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hnRNP A1B, a Splice Variant of HNRNPA1, is Spatially and Temporally Regulated

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ABSTRACT

RNA binding proteins (RBPs) play a key role in cellular growth, homoeostasis and survival and are tightly regulated. A deep understanding of their spatiotemporal regulation (Spatiotemporal gene expression is the activation of genes within specific tissues of an organism at specific times during development.Gene activation patterns vary widely in complexity. Some are straightforward and static, such as the pattern of tubulin, which is expressed in all cells at all times in life.) is needed to understand their contribution to physiology and pathology. Here, we have characterized the spatiotemporal expression pattern of hnRNP A1 and its splice variant hnRNP A1B in mice. We have found that hnRNP A1B expression is more restricted to the CNS compared to hnRNP A1, and that it can form an SDS-resistant dimer in the CNS. Also, hnRNP A1B expression becomes progressively restricted to motor neurons in the ventral horn of the spinal cord, compared to hnRNP A1 which is more broadly expressed. We also demonstrate that hnRNP A1B is present in neuronal processes, while hnRNP A1 is absent. This finding supports a hypothesis that hnRNP A1B may have a cytosolic function in neurons that is not shared with hnRNP A1. Our results demonstrate that both isoforms are differentially expressed across tissues and have distinct localization profiles, suggesting that the two isoforms may have specific subcellular functions that can uniquely contribute to disease progression.

Keywords: RNA Binding Protein, Central Nervous System, Motor Neuron, Amyotrophic Lateral Sclerosis (ALS), hnRNP

Introduction:

The heterogeneous ribonucleoprotein (hnRNP) family is composed of multiple proteins regrouped into 13 families. hnRNP family diversity derives from successive gene duplication events and family members that are more closely related among higher eukaryotes permits diversification of underlying phenotypic differences hnRNPs are characterized by their ability to bind RNA and DNA via their RNA binding domains (RBDs). The function of di hnRNP family members varies by the specificity of their RNA binding properties, the composition of their different domains and their subcellular localization (Krecic and Swanson, 1999; Han et al., 2010b; Geuens et al., 2016; Bampton et al., 2020). Most hnRNP family members contain a nuclear localization sequence (NLS) which mediates their nucleocytoplasmic shuttling. At steady state, hnRNPs are predominantly localized to the nucleus, but are translocated to the cytoplasm following di stimuli or via their recruitment by other RBPs. hnRNPs are also enriched in intrinsically disordered regions (IDRs) which mediate protein-protein interactions and facilitate liquid-liquid phase separation (LLPS), a key event that governs the formation of biomolecular condensates (Geuens et al., 2016; Bampton et al., 2020; Milkovic et al., 2020). RNA binding proteins (RBPs) are critical to all cellular processes and their expression is tightly regulated during cellular diKishore et al., 2010). The misregulation of RBP expression and function is often linked to disease (Lukong et al., 2008; Mittal et al., 2009; Ramakrishnan and Janga, 2019). A further complexity is that many RBPs can be alternatively spliced to generate conserved isoforms having unique functions or localization, even though they share common features (Krecic and Swanson, 1999; Xu et al., 2001, 2019; Sarkar et al., 2003; Liu et al.,

