Differential Subcellular Distributions of hnRNP A2/B1 Spliceoforms

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Introduction

Background

mRNA trafficking in neural cells is extremely dynamic and controlled (reviewed in 1,2). One factor involved in neural mRNA trafficking is heterogeneous nuclear ribonucleoprotein (hnRNP) A2/B1 (3).

hnRNP A2/B1 is needed for myelination and synaptic regulation (4), and is heavily involved in mRNA processing (reviewed in 5,6). This makes hnRNP A2/B1 isoforms essential in post-transcriptional regulation in both the nucleus and cytoplasm.

Alternative splicing of A2/B1 pre-mRNA produces 4 isoforms as shown in Figure 1. The isoforms are differentially expressed in different tissue types (7), at different points of the cell cycle (8,9), and in various disease states (10-13). This distinction in expression may indicate isoform-specific functions and regulatory mechanisms.

Current research mainly focuses on hnRNP A2/B1 without discerning between isoforms, or only centres on isoform A2. Hence, this paper aims to investigate the subcellular localization of the 4 isoforms.

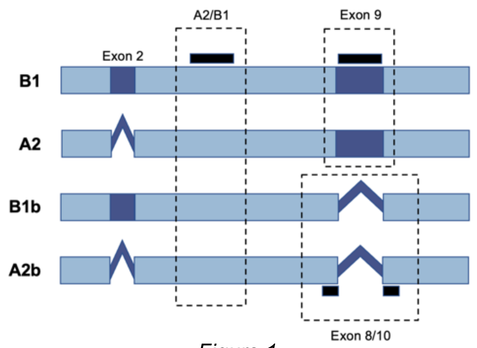


Figure 1

Materials and Methods

Immunostaining with isoform specific Ab

The intracellular distribution of A2/B1 proteins in either nuclear or cytoplasmic compartments were examined by immuno-staining with isoform-specific antibodies recognizing A2/B1 (all isoforms), Exon 8/10 (A2b and B1b) or Exon 9 (A2 and B1).

In Figure 1, the dark blue segments represent spliced exons 2 and 9. Black bars represent locations of epitopes and dashed boxes span isoforms that are detected by each antibody.

GFP-fusion protein transfection and immunostaining

Individual exogenous GFP-fusion proteins for each of the A2/B1 isoforms in hippocampal neurons, and in B104 neuroblastoma cells were expressed; cells were transfected with pEGFP-N1 or A2/B1-GFP fusions and subsequently immuno-stained for GFP 24h post-transfection

This was to circumvent the difficulty of differentiating the A2b and B1b isoforms from immunostaining alone, given that they shared a common epitope recognized by anti-exon 8/10.

Fluorescence correlation spectroscopy measurements of A2/B1 isoforms

B104 cells were microinjected with vectors expressing A2/B1-GFP, and fluorescence correlation spectroscopy (FCS) was used to determine the proportions of freely diffusing, slow and immobile components for each of the A2/B1 isoforms in different cells.

1) FCS measurements were made with the observation volume positioned in the nucleus
2) FCS measurements were made with the observation volume positioned in the cytoplasm.
3) Cytoplasm/ nuclear partition coefficients ([C]/[N]) were obtained for different A2/B1 isoforms, determined from FCS data

