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Computational analysis of human and mouse CREB3L4 Protein

Kiran Kumar Velpula[1§], Azeem Abdul Rehman[2§], Soumya Chigurupati[1], Ramadevi Sanam[3], Krishna Kishore Inampudi[4] & Chandra Sekhar Akila[5*]

[1]Burnett School of Biomedical Sciences, College of Medicine, University of Central Florida, 4000 Central Florida Blvd, Orlando, FL 32816; [2]Bradley University, Peoria, IL, 61615; [3]Informatics Division, GVK Biosciences Pvt Ltd, Hyderabad, India; [4]Department of Chemistry, University of Hyderabad, India 500046; [5]Department of Biotechnology, School of Life Sciences, Yogi Vemana University, Kadapa, Andhra Pradesh, India -516003; Chandra Sekhar Akila – Email: chandrasekhar9@yahoo.com, acsekhar@yogivemanauniversity.ac.in; Phone: 0091-8562-225426 & 225435 (O); Fax: 0091-8562-225419; *Corresponding author
§Both authors contributed equally

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Abstract:
CREB3L4 is a member of the CREB/ATF transcription factor family, characterized by their regulation of gene expression through the cAMP-responsive element. Previous studies identified this protein in mice and humans. Whereas CREB3L4 in mice (referred to as Tisp40) is found in the testes and functions in spermatogenesis, human CREB3L4 is primarily detected in the prostate and has been implicated in cancer. We conducted computational analyses to compare the structural homology between murine Tisp40α human CREB3L4. Our results reveal that the primary and secondary structures of the two proteins contain high similarity. Additionally, predicted helical transmembrane structure reveals that the proteins likely have similar structure and function. This study offers preliminary findings that support the translation of mouse Tisp40α findings into human models, based on structural homology.

Background:
The CREB/ATF family contains transcription factors that regulate various processes, including cell proliferation, differentiation and apoptosis **[1-4].** Members of the CREB/ATF family are characterized by their control of gene expression through the cAMP-responsive element sequence **[5].** Moreover, these proteins contain a conserved transmembrane region and basic region-leucine zipper (bZip) domain on the C-terminus **[5-8]**. Although particular proteins are ubiquitously expressed in tissues, certain members are tissue specific and organism specific. For instance, the CREB3L4 protein is primarily found in the human prostate **[6]**, whereas mice express CREB3L4, referred to as Tisp40, almost exclusively in the testis **[9-11]**. Nonetheless, CREB3L4 isoforms are cytoplasmic proteins, found embedded in the endoplasmic reticulum **[6, 10]**. Upon activation of CREB3L4, via Golgi protease S1P cleavage, CREB3L4 translocates to the nucleus to regulate DNA targets **[7, 11, 13]**.

Two isoforms of the mouse CREB3L4 protein have been identified, namely Tisp40α and Tisp40β **[10-12]**. Both transcripts vary in size, where Tisp40α contains 315 amino acids and Tisp40β possesses 370 amino acids. This transcript difference in size is due to differing transcription start sites **[11].** Moreover, this difference could result in varying secondary structure elements, ultimately promoting alternative structures. Although Tisp40α is the more abundant form of the CREB3L4 protein in mice testes, Tisp40β is the more potent transcriptional activator **[13].** In contrast, human CREB3L4 contains 395 amino acids, with a similar transcription start site to that of Tisp40β [GenBank AB057281.2]. Furthermore, elevated CREB3L4 expression in humans has been linked to a variety of cancers, including prostate and hepatocellular carcinomas **[6, 13-14].**

Mice offer a valuable experimental model organism for analyzing signaling pathways implicated in human cancer development. However, preliminary examinations must be

conducted in order to insure that results from murine studies can be translated into a human model. Computational approaches involving bioinformatics offer a method for deducing protein homology when comparing factors across organisms. The aim of the current study is to analyze the similarities and differences of CREB3L4 in mice and humans, using tissue location, sequence length, sequence homology, protein binding sites and folding patterns in active sites as parameters for assessing whether knowledge regarding Tisp40α in mice can be extrapolated for human CREB3L4. Structural similarities can reveal functional similarities, as most protein function is ultimately determined by structure.