2008; Han et al., 2010a,b; Gueroussov et al., 2017). For example, the inclusion of one exon in PTBP1 or hnRNP D modifies their eGueroussov et al., 2015, 2017). Interestingly, most of the alternative splicing events observed in hnRNPs are located in their IDR, and these events are usually mammalian-specific. For example, hnRNP A and D subfamilies contain mammalian specific alternative exons within their IDRs that are enriched for glycine-tyrosine (GY) in the C-terminus. Those isoforms have different alternative splicing and have altered phase separation properties (Gueroussov et al., 2017). Thus, alterations in RBP isoform usage can impact multiple targets. Moreover, mutations in the IDRs of hnRNP family members are associated with several diseases and frequently result in protein mislocalization and aggregation (Kim et al., 2013; Conlon and Manley, 2017; Purice and Taylor, 2018; Bolognesi et al., 2019; Gui et al., 2019; Batlle et al., 2020). However, studies of RBPs typically focus on only one isoform and the di overlooked. Thus, understanding the function of these alternative isoforms will inform how their misregulation may contribute to di As an RBP, hnRNP A1 contains two RNA recognition motifs (RRM1 and RMM2, collectively referred to as the UP1 domain) each composed of two RBDs, and an RGG box which features repetition of RGG which serves to mediate nucleic acid binding while promoting phase separation.In addition, hnRNP A1 has IDRs that are enriched in glycine glycine-rich domain; GRD) and mediate protein-protein interactions and phase separation, and a C-terminal M9 sequence which determines its nucleocytoplasmic shuttling (Siomi and Dreyfuss, 1995; Ding et al., 1999; Ghosh and Singh, 2020; Martin et al., 2021). HNRNPA1 can be alternatively spliced to create several di ENSG00000135486.17; NCBI, Gene ID 3178), including two that encode for the isoforms hnRNP A1 (NP_002127.1, CCDS: 41793.1, UniProt: P09651-2) and hnRNP A1B (NP_112420.1, CCDS: 44909.1, UniProt: P09651-1). The coding sequence of these two isoforms di spliced exon, referred to as exon 7B, which elongates the GRD by 52 amino acids. This splicing event can be modulated by hnRNP A1 itself and we have also previously shown that it is repressed by TDP-43 in cellular models (Chabot et al., 1997; Deshaies al., 2018). hnRNP A1 is central to RNA metabolism including transcription, splicing, nuclear export, translation, and turnover (Jean-Philippe et al., 2013). However, very little is known about its alternative isoform hnRNP A1B. The tight regulation of RBP expression is crucial for spatiotemporal regulation of RNA and is indelibly linked to development, diKamma et al., 1995; Bronstein et al., 2003; Blanchette et al., 2006; Huang et al., 2010; Sephton et al., 2012; Geuens et al., 2016; Kemmerer et al., 2018). Importantly, the mislocalization of RBPs, such as TDP-43, FUS and hnRNP A1, is a histopathological feature of several neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) (Deshaies et al., 2018; Aksoy et al., 2020; de Boer et al., 2020). However, while the spatial and temporal regulation of TDP- 43 and FUS have been reported (Sephton et al., 2010; Huang et al., 2013), hnRNP A1 and its isoform hnRNP A1B have not. Determining the spatial and temporal expression patterns of hnRNP A1B and hnRNP A1 in tissues will facilitate a better understanding of the function of these two isoforms and enable a better consideration of their relative contributions to disease.Here, we demonstrate that hnRNP A1B and hnRNP A1 are Di development and throughout aging.

Materials and Methods:

Animals

C57BL6/N mice of both sexes were used in this study at various ages. For each time point, three mice were collected for protein and RNA extraction and two mice were perfused for imaging. For protein and RNA extraction, tissues were dissected, snap frozen in liquid nitrogen and stored at -80 c until use.

Cell Culture

Primary cortical neurons were prepared from E18.5 C57BL/6N mouse embryos, exactly as previously published (Khalfallah et al., 2018). CB3 cells are mouse erythroleukemia cells where the endogenous HNRNPA1 allele is inactivated due to a retroviral insertion. These cells were stably transfected

to uniquely express mouse hnRNP A1 or hnRNP A1B cDNA, as previously described (Yang et al., 1994). CB3 cells were cultured in Minimum Essential Media (MEM) Alpha Modification (Fisher Scientific) supplemented with 10% FBS (Life Technologies), 1% Penicillin-Streptomycin (Wisent) and 50 mg/ml geneticin (Life Technologies) to maintain stable expression.

Characterization of hnRNP A1B Antibody

Polyclonal antibodies were commercially generated by Medimabs (Montreal, QC) in rabbits immunized with a synthetic peptide (C-YGGSGSYDSYNNGG), corresponding to amino acids 281- 294 (encoded by exon 7B) of hnRNP A1B (NP_112420.1) (Supplementary Figure 1A). Sera were a the same immunization peptide. To demonstrate specificity, a recombinant mouse hnRNP A1 or hnRNP A1B protein (generously provided by Dr. Benoit Chabot.

Immunohisto chemistry

Antigen retrieval was performed on sections via heating to 90C for 20 min in citrate buffer ph 6.0.Sections were then blocked with 5% donkey serum (Jackson ImmunoResearch) and then sequentially labeled with the indicated primary antibodies: anti-hnRNP A1B (custom, 1:100), anti-hnRNP A1 (4B10,santa cruz,1:100) and donkey anti mouse/rabbit secondaryantibody conjugated with horseradish peroxidase (HRP, Jackson Immuno Research, 1:500). The signal was visualized with the DAB substrate-chromogen kit (Jackson Immuno Research) and slides were imaged with a bright field microscope (Leica DM4000B).

