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Molecular characterization of RNA binding motif protein 3 (RBM3) gene from Pashmina goat

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ABSTRACT

Pashmina goat inhabits the high altitude cold arid desert of Ladakh, India. This goat is known for its finest and costliest under fiber. Though the under fiber may be a part of its complex thermoregulation mechanism, the genetics of its adaptability under cold conditions is not known. As an attempt to understand its adaptive genetics, and the role of RNA-binding proteins at the cellular response, this study was conducted to characterize the RBM3 gene in Pashmina goat and its expression during hypothermia. The ORF of Pashmina RBM3 gene was 273 bp. Phylogenetic analysis revealed that Pashmina RBM3 is closely related to *Bos taurus* RBM3. Pashmina RBM3 was characterized by comparative modeling studies. The final 3-D model contained two α-helices and four β-sheets. qRT-PCR data showed that Pashmina RBM3 gene expression was significantly higher ($P < 0.05$) at moderate (30 °C) hypothermic stress conditions as compared with deep (15 °C) hypothermia.

1. Introduction

Pashmina (Cashmere) goat (*Capra hircus*) is found at higher altitudes of the Himalayas in Northern India, Nepal and Pakistan. In India, Pashmina goat is mainly found in the Ladakh region of Jammu and Kashmir (Banerjee, 1991). Ladakh is located in Himalayan range of mountains at an altitude of about 3000–6000 m above the mean sea level. The annual rainfall in this area is quite low (up to 8–9 cm) and the temperature levels fluctuate between +35 °C in summers and –40 °C in winters. The high wind velocity with low precipitation rate, low humidity, low oxygen tension and fluctuating temperature makes this cold arid high altitude region hostile for man as well as to livestock species. Under such harsh environmental conditions, Pashmina goats still manage to survive by adapting different adaptation strategies.

In general organisms have developed sophisticated strategies to adapt themselves to various environmental temperature shifts. In mammals as well as other organisms, cold stress changes the lipid composition of cellular membrane, suppresses the rate of protein synthesis and ultimately the cell growth (Aloia and Raison, 1989; Burdon, 1987; Rao and Engelberg, 1965; Watanabe and Okada, 1967). In eukaryotic cells, gene expression is regulated both at transcriptional as well as post transcriptional level (Burd and Dreyfuss, 1994). All these processes are mediated by various RNA-binding proteins and by small RNAs as stable ribonucleoprotein (RNP) complexes (Dreyfuss, 1986; Gorlach et al., 1993; Mattaj, 1990). RNA-binding proteins have been known to play important roles in the cellular response at low temperatures. In case of *E. coli*, the CspA destabilizes RNA secondary structures during hypothermia and thereby facilitate translation (Bae et al., 1997). In *Xenopus* oocytes, Y-box binding proteins contain a cold-shock domain which controls translation during hypothermia (Matsumoto and Wolffe, 1998) whereas in case of mammals, two cold-induced RNA-binding proteins, CIRBP and RBM3, were found to function during hypothermia (Dresios et al., 2005; Lleonart, 2010; Smart et al., 2007). Both proteins are structurally highly similar and belong to the glycine rich RNA-binding protein family. Members of this family are characterized by one RNA recognition motif adjacent to a C-terminal arginine and a glycine-rich domain. Moreover, both CIRBP and RBM3 are thought to be the modulators of gene expression during mild hypothermic conditions and have been proposed to function as mRNA chaperone molecules to help maintain or enhance protein translation during periods of cellular stress (Danno et al., 1997; Nishiyama et al., 1997; Smart et al., 2007). Hypothermia generally down-regulates protein synthesis and metabolism in mammalian cells (Fujita, 1999; Giuliodori et al., 2004; Golovlev, 2003; Gualerzi et al., 2003; Homma et al., 2003; Murata et al., 2006; Phadtare, 2004; Phadtare et al., 1999; Ulusu and Tezcan, 2001; Weber and Marahiel, 2003) but the

Abbreviations: RBM3, RNA binding motif protein 3; RBP, RNA binding protein; CIRBP, cold induced RNA binding protein; qRT-PCR, quantitative real-time PCR; ORF, open reading frame; RNP, ribonucleoprotein; cDNA, complementary DNA; RMSD, root mean square deviation; CDD, Conserved Domain Database; PDB, Protein Database

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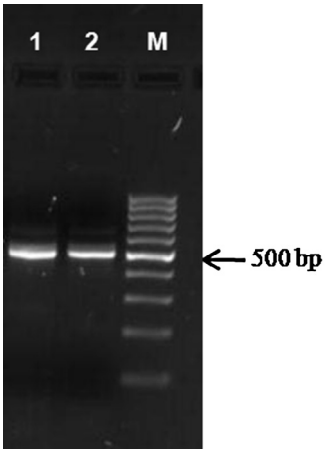

Fig. 1. PCR amplification of Pashmina RBM3 gene. Lane 1–2: RBM3 PCR Amplicon; lane M: 100 bp DNA Ladder.

expression levels of CIRBP and RBM3 was found to be induced and up-regulated under these conditions (Danno et al., 2000; Dresios et al., 2005; Nishiyama et al., 1998; Smart et al., 2007). Additionally RBM3 transcripts were also found to be elevated in hibernating animals during winter (Williams et al., 2005).