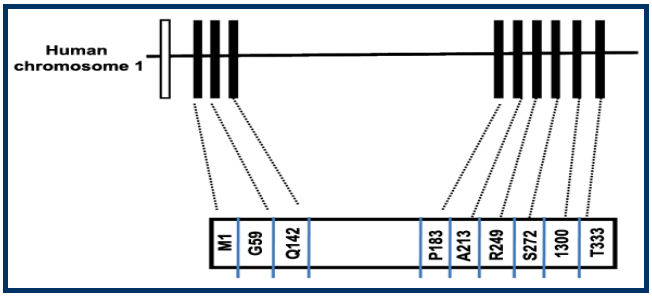

Figure 1: Legend – Structure of the human CREB3L4 gene with the encoded polypeptides. The solid black rectangles illustrate the coding exons while the solid line depicts the non-coding exons. The Genbank accession number for the human CREB3L4 gene is AB052781.2

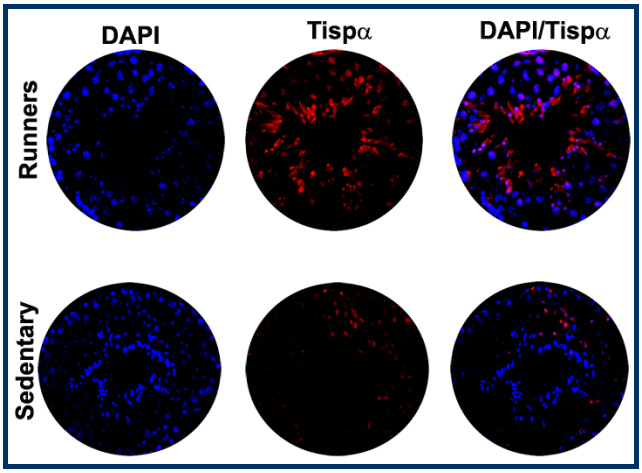

Figure 2: Expression of the Atce1/Tisp40α isoform of CREB3L4 in mouse spermatids in life long runners and sedentary mice.

Methodology:

For immunostaining, frozen testis sections (5 microns) were exposed for 60 minutes to PBS containing 10% normal goat serum (Sigma, St. Louis, MO) and 0.1% Triton X-100 (Research Organics Inc, Cleveland, OH) to block nonspecific antibody binding, followed by incubation overnight with primary antibody for mouse Atce1/Tisp40a Isoform of CREB3L4 at 4°C. After being incubated with Alexa Fluor 568-conjugated IgG (1: 500) secondary antibody and counterstained with 4,6-diamidino-2-phenylindole (DAPI), images were acquired by using Nikon Eclipse E600 fluorescence microscope. Images were processed by using SPOT advance software, Diagnostic Instruments, Sterling Heights, MI and Photoshop CS3 (Adobe Systems, San Jose, CA), with the input levels adjusted to span the range of acquired signal intensities exactly.

Full-length cDNA of mouse and human CREB3L4 were obtained from NCBI GenBank (accession numbers AF287260 and AB052781.2 respectively) while protein sequences were downloaded from Uniprot database (Q9D2A5 and Q8TEY5 respectively). Sequence alignment was conducted using a ClustalW program to identify homologous regions **[15].** Secondary structural similarities were assessed using PHD, a neural network method **[16].** Transmembrane helices were predicted using PHD Helical Transmembrane prediction **[17].**