Immunofluore science

Tissue sections were blocked in 3% bovine serum albumin (BSA) in PBS and permeabilized with 0.5% Tween-20 in PBS, before incubation with primary antibody overnight at 4C. Cells grown on coverslips were fixed with 4% PFA/PBS, permeabilized with 0.2% Triton X-100/PBS and blocked with 1%BSA/PBS before incubation with indicated primary antibody (anti-hnRNP A1B). Sections or coverslips were then incubated with the appropriate fluorophore conjugated secondary antibodies for 1h at RT, and then mounted using Prolong antifade reagent (ThermoFisher).

Lysate Preparation

For P28 to 18M mice, cervical and lumbar spinal cords were microdissected on ice and lysed to extract protein and RNA using the Norgen extraction kit (Norgen Biotek, 47700), according to manufacturers protocol .These samples were lysed with the Norgen extraction kit to extract RNA. For protein extraction, samples were lysed in 50 mM Tris pH 7.5, 1 mM EDTA, 150 mM NaCl, to the manufacturer's protocol. Cells were lysed in RIPA buffer with protease inhibitors.

Immunoblot

After protein quantification by BCA (Pierce), equal amounts of 15 µg of lysates were subjected to 12.5% SDS-PAGE separation. Proteins were transferred to nitrocellulose membranes which were then blocked with 5% powdered milk/PBS-T before incubation with primary antibodies: anti-hnRNP A1B (custom; 1:2000), anti-actin (MP Biomedical, 1:10 000), anti-hnRNP A1 (4B10, Santa Cruz, 1:1 000), anti-TDP-43 (Proteintech, 1:10 000), anti-tubulin (Abcam, 1:1000). Membranes were then incubated with appropriate HRP-conjugated secondary antibody (Jackson ImmunoResearch, 1:5000) and signal was revealed by ECL (ThermoFisher Scientific). Acquisition was done on CL- Xposure radiography films (ThermoFisher Scientific), BIO-RAD ChemiDOC MP imaging system, or LiCor. Mean intensity was measured using Photoshop (Adobe) and normalized to a loading control.

qRT-PCR

Equal amounts of RNA were reverse transcribed using the QuantiTect Reverse Transcription kit (Qiagen).

Size Exclusion Chromatography

Size exclusion chromatography (SEC) was performed as previously described (Sévigny et al., 2020). Mouse brains of P18 mice were processed using a Dounce homogenizer in homogenization buffer.

