Title: Identifying PUF Co-Regulators by RNA-Seq Coupled with Conserved Motif Search

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PUF proteins regulate abundance of mRNAs by binding a conserved 3'UTR motif (UGUANAUA). Due to the diversity of mechanisms seen, we hypothesized that PUF co-regulators have significant impacts on PUF function. We identified PUF target mRNAs by knocking down PUF, identifying differentially abundant mRNAs by RNA-Seq, and identifying the subset of PUF response genes that have PUF binding motifs. This process yielded 99 likely direct (binding) targets of PUF. We screened these mRNA sequences for the canonical binding motifs of known RNA-binding proteins and microRNAs, then used MEME to search for novel over-represented motifs. Two interesting results include conserved poly-A sites, and a site that most closely matches the binding site for the AIRE transcription factor. Poly-A binding proteins bind the poly-A tails of mature mRNAs and have multiple effects on those mRNAs, including influencing abundance. We previously identified a potential association between PUF and poly-A tails, so identifying poly-A binding motifs in other segments of the mRNA may expand our understanding of this mechanism. The mechanism for a transcription factor such as AIRE to act as a PUF co-regulator is much less clear, though at least four transcription factors have been shown to bind both DNA and RNA in a sequence specific manner (MDM2, WT1, SmZF1, and Spi-1/PU.1). Beyond regulation of transcription, the role(s) that transcription factors may play in regulation of mRNAs are not well understood. However, this result provides tantalizing evidence that AIRE and other known regulatory proteins could serve as co-regulators with PUF proteins.