Caprin-1 binding profile to target RNAs via enhanced CLIPseq

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Caprin-1 is a cytoplasmic RNA binding protein (RBP) expressed in most mammalian tissues and is particularly abundant in neurons as part of RNA granules. This RBP binds to RNA targets in mRNPs complexes and contains a conserved RG-rich domain, which is related to cellular granules formation and aggregation of proteins. Caprin-1 is also a component of high-density cytoplasmic granules called stress granules (SG), a structure comprised of RNA and protein in a tightly packed aggregate, which has been associated with regulation of protein expression via stalling of translation pre-initiation complexes in target RNAs. Several neurodegenerative diseases have been associated with formation of stress granules. While toxicity of granules is still under debate, we wanted to better understand Caprin-1 function in SGs and its implications in the cell. Since ectopic expression (EE) of CAPRIN1 in cells is sufficient to induce the formation of SGs, we performed enhanced crosslink immunoprecipitation sequencing (eCLIPseq) on human HEK293T cells subjected to CAPRIN1 EE. This approach allowed us to define the target RNAs bound to the protein of interest and to profile potential binding sites to the target RNA (binding sites). We complemented this approach with other high-throughput techniques such as RNA-immuprecipitation sequencing (RIPseq) and standard RNA sequencing (RNAseq) to allow a biologically relevant set of target genes to be used in further wet lab experiments. Our results revealed that Caprin-1 binds to 1720 different RNA targets, comprised mostly of mRNAs, in multiple locations, indicating a " coating " behavior in the binding activity, with a bias towards the CDS and 3'UTR regions. Based on over-representation of sequences capable of forming G-quadruplex structures in binding sites, we propose that Caprin-1 binds to RNA via this secondary RNA structural motif. We also observed that 174 binding targets were abundant in the RIPseq, gene ontology profiling of this subset showed enrichment in nucleotide binding and RNA-metabolism related classes. Complementary analysis from RNAseq also indicates that binding targets of Caprin-1 are mostly up-regulated after stress granule induction. In summary our findings suggest that Caprin-1 binds to a structural RNA motif, which can lead to stabilization of a set of mRNA transcripts enriched in RNA-related targets and regulate their expression.

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