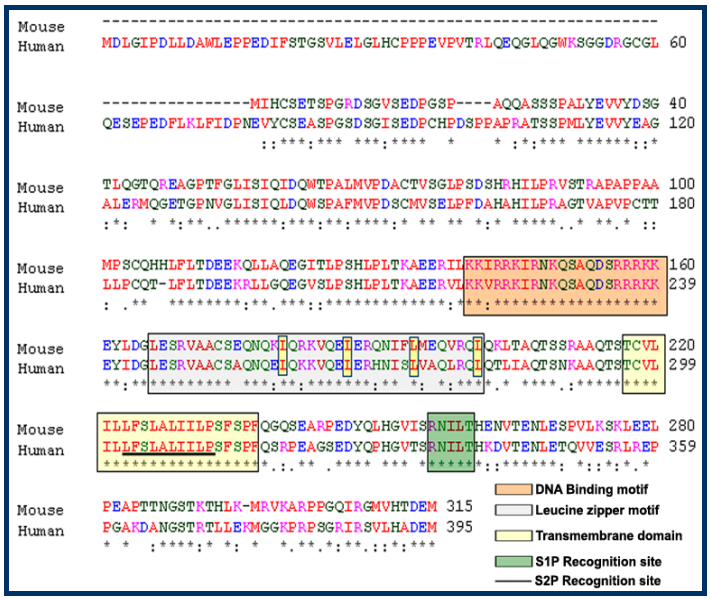

Figure 3: Alignment of the deduced amino acid sequences of CREB3L4 from mouse and human species. '*' represents conserved amino acid acids, ':' represents high similarity, '.' represents low similarity. The deduced, basic regions, leucine zipper motifs and transmembrane regions are indicated in the above sequences. The conserved, repeated leucine residues in the leucine zipper motif are highlighted and the putative S1P recognition sites are boxed.

Discussion:

Similar to the murine CREB3L4 as shown by El-Alfy et al. (2006), the human isoform also contains nine exons **(Figure 1).** However, the human CREB3L4 has only one isoform while the mouse contains two isoforms. Specifically, the human isoform is more similar to mouse Tisp40β as it contains the initial 55 residues which are absent in Tisp40α. Nonetheless, the current study utilized Tisp40α because this particular isoform is more prevalent **[13].**

DAPI staining and fluorescence microscopy reveal that active mice had higher Tisp40α expression in their spermatids compared to sedentary mice. Images suggest that Tisp40α operates as a stress-response molecule during murine spermatogenesis **(Figure 2)**. Detection of the Tisp40α isoform is consistent with a prior study in which only Tisp40α was present in the mice testes **[11].** Zhang and Kaufman (2004) propose that factors containing a basic leucine zipper domain (bZIP) support the maintenance of the endoplasmic reticulum (ER) **[18].** Specifically, bZIP factors initiate the production of proteins utilized by the ER for the synthesis of peptides. Thus, if the onset of activity instigates stress and elevated protein production, greater expression of bZIP factors such as Tisp40α

would likely occur. This reasoning provides an explanation for the elevated Tisp40α shown in the active mice. Moreover, Chigurupati *et al.* (2008) report that exercise in mice alleviates oxidative stress and promotes spermatogenesis and testosterone production. The Tisp40α isoform possibly mediates this effect, as demonstrated by the elevated expression of Tisp40α in running mice **[19].**

