PRINCIPE FELIPE
CENTRO DE INVESTIGACION

PhD Thesis Defense

Functional characterization of the yeast RNA binding protein Mip6

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RNA binding proteins (RBPs) participate in virtually every step of gene expression, demonstrating a potential role as a quality control system contributing to RNA homeostasis. One of the proteins with an essential role in mRNA export in yeast is the evolutionary conserved Mex67 transporter. Although Mex67 binds to RNAs, it requires adaptor proteins to interact with quality-controlled mRNAs and undergo export through the nuclear pore complex. During heat shock, Hsf1 and Msn2/Msn4 transcription factors produce stress-responsive mRNAs. The export of Hsf1-dependent mRNAs depends on direct interaction with Mex67, without quality control mechanisms. However, we know less regarding the export of Msn2/Msn4-dependent transcripts, although various relatively unstudied RBPs may participate in this process. Previous yeast two-hybrid assays revealed an interaction between Mex67 and the putative RBP Mip6. Mip6 contains four RNA recognition motifs (RRMs) and has been described as a modulator of mRNA metabolism during sporulation.

In this thesis, we report the functional characterization of Mip6 under optimal and stressful growth conditions through a study of the functional interaction with the essential exporter Mex67, its mRNA targets, and the required elements for its subcellular localization.

Mip6 directly interacts with the ubiquitin-associated domain of Mex67 through tryptophan-442 of Mip6 RRM4, whose mutation leads to the slow growth of yeast cells in vivo. Mip6 shuttles between the nucleus and the cytoplasm in a Mex67-dependent manner and concentrates in cytoplasmic foci in response to insult by various stressors. Mip6 copurifies with Pab1 in stress granules (SGs), and cells lacking MIP6 display altered Pab1-SGs behavior. Confocal microscopy studies established that deletion of the Mip6 RRMs leads to Mip6 nuclear retention. Moreover, the export of Mip6 is also regulated by a Crm1-independent Nuclear Export Signal (NES) in RRM4 that is controlled by the karyopherin Msn5. Photoactivatable ribonucleoside-enhanced crosslinking and immunoprecipitation (PAR-CLIP) experiments demonstrated enriched Mip6 binding to Msn2/Msn4 dependent transcripts under stress-free conditions; however, Mip6 enrichment changes during heat shock showing a preferential binding to ribosomal protein genes (RPGs). Consistent with PAR-CLIP results, MIP6 deletion and the mutation of tryptophan-442 augment the expression levels of Msn2/Msn4 targets (HSP12 and CTT1). Deletion of both MIP6 and the RRP6 nuclear exosome component further increases HSP12 and CTT1 levels.

These results reveal a novel role for Mip6 in mRNA homeostasis through direct interaction with Mex67. We envision a model in which the initial interaction of Mip6 with RNA contributes to the Mex67- and/or NES-dependent export of Mip6. This transport helps to regulate the levels of Msn2/Msn4-dependent mRNAs under stress-free conditions. However, in response to stressors, Mip6 accumulates in SGs aid the repression of RPG mRNAs.

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