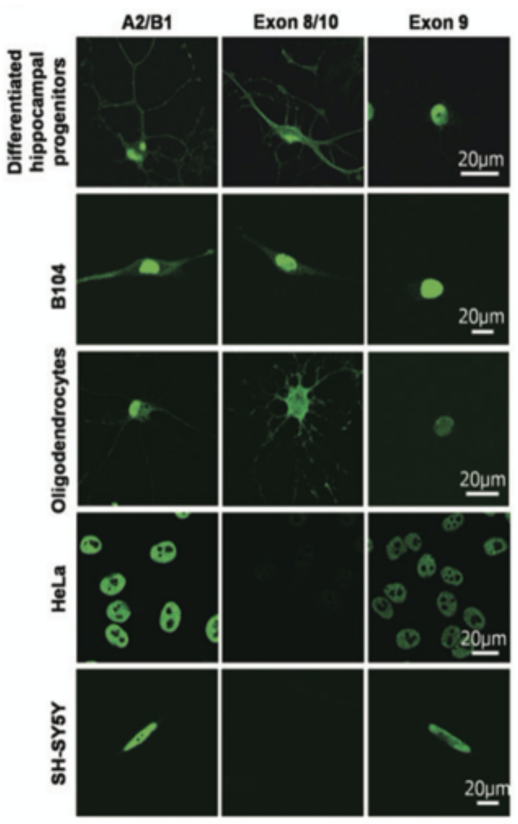
Results and Discussion

Differential Intracellular Distribution of Endogenous hnRNP A2/B1 isoforms

In rat neural cells, anti-A2/B1 strongly stained nuclei with granular staining of cytoplasmic processes. Anti-exon 8/10 exhibited a similar staining, while anti-exon 9 staining was restricted to nuclei.

In human cells, only the A2 and B1 isoforms were expressed at detectable levels. Anti-exon 8/10 did not stain the cells, and both anti-A2/B1 and anti-exon 9 staining were exclusively nuclear.

These findings support the conclusion that A2 and B1 are mainly localized to nuclei, and the predominant extranuclear isoforms in rat neural cells are A2b and B1b



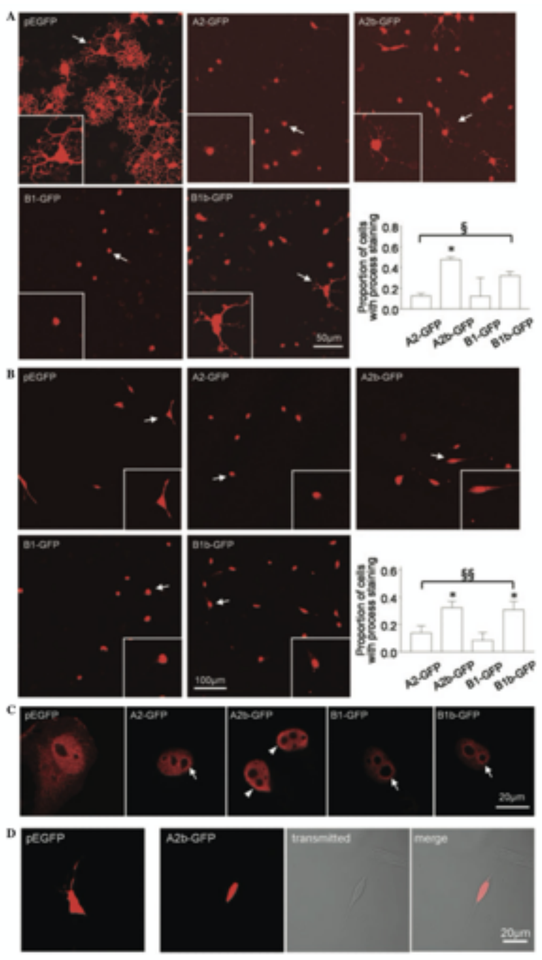
Differential intracellular and intranuclear distribution of exogenous A2/B1 GFP-fusion proteins in cells.

For hippocampal progenitor and B104 cells, A2b-GFP and B1b-GFP were expressed in the processes, while A2-GFP and B1-GFP were expressed only in the nuclei.

In HeLa cells, all GFP-fusion proteins localized to the nucleus, with a unique distribution pattern of A2b-GFP at the nuclear envelope, and other isoforms around the nucleoli.

In SH-SY5Y cells, pEGFP was expressed in processes, but A2b-GFP was localized completely to the nucleus.

These findings support the conclusion that A2b (and also B1b in the case of B104 cells) is exported from the nucleus/ retained in the cytoplasm to a greater extent, and A2/B1 isoforms are mostly localized to nuclei.