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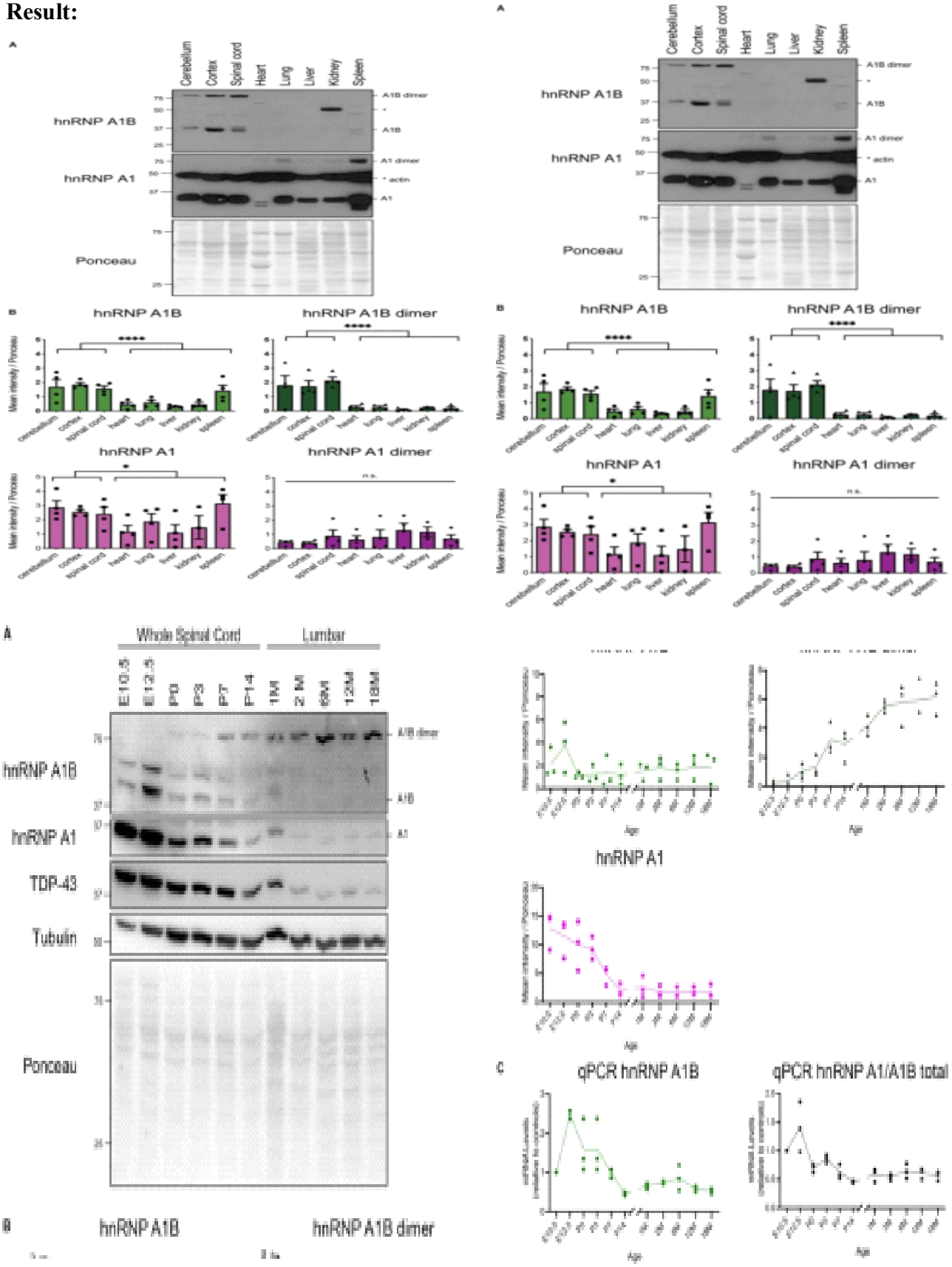
Result:



Analysis and Statistics:

For immunohistochemistry images, the intensity of hnRNP A1 and hnRNP A1B was measured in arbitrary units across a segment with FIJI

Conclusion :

This study provides the first detailed spatiotemporal characterization of two isoforms of hnRNP A1 throughout development and aging. The sustained presence of hnRNP A1B in the processes of neurons suggests that hnRNP A1B has a novel function that is not shared with hnRNP A1. It will inform studies of their misregulation in disease contexts. Finally, our results reinforce the idea that RBP isoform usage, expression and localization are continuously regulated and that a deeper understanding of RBP spatiotemporal regulation is critical to inform on physiology and pathology.

Ethics Statement:

The animal study was reviewed and approved by Institutional Committee for the Protection of Animals (CIPA) of the Centre de Recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM).

References:

1. Aksoy, Y. A., Deng, W., Stoddart, J., Chung, R., Guillemin, G., Cole, N. J., et al. (2020).
2. "STRESSED OUT": The Role of FUS and TDP-43 in Amyotrophic lateral sclerosis. Int. J. Biochem. Cell Biol. 126:105821. doi: 10.1016/j.biocel.2020. 105821
3. `Allemand, E., Guil, S., Myers, M., Moscat, J., Cáceres, J. F., and Krainer, A. R. (2005). Regulation of heterogenous nuclear ribonucleoprotein A1 transport by phosphorylation in cells stressed by osmotic shock. Proc. Natl. Acad. Sci. U.S.A. 102, 3605–3610. doi: 10.1073/pnas.0409889102
4. Arbour, D., Vande Velde, C., and Robitaille, R. (2017). New perspectives on amyotrophic lateral sclerosis: the role of glial cells at the neuromuscular junction. J. Physiol. 595, 647–661. doi: 10.1113/jp270213
5. Bampton, A., Gittings, L. M., Fratta, P., Lashley, T., and Gatt, A. (2020). The role of hnRNPs in frontotemporal dementia and amyotrophic lateral sclerosis. Acta Neuropathol. 140, 599–623. doi: 10.1007/s00401-020-02203-0
6. Barbosa-Morais, N. L., Irimia, M., Pan, Q., Xiong, H. Y., Gueroussov, S., Lee, L. J.,et al. (2012). The evolutionary landscape of alternative splicing in vertebrate species. Science 338, 1587–1593. doi: 10.1126/science.1230612