Functional implications of the emergence of alternative splicing in hnRNP A/B transcripts

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INTRODUCTION

hnRNPs participate in many aspects of nucleic acid metabolism, hence their functional diversification through generations of alternatively spliced isoforms has far-reaching implications for the entire transcriptome. Alternate splicing in hnRNPs has revealed previously unrecognised interspecies variation in the the alternative splicing of hnRNPs A/B, as well as iso-form specific expression and function. This reveals a functional diversity through the evolution of dynamic and highly regulated, species-specific alternative splicing patterns, with a trend toward increasing splicing variation in mammals. This adds an unexpected layer of regulatory complexity in transcription and the eventual output in vertebrates.

AIM

Detailed study of hnRNP A/B sequences was conducted across six vertebraes, with the aim of exploring the extent of splicing variation and regulatory significance, in particular species-specific variations in splicing patterns and isoform expressions.

METHODS

1 Pairwise alignment of hnRNP A/B genes according to regions of high similarities was done to compare level of conservation across genes and identify regulatory elements.

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2 Computational tools were used to search for and compare the strength and frequency of splice sites

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3 RT-PCR analysis on brain cDNA from six species (mouse, rat, chicken, Xenopus laevis, cow and human) using species-specific primers was performed.

DISCUSSION

HnRNPs are important regulators of RNA metabolisms and different expressions of hnRNP isoforms imply associated changes in the cell's transcriptional outcome. Hence the findings suggest a new level of specific variation in the key RNA processing proteins.

Evolution of alternative exons in hnRnPs A/B

Variety of mechanisms including cis-based and trans-based apply - increasing protein diversity without increasing gene numbers.

Variation in alternative splicing across species

Different isoforms of hnRNPs A/B have different functional characteristics - this affects how we extrapolate experimental results between species

Functional significance of alternative exons

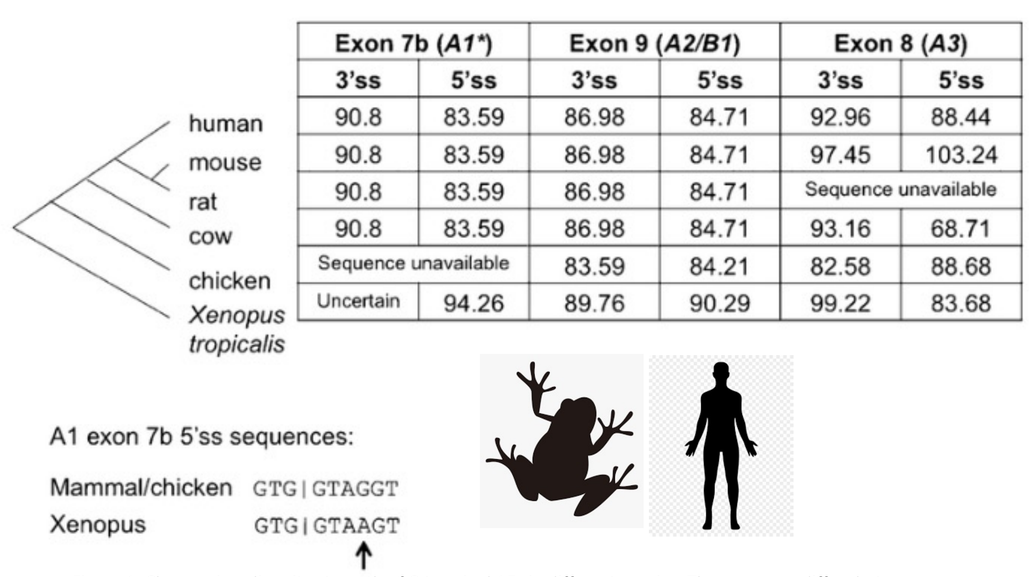
Presence or absence of alternate exons in the hnRNP A/Bs modifies their function

hnRNPs are spliced differently in different species - there are different functions for each RNP

CONCLUSION

Modification of their structural properties by the presence of alternative exons can generate isoform-specific differences in nucleic acid-binding properties, which in turn would impact upon multiple steps in nucleic acid processing. There is increasing evidence that the presence or absence of alternate exons in the hnRNP A/Bs modifies their function. This study has revealed at functional diversification of the hnRNPs A/B is particularly pronounced in mammals, which is likely to have con- tributed to the generation of increased post-transcriptional complexity. In conclusion, the hnRNPs A/B contribute an additional layer of complexity to species-specific reg- ulatory control and the networks that underlie alternative splicing. Future research based on this can be used to focus on the differences between humans and other species.

RESULTS



	Exon 7b (*A1**)		Exon 9 (*A2/B1*)		Exon 8 (*A3*)	
	3'ss	5'ss	3'ss	5'ss	3'ss	5'ss
human	90.8	83.59	86.98	84.71	92.96	88.44
mouse	90.8	83.59	86.98	84.71	97.45	103.24
rat	90.8	83.59	86.98	84.71	Sequence unavailable	
cow	90.8	83.59	86.98	84.71	93.16	68.71
chicken	Sequence unavailable		83.59	84.21	82.58	88.68
Xenopus tropicalis	Uncertain	94.26	89.76	90.29	99.22	83.68

Figure 1: Changes in splice site strength of C-terminal AEs in different species. The sequence differs between Xenopus and the mammal/chicken at the +4 position

RT-PCR was performed on brain cDNA on 6 species, splice site sequence is as shown (Figure 1). There was a novel discovery that exon 8 of human A3 is alternatively spliced at very low levels whilst chicken A3 was alternatively spliced. A3 was also shown to be alternatively spliced at exon 1b for all mammals, inclusion favored in human and cow whereas exon exclusion favored in rodents.

The ratio of band intensities of larger/smaller transcript for cows (0.65) was the highest compared to the other mammals. Of note, the C-terminal AEs of A2/B1 were alternatively spliced only in rodents (Figure 2). This was consistent with prior computational analysis, where comparison of mouse A2/B1 and A3 revealed no similarity between their N-terminal AEs, showing that these exons arose independently (Table 1).

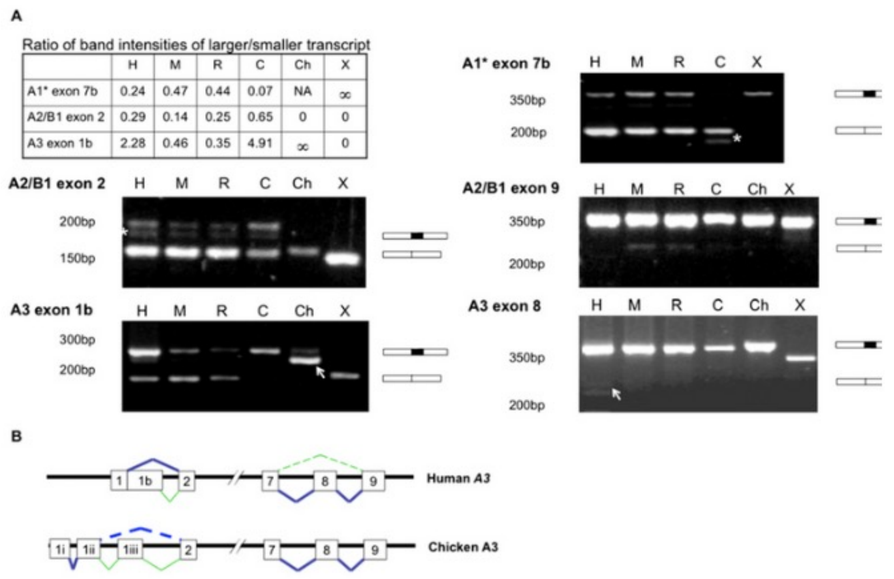


Figure 2: (A) RT-PCR analysis of inclusion levels of alternative exons of hnRNPs A/B in brain cDNA from different species . Arrows point to novel amplicons not previously described, while asterisks indicate presence of double bands due to band-splitting. Ratios of band intensities of amplicons that have variable proportions across mammal (B) Splicing of alternative exons in hnRNP A3 in human and chicken. Boxes represent exons and black horizontal lines represent introns. Bold blue and faint green lines represent major and minor splicing patterns, respectively. Novel splicing patterns identified in the RT-PCR analysis are dashed.

TABLE 1. Percentage conservation level of alternative exons in hnRNPs A/B

Alternative exon	A1	A2/B1	A3
N-terminal AE (across mammals)	NA	100% (36/36)	97% (64/66)
C-terminal AE (across mammals)	97% (155/159)	99% (119/120)	86% (126/147)
C-terminal AE (across all species)	42% (89/213)	77% (92/120)	62% (101/162)

Numbers in parentheses refer to number of conserved nucleotides out of total nucleotide positions; AE, alternative exon; NA, not applicable.

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