Alternative Splicing of Splicing Regulator Protein, Polypyrimidine Tract Binding Protein 1

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Background

Polypyrimidine-tract binding protein 1 (PTBP1) is essential for embryonic development before gastrulation. It is an important cellular regulator in pre-mRNA splicing as well as in the regulation of alternative splicing events. During neuronal differentiation, PTBP1 regulates many splicing events. PTBP1 has expression in the following tissues: brain, and spinal cord, placenta, bone marrow, colon, kidney, prostate, and spleen. Disease associated with PTBP1 include Alzheimer's disease, ovarian tumors, Parkinson's disease, and glioma. It also contributes to strokes and ALS. PTBP1 contributes to the neuronal differentiating process by switching expression from PTBP1 to PTBP2 during the development of the brain. This is interesting and contributed to the decision of choosing PTBP1 for the reason that it helps understand what makes a neuron a neuron.

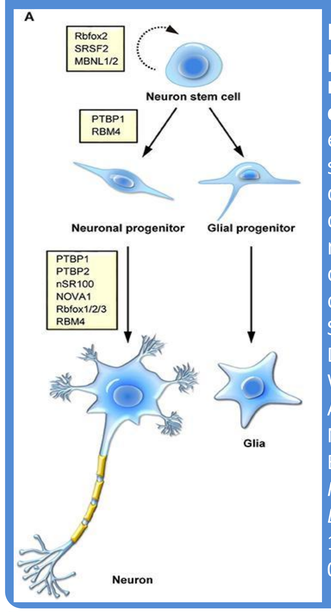


Figure 1. PTBP1 participation in neuronal stem cells. PTBP is expressed in the self-renewing division and fate determination of neuronal stem cells and neuronal cell differentiation. Su Chun-Hao, D Dhananjaya, Tarn Woan-Yuh. 2018. Alternative Splicing in Neurogenesis and Brain Development. *Frontiers in Molecular Biosciences*. 10.3389/fmolb.2018.00012. 2296-889X.

Aim

PTBP1 gene regulates alternative splicing events by operating exon skipping during muscle cell differentiation in its own pre-mRNA and exists in tissues including total brain and the placenta.

Results

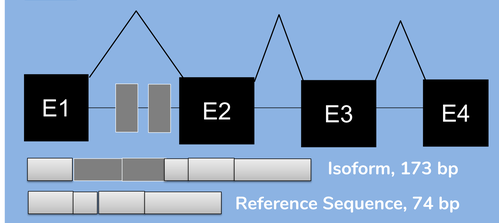


Figure 2. Gene Map: This gene map was derived from Aceview and the Santa Cruz genome browser. Exons and introns of the gene are shown as boxes and lines.

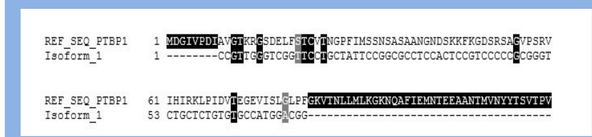


Figure 3. Multiple Alignment. The reference protein sequence and isoform protein sequence for PTBP1 are aligned. Differences between the two protein sequences are represented by the shaded areas.

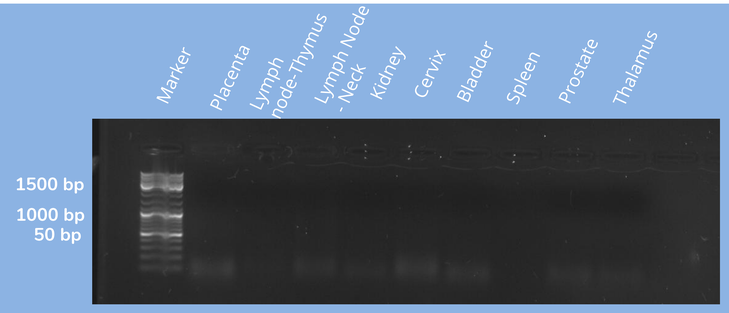
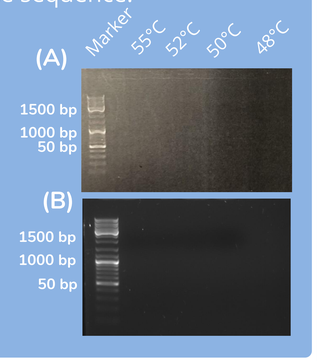


Figure 4.RT-PCR: Gel Electrophoresis shows that PTBP1 is expressed in placenta (35 bp), cervix (32 bp), and bladder (30 bp) for the reference sequence.