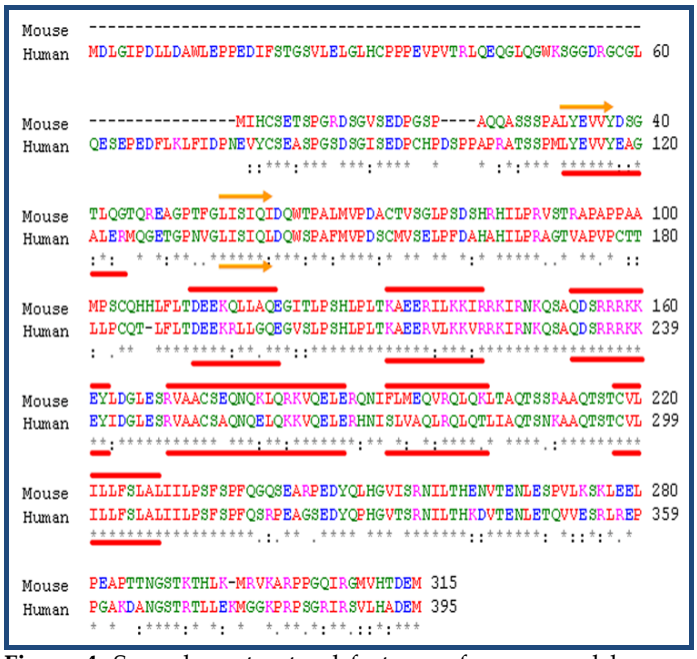

Figure 4: Secondary structural features of mouse and human CREB3L4 protein predicted by the PHD program. Yellow arrows indicate the region of beta strand conformation while red color lines indicate regions with alpha helices.

Human CREB3L4 contains 69% identity and 80% similarity with Mouse Tisp40α **(Figure 3).** Furthermore, three notable features were found in both isoforms: the DNA binding basic region, the dimerized leucine zipper and the putative transmembrane region containing 20 hydrophobic amino acids. The conserved sequence (RXXL), which is speculated to be the consensus recognition motif of S1P, was also prominently present.

According to PHD, both proteins contain a secondary structure that primarily consists of coils and alpha helices. Specifically, mouse Tisp40α showed 31.75% alpha helices, 11.43% beta strands and 56.83% random coils whereas human CREB3L4 showed 34.80% alpha helices, 9.72% beta strands and 55.49% random coils **(Figure 4).** Transmembrane helices, as predicted by the PHD Helical transmembrane (PHDhtm) program, also showed similar secondary structural features **(Figure 5).** Conserved transmembrane helices suggest that the overall folding and resultant function of these proteins are likely similar.

Our results reveal that the genomic organization of human CREB3L4 is very similar to mouse Tisp40α. Although the two proteins are found in different organisms and tissues, the isoforms display very similar secondary structural homology. Comparisons of 3D structures for these proteins are unavailable because the current RCSB database does not contain the structures. Our computational results suggest that the important domains necessary for the function of the protein are well conserved. These proteins likely carry out similar functions, acting as membrane-associated transcription factors with a bZIP domain that mediate DNA binding and dimerization.