Keeping in view the uniqueness of the Pashmina goat for its commercial fine fiber, its adaptability to extreme cold arid conditions and the importance of the RBM3 as a molecular chaperon in hypothermia, this study was aimed at molecular characterization of the RBM3 gene from Pashmina goat and its comparative expression profile under moderate and deep hypothermic conditions. The Bakerwal breed of goat from sub-tropical climatic region was included as a contrast for comparative gene expression under cold stress.

2. Materials and methods

2.1. Animal and blood sample collection

Pashmina and Bakerwal goats maintained at Mountain Sheep and Goat Research Station of Sher-e-Kashmir University of Agricultural

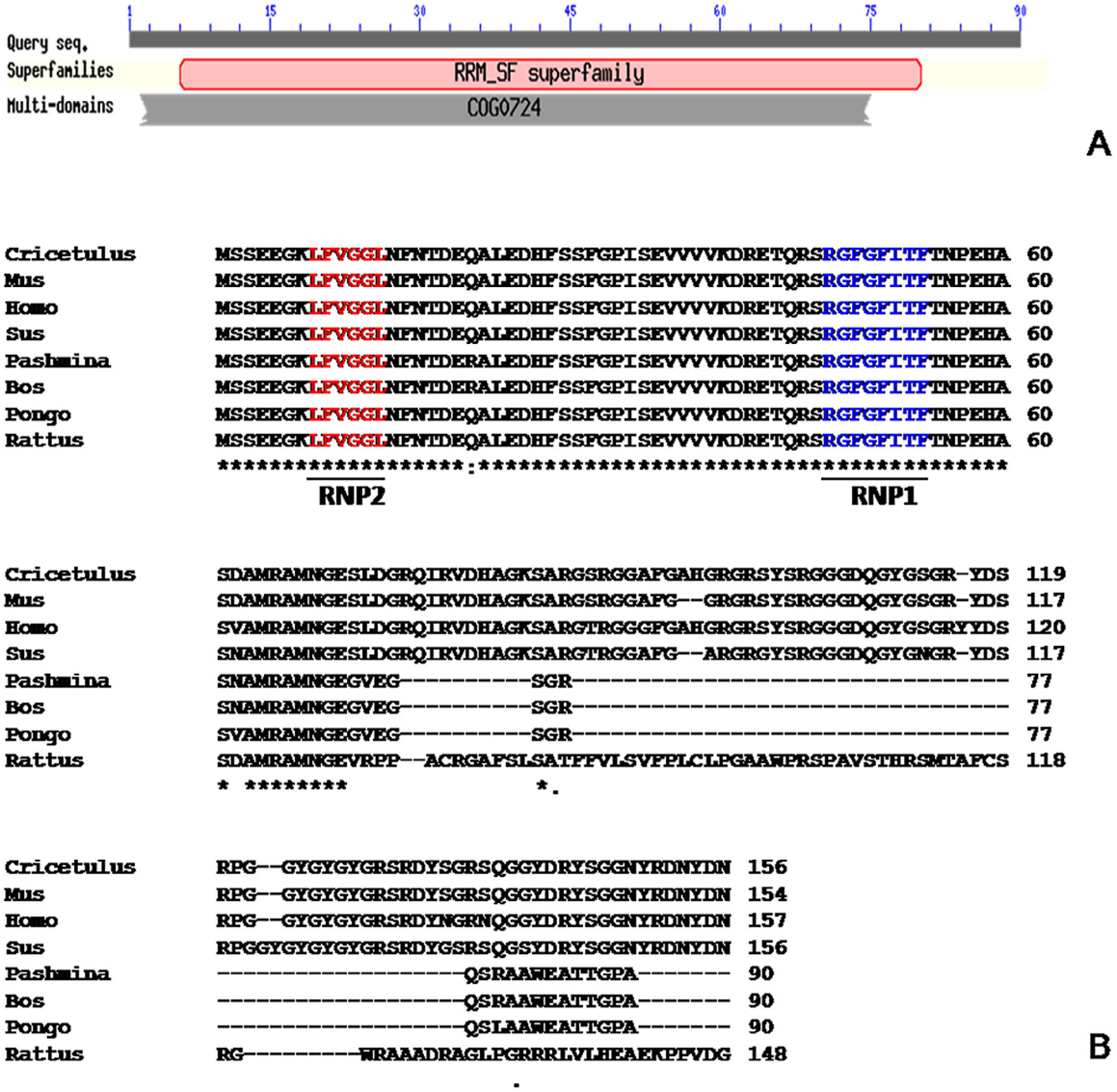

Fig. 2. (A) Conserved Domain Database (CDD) search for Pashmina RBM3 on the NCBI server. (B) Multiple sequence alignment of deduced amino acid sequence of RNA binding motif protein 3 from different animals. RNP1 and RNP2 Conserved motifs were highlighted with red and blue colors respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)