A) = Hippocampal Progenitor

B) = B104 cells

C) HeLa cells

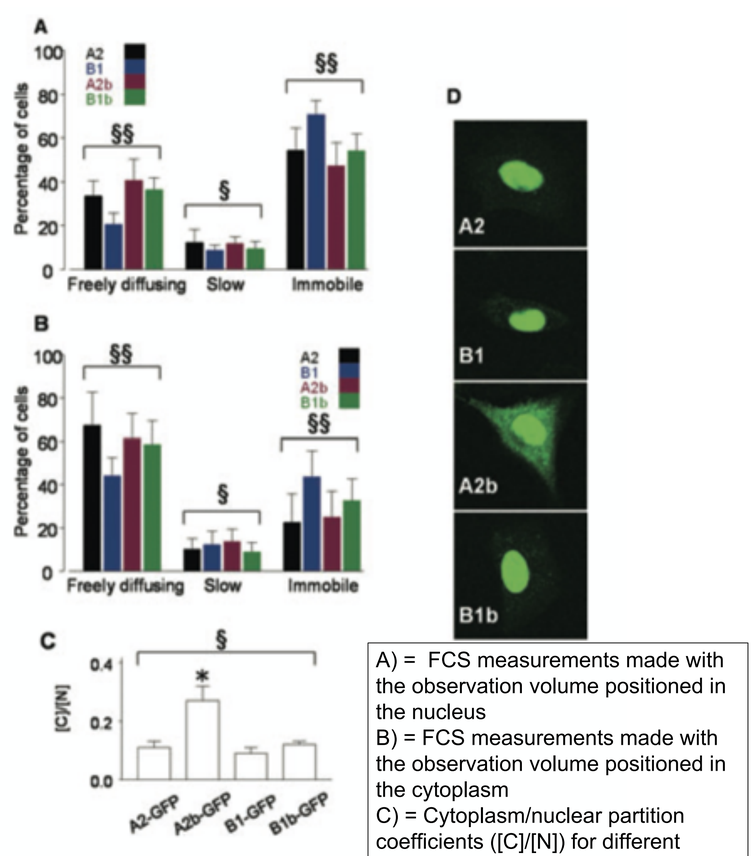
D) SH-SY5Y cells

Dynamics of A2/B1 GFP-fusion proteins in B104 cells

In most cells, A2/B1 isoforms were predominantly immobile in the nucleus and freely diffusing in cytoplasm.

This suggests that most A2/B1 molecules in the nucleus are bound to relatively immobile binding partners (e.g RNA transcripts or telomeres), whereas most A2/B1 molecules in the cytoplasm are freely diffusing.

Additionally, the comparatively greater cytoplasm/ nuclear partition coefficients for A2b reflect the reduced association of A2b with immobile binding partners in the nucleus, allowing for increased export of freely diffusing A2b to the cytoplasm.



A) = FCS measurements made with the observation volume positioned in the nucleus
B) = FCS measurements made with the observation volume positioned in the cytoplasm
C) = Cytoplasm/nuclear partition coefficients ([C]/[N]) for different A2/B1 isoforms

Conclusion

In this study, we found that A2b was the predominant cytoplasmic isoform in rat neural cells. Fluorescence correlation spectroscopy (FCS) in live cells revealed different dynamic properties for different isoforms.

Also, the subcellular localization of hnRNP A2/B1 was dependent on RNA integrity and the inclusion of alternative exons 2 and 9. Furthermore, the transcript and protein levels of the different isoforms varied with developmental stage and species.

Finally, we detected isoform-specific differences in cytoplasmic functions. This is the first study to establish that there are differences among the hnRNP A2/B1 isoforms in terms of their subcellular localizations, dynamic properties and functional roles in RNA trafficking. This will have important implications for understanding their differential roles in mRNA processing, with A2b being the major player in mRNA trafficking.

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