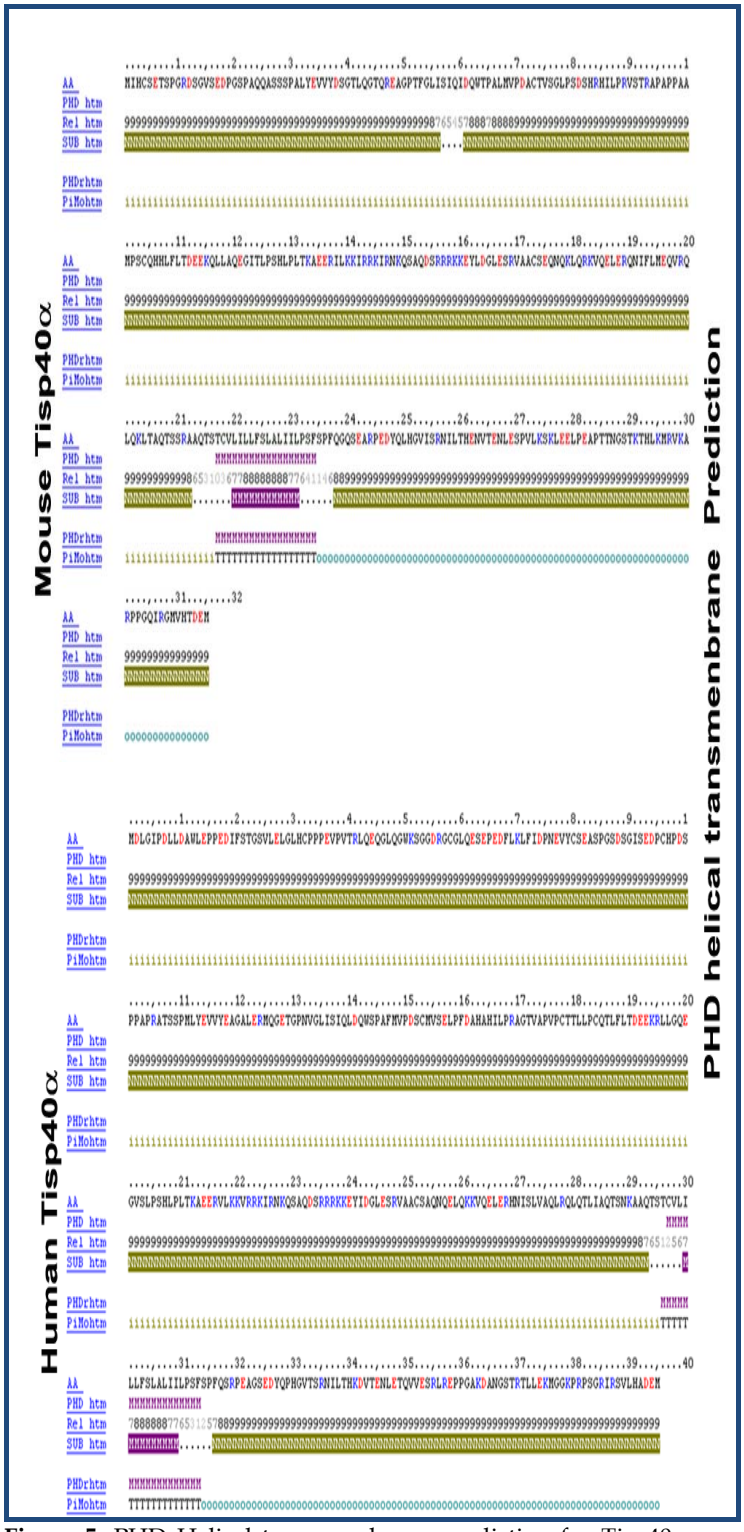

Figure 5: PHD Helical transmembrane prediction for Tisp40α and human CREB3L4. A similar topological prediction for both mouse and human protein is shown, indicating that overall 3D fold might be similar in for both isoforms.

Conclusion:
The present study offers the first preliminary results depicting the homology of mouse Tisp40α to human CREB3L4. We conclude that although both proteins are found in different organisms and tissues, the isoforms likely demonstrate similar mechanisms in regulating gene expression due to their high structural homology. Other studies have mentioned the presence of a pig CREB3L4 that contains a similar genomic organization to the human CREB3L4 **[5].** Further studies examining the homology of the porcine gene would be useful in constructing an evolutionary tree for the CREB3L4 gene. Although mouse Tisp40α is commonly identified in the mouse testes, RT-PCR results have revealed the presence of mRNA transcripts of the gene in the mouse prostate **[10].** Moreover, another study detected human CREB3L4 mRNA transcripts in the human testes **[20],** suggesting that this protein may be expressed in a variety of tissues within a single organism.

Understanding the homology across different isoforms is vital to extrapolating information across organisms. Numerous studies utilize a murine model to analyze interactions in vivo. However, murine models exhibit little use if observations cannot be related to the human population. Structural evidence suggests the similarity existing between mouse Tisp40α to human CREB3L4, despite the two proteins being present in different organisms and tissues. Thus, translating observations collected on Tisp40α in a mouse-based model to human CREB3L4 is plausible, as supported by the results of the study.

Conflict of Interest:
The authors declare no conflict of interest exists with this manuscript.

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