Figure 5.RT-PCR: Gel electrophoresis for (A) and (B) did not indicate annealing temperatures for PTBP1. There were not annealing temperatures to be determined for PTBP1. The bp of interest was 174.



Results

Figure 6. Domain structure. The RRM domain is a putative RNA-binding domain of about 90 amino acids known to bind single-stranded RNAs. The reference sequence for PTBP1 has four of them. The predicted isoform is truncated. Therefore, it has none of the four.

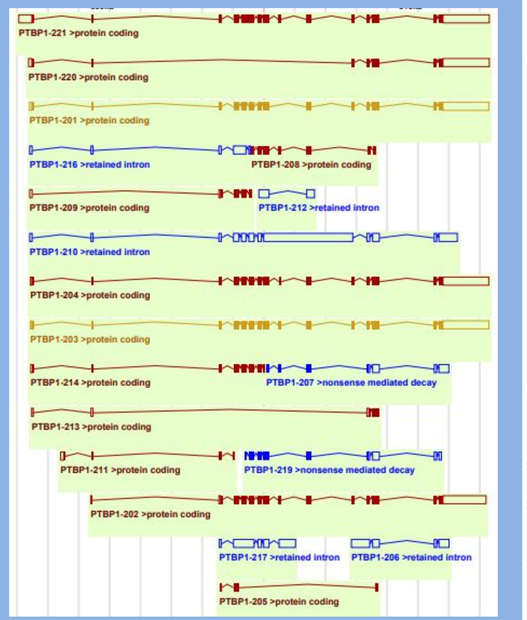


Figure 7. Reference Sequence of PTBP1

Methods

Testing Primers and Determining Annealing Temperatures with cDNA (3 PCR's ran)

1. **Gradient Polymerase Chain Reaction on cDNA using Phusion Master Mix**
   - Annealing temperatures: 43.6°C – 56.0°C
   - PTBP1 forward and reverse primers
   - Total brain in cDNA
2. **Gradient PCR on genomic DNA using Phusion with Betaine, Ethylene Glycol, and DMSO/DTT**
   - 5M Betaine
   - 100% Ethylene glycol
   - 100% DMSO and 200 mM DTT
   - PTBP1 forward primer
3. **PCR on cDNA using Phusion**
   - Same primer used PTBP1
   - DNAs used: Placenta, LN-T, LN-N, Kidney, Cervix, Bladder, Spleen, Prostate, and Thalamus

Reverse Transcription on RNA

1. Reagents used: dNTP mix, Oligo dT, RNase inhibitor, RNase-free water, and RNA
2. Samples: Primer Extension, CDNA synthesis, and termination of reaction

Agarose Gel Electrophoresis

1. Ran on 1.5% Agarose gel
2. For the first and third PCR, gel ran on 30 minutes at 100V; For the second PCR, ran on 45 minutes at 120 V.

Conclusion and Future Directions

The NCBI data predicted one isoform that was missing sequences in comparison to the reference sequence. I was not able to determine the best annealing temperature for PTBP1 because both results for the annealing temperature did not find anything in the gel electrophoresis. I was not able to determine any alternative splicing in the brain region. Although, I observed PTBP1 alternative splicing occurring in the placenta, bladder, and cervix. For future direction, I can test tissues closer to the areas where PTBP1 is located. The predicted RNAs make different proteins depending on the tissue the alternative splicing is occurring in by PTBP1. There must be more tests done to determine if PTBP1's function is affected.

Literature Cited

(1) Su Chun-Hao, D Dhananjaya, Tarn Woan-Yuh. 2018. Alternative Splicing in Neurogenesis and Brain Development. *Frontiers in Molecular Biosciences*. 10.3389/fmolb.2018.00012. 2296-889X.

(2) Liu, G., Bao, X., & Wang, R. (2015). Expression quantitative trait loci regulate HNF4A and PTBP1 expression in human brains. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(30), E3975. doi:10.1073/pnas.1509048112

(3) Fontana, L. 2012. PTBP1 (polypyrimidine tract binding protein 1). *Atlas of Genetics and Cytogenetics in Oncology and Haematology.*

(4) Patton, JG, Mayer, SA, Tempst, P, Nadal-Ginard, B. Genes and development. *Characterization and molecular cloning of polypyrimidine tract-binding protein: a component of a complex necessary for pre-mRNA splicing.* 10.1101/gad.5.7.1237.