Cellular Fractionation to Characterize the Interaction of Nucleolin with Alpha-COP

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Background and Hypothesis:

The goal was to further characterize the interaction between Nucleolin and the alpha subunit of the COPI coatomer complex. Nucleolin contains a C-terminal dilysine motif, which mediates interactions between the WD40 domain of alpha-COP and COPI-interacting proteins. Previous work in the lab showed that this C-terminal dilysine is required for co-immunoprecipitation of alpha-COP with Nucleolin. Because alpha-COP is exclusively found in the cytoplasmic compartment, we hypothesized that the interaction between alpha-COP and Nucleolin is exclusively cytoplasmic but previous co-immunoprecipitations had only been performed from whole cell lysates. Alpha-COP has been shown to bind mRNA, but it was unclear whether this interaction was direct or whether alpha-COP was binding an RNA binding protein (RBP) which would act as a bridge between the mRNA and alpha-COP. Nucleolin acts as an RBP in both the nuclear and cytoplasmic compartments. We hypothesize that some of the mRNA bound to alpha-COP are present due to their association with Nucleolin.

Experimental Design or Project Methods:

The first aim of the project was to optimize a reproducible cell fractionation protocol to reliably separate nuclear and cytoplasmic compartments. The second goal of the project was to identify mRNA that would co-immunoprecipitated with Nucleolin in HEK293T cells where we can easily express tagged versions of alpha-COP. Nucleolin-bound mRNA had previously been identified in Hela cells. We began by testing for the expression of these mRNAs in 293-TT cells using the published RT-PCR primers.

Results:

After these tests identified Ftl (Ferritin light polypeptide) as a highly abundant transcript in 293-TTs, we performed RNA immunoprecipitation from cells expressing epitope tagged Nucleolin or alpha-COP. We confirmed that both Nucleolin and alpha-COP are in complex with Ftl mRNA.

Potential Impact:

Future experiments will use short-hairpin RNA to knockdown Nucleolin and determine whether the levels of Ftl mRNA that co-immunoprecipitated with alpha-COP are reduced in the absence of Nucleolin.

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