Application Note

Detection of alternative Tra2β regulated splicing

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This application note describes how the QIAxcel® system was used to successfully determine the splicing pattern of exonic sequences targeted by Tra2β protein isoforms.

Introduction

Tra2β (*Sfrs10*) is an evolutionarily conserved splicing protein that is crucial for mouse embryogenesis (1), but its biological role is not fully understood. It has a modular structure with domains rich in arginine and serine (RS1 and RS2) and a central RNA recognition motif (RRM) that binds to target RNA sequences (2, 3). Furthermore, at least 3 isoforms of Tra2β have been identified. Tra2β is known to splice the *Nasp* histone chaperone gene, which monitors DNA double strand breaks (4). An evolutionarily conserved cassette exon (annotated *Nasp*-T) may play a crucial role in developmental processes. Tra2β splices *Nasp* via a number of binding sites, but the exact role of these interactions is not known.

Because of the high levels of splicing inclusion observed for the wild type *Nasp*-T exon at endogenous cellular concentrations of Tra2β, we tested a mutated exon ("M3+M4"), which is less efficiently spliced, to find out whether the Tra2β binding sites are necessary for splicing activation. We also investigated the need for the Tra2β RRM and RS1 domains in these interactions (5).

The QIAxcel system provides rapid, sensitive, and reproducible analyses of Tra2β regulated splicing. This system may also prove advantageous for studying the role of other splicing proteins and their target sequences.

Materials and Methods

HEK 293 cells were cotransfected with a mutated *Nasp*-T construct (M3+M4) and one of 3 Tra2β–GFP constructs encoding full length Tra2β, Tra2βΔRRM, or Tra2βΔRS1. Control cells were cotransfected with the *Nasp*-T construct (M3+M4) and GFP only. ▷

The extracted pre-mRNAs were subjected to RT-PCR and subsequently analyzed using the QIAxcel system. Samples of low DNA concentration were analyzed using Method OL400. Samples were injected at 8 kV for 20 s, and separation was performed at 6 kV for 400 s. Alignment marker, with fragments of 15 bp and 3 kb, was injected at 4 kV and 20 s and run simultaneously with the samples. QX DNA size marker, with fragments ranging from 50–800 bp, was used for size and concentration estimation.

High-throughput sequencing of RNA isolated by crosslinking immunoprecipitation (HITS-CLIP) was performed as previously described (6) using an antibody specific to Tra2β (7).

Results and Discussion

As expected, analyses on the QIAxcel system demonstrated very high splicing activation by full length Tra2β, but also significant percentage splicing inclusion (PSI) activation by the Tra2βΔRRM–GFP protein (Figure 1). These results indicate that for some exons, Tra2β can act as a coactivator as well as a splicing activator.

Interestingly, Tra2βΔRS1 seems to behave as a potent splicing repressor. This indicates that the endogenous Tra2βΔRS1 isoform acts a splicing repressor and/or that the RS1 domain plays a central role in splicing activation.

Conclusions

- The QIAxcel system is a valuable tool for revealing exon splicing patterns.
- Analyses of the splicing patterns using the QIAxcel system were both qualitative and quantitative.
- The QIAxcel system can help to identify the roles of other splicing proteins as activators, coactivators, or repressors.

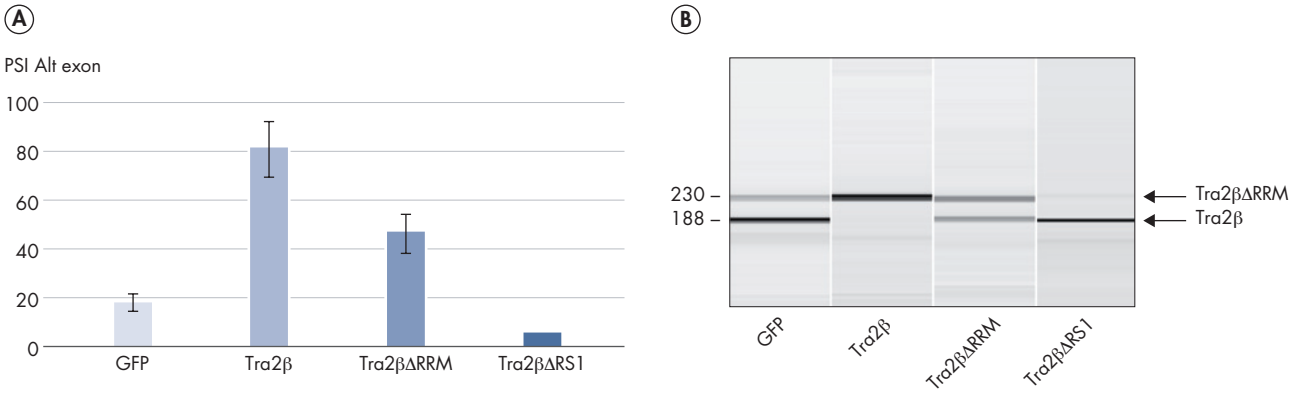

Figure 1. Splicing of *Nasp*-T M3+M4 (mutant) exon. A. Percentage splicing inclusion (PSI) of a panel of exons identified through HITS-CLIP in response to GFP and Tra2β-GFP fusion proteins. Data represent at least 3 biological replicates, and the error bars are shown as standard errors. **B.** Representative image from RT-PCR analysis on the QIAxcel system. Probability (***p***) values were calculated using an independent two-sample T-test between the PSI levels for cells cotransfected with GFP and each of the different Tra2β-GFP constructs ($p \leq 0.05$, $p \leq 0.01$) (adapted from reference 5).

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