FILE S2

Supporting Results

CLIP Specificity: Multiple control experiments were conducted to ensure that CLIP specifically identified *in vivo* RNA targets of Khd1. As part of this analysis, Khd1-RNA complex formation was visualized by end labeling RNAs following immunoprecipitation of Khd1-TAP and treatment with RNAse A as previously described (ULE *et al.* 2005). Non-specific RNA contaminants migrate at a much lower molecular weight than protein-RNA complexes, and can be separated using SDS-PAGE. Additionally, transfer to nitrocellulose membrane retains the protein-RNA complexes, but not free RNA (SANFORD *et al.* 2008; ULE *et al.* 2003). Khd1-RNA complexes were then visualized using autoradiography.

Khd1-RNA complex formation requires UV crosslinking (Figure S2) as has been previously demonstrated using mammalian RNA-binding proteins (SