIF4E	eukaryotic translation initiation factor 4E (217 aa)					● ● ●	0.961
	AKT1S1	AKT1 substrate 1 (proline-rich) (256 aa)					● ● ●	0.957

Figure 3 Raini Cashmere goat Rheb interaction with other predicted proteins and description of predicted functional partners using the STRING program

Different line colors represent the types of evidence for the association

Line thickness relates to combined score

Colors describe the type of evidence

The replacement leads to lower basal GTPase activity in Rheb than that of Ras and result in a higher GTP level, which is required for S6K1 phosphorylation via mTOR because the Rheb is an upstream regulator of S6K1 and mTOR. Meanwhile, the goat Rheb protein has a prenyl group binding site (CAAX box) where X is methionine at the C-terminal. The CAAX box in Rheb is farnesylated and the membrane localization of Rheb through farnesylation is important for upstream regulation of S6K1 activity in mTOR signaling pathway (Tee *et al.* 2003), that this replacement was seen in results of this study and confirmed results of other researchers. Besides, the deduced goat Rheb protein has G1 box to G5 box and two switch regions in its Ras family conservative domain (Figures 2 and 3). All indicated features are very similar to reported results about Rheb in Inner Mongolia Cashmere goat and other species, indicating that Rheb gene has been correctly studied from Raini Cashmere goat.

The Rheb switch II is also critical for signaling to the target of mTOR although the mechanism is unclear. As shown above, goat Rheb has a Ras family conservative domain containing switch I region from amino acid 36 to 43. The switch I region and FKBP-C domain may have interaction and regulate the activity of mTOR signaling path way in goat cells, but need to be further verification.

CONCLUSION

In conclusion, Rheb cDNA is 555 bp in length, including a complete ORF corresponding to a polypeptide of 184 amino acids. Rheb employs an autoinhibitory mechanism maybe maintain a high activation state in cells essential for the proper maintenance of mTORC1 signaling and cellular growth. This is the first proposed bioinformatics study of Rheb in Raini Cashmere goat within the Ras subfamily, which may be relevant to some other Ras superfamily and provides a view into the molecular and bioinformatic mechanism of Rheb.

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