A128 · A novel RNA motif discovery pipeline to elucidate GLDR-2 target recognition

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RNA tailing is the non-templated, post-transcriptional addition of nucleotides to the 3′-ends of transcripts carried out by terminal nucleotidyl transferases (TNTs). GLDR-2 is a non-canonical TNT, lacking an RNA recognition domain, and is ubiquitously expressed in C. elegans. GLDR-2′s closest homolog is GLD-2, a cytoplasmic poly(A) polymerase expressed in both C. elegans and mammals that plays a crucial role in gametogenesis and development. GLD-2-depleted C. elegans are completely sterile. Similarly, GLDR-2-depleted male C. elegans are sub-fertile. The exact mechanism underlying GLDR-2 targeting to specific RNAs remains unknown. We hypothesize that RNA-binding proteins (RBPs) mediate interactions between GLDR-2 and specific RNAs by binding conserved motifs shared among targets. We combine computational and experimental approaches to identify these motifs using a novel in silico motif-discovery pipeline. We developed a computational pipeline to identify candidate RNA motifs that mediate GLDR-2 association with its target RNAs, as identified by Vieux et al., 2021. We combine HMM-based sequence searches with structural modelling to identify recurring motifs among RNAs that interact with GLDR-2. Motifs are ranked based on their prevalence among targets and the conservation of their predicted secondary structures. The highest-ranking motifs will be experimentally validated using immunoprecipitation coupled with high-throughput sequencing to assess their association with GLDR-2. We will then disrupt candidate RBPs associated with these motifs using CRISPR/Cas9-based genome editing to evaluate their requirement for GLDR-2 targeting to specific RNAs. Ultimately, establishing the molecular mechanisms that determine GLDR-2 target recognition will advance our understanding of its roles in fertility and development.

A novel RNA motif discovery pipeline to elucidate GLDR-2 target recognition

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