

The Role of Transcriptional Pausing in Alternative Splicing and Polyadenylation

Corey Nemec

Marquette University

Program Mentor: Dr. Stephen Munroe

The thyroid hormone nuclear receptor gene ($TR\alpha$) encodes two functionally antagonistic proteins through alternative processing. $TR\alpha 2$ is the more abundant isoform in most tissues; however, several tissues produce more $TR\alpha 1$. Previous studies have identified several splicing regulatory elements including intronic splicing enhancers (ISEs) and exonic splicing enhancers (ESEs) that promote splicing of $TR\alpha 2$ mRNA. Moreover, recent work demonstrates a link between transcription and pre-mRNA processing that is crucial for alternative splicing and polyadenylation.

A guanosine-rich (G-rich) region between the closely spaced $TR\alpha 1$ polyA site and the $TR\alpha 2$ splice site has been shown previously to promote splicing. However, G-rich sequences known as MAZ elements are also known to be involved in transcriptional pausing. Therefore, such sequences may also promote polyadenylation at sites upstream. In these studies I plan to exam the region downstream of the $TR\alpha 1$ polyadenylation to identify and characterize potential pause sites and determine their role in the alternative processing of thyroid hormone receptor pre-mRNA.

For our initial studies, I have used the polyA competition (PAC) vector, developed by Nicholas Proudfoot, in which a multiple cloning site (MCS) is flanked by a weak upstream polyA signal and a strong downstream synthetic polyA signal. The G-rich region was inserted into the MCS of the PAC vector in both orientations. These two constructs, along with the PAC vector and a MAZ construct were transfected into HEK 293 cells. The mRNA was collected and quantified using real-time PCR and an RNase protection assay. As expected, transcripts from the the PAC vector primarily polyadenylated downstream due to the strong polyA site, whereas the MAZ construct preferred the upstream site. In its forward direction, but not the reverse direction, the G-rich region displayed a significant increase in upstream polyadenylation over the PAC vector, which suggests that this region causes MAZ-like transcriptional pausing. Future research will include deleting the G-rich region and/or inserting MAZ elements into a $TR\alpha$ minigene to explore further the role this region plays in determining the alternative processing of $TR\alpha$ *in vivo*.