DIFFERENTIAL QUANTIFICATION BY Real Time-PCR OF TWO LY6G5B TRANSCRIPTS GENERATED BY AN INTRON RETENTION EVENT

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Introduction

Alternative splicing is an important tool to generate proteome complexity, starting from a relatively limited number of genes.

Major Histocompatibility Complex (MHC) class III region genes present a high rate of different splicing events and has a particular biomedical interest due to the several immune-related diseases mapped in this genomic region.

This study aims the precise quantification of the two transcripts of the MHC *LY6G5B* gene, one generated by an intron retention event. As this transcript has a premature stop codon, it should be degraded quickly by Non-sense Mediated Decay. Nevertheless, it seems to be stable and even the most abundant transcript, especially in tissue samples. This could indicate that this mis-spliced form is a real transcript which could have a potential regulatory function.

LY6G5B retains first intron

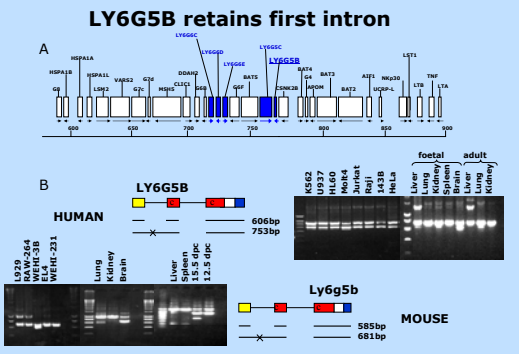

Figure 1. **(A) The MHC class III region containing the LY6 cluster between *HSP* and *TNF* genes, where some loci for immune related pathologies have been mapped. (B) *LY6G5B* gene presents a splicing form which retains the first intron of 148 bases. Sequence and RT-PCR analysis showed that the intron-retained form, contained a premature stop codon, was the most abundant and, was conserved in mouse.**

Differential detection of the two isoforms by real time PCR

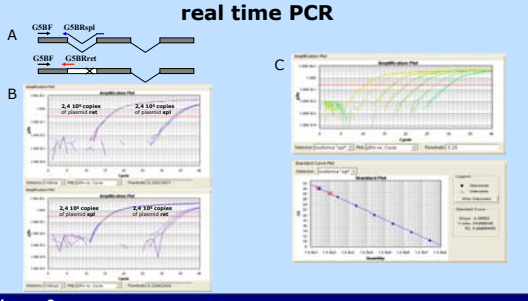

Figure 2. **Experimental Real Time-PCR assay set-up: (A) Primers for SYBR-Green Real Time-PCR, with the reverse primers located at the exon-exon or exon-intron junctions. (B) Primer pairs were tested for cross-amplification. (C) Standard dilutions were performed with cloned cDNAs for absolute quantifications. RT-minus was performed in each sample to exclude amplification of genomic DNA.**

Relative expression of the two *LY6G5B* isoforms

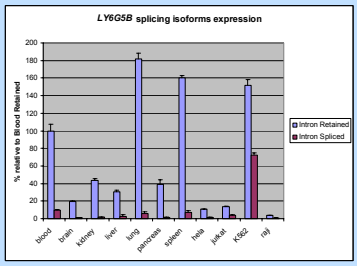

Figure 3. **Quantification the two isoforms in different cell lines and tissues. Intron retaining isoform is the most abundant in all RNA samples. The data are normalised with respect to GAPDH.**

Splicing isoforms stability studies

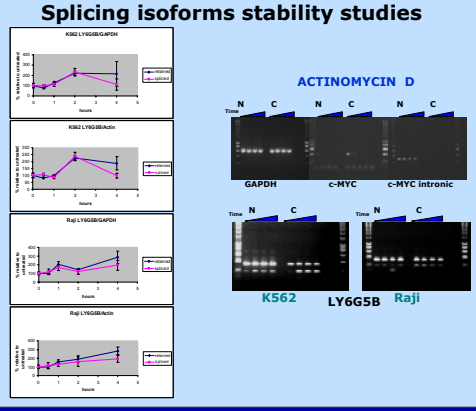

Figure 4. **Treatment with the transcriptional inhibitor Actinomycin D (5 mg/L) during 1/2, 1, 2 and 4 hours was performed to investigate stability of the two LY6G5B transcripts. The assay indicates increased stability of the LY6G5B transcripts relative to the controls GADPDH and Actin.**

Conclusions

- **We designed a differential and specific Real Time-PCR assay to detect the two splicing isoforms of the *LY6G5B* gene.**
- **With this assay we absolutely quantified the two isoforms to compare their levels in each analysed sample.**
- **The intron-retained isoform is the most abundant in all cell lines and tissues studied.**
- **The two LY6G5B isoforms showed to be more stable than the controls GADPH and actin, after treatment with Actinomycin D.**