

Cis-Acting Elements Regulating TR α Splicing in *Monodelphis domestica*

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The TR α gene locus encodes two functionally antagonistic nuclear receptors, distinct only in their 3' terminal exons. The 3' terminal exon of TR α 2 overlaps the 3' terminus of Rev-erb α , another nuclear receptor gene. This overlap results in a 67 codon bidirectional coding sequence (BCS) in the eutherian locus. Both TR α 1 and TR α 2 mRNAs are expressed at all developmental stages and in all tissues. Yet, no TR α 2 has been found in *Monodelphis domestica*—the South American Opossum (SAO)—in any of the tissues or developmental stages examined. Interestingly, the BCS architecture found in eutherians is absent in the SAO; instead, the stop codons of TR α 2 and Rev-erb α are directly adjacent to each other. We aim to identify the cis-acting elements that regulate TR α 2 splicing.

Initially, the splicing locus was split into two pieces: an upstream fragment containing the 5' splice site and a downstream fragment containing the 3' splice site. These fragments were joined to constitute minigenes with an upstream fragment and a downstream fragment from rat, SAO, or both. Three of these minigene constructs have been completed: upstream rat/downstream rat, upstream SAO/downstream rat, and upstream SAO/downstream SAO. These minigene constructs were transfected into HEK 293 cells. The total RNA was collected and reverse transcribed, producing the template to be used in real time RT-PCR reactions. TR α 1 and TR α 2 splicing were assayed. As expected, the upstream rat/downstream rat minigene spliced TR α 2 very efficiently. The upstream SAO/downstream rat minigene spliced TR α 2 comparably. However, the upstream SAO/downstream SAO minigene did not splice TR α 2 appreciably. These results suggest that the SAO 5' splice site is competent for TR α 2 splicing, but the SAO 3' splice site is not conducive to TR α 2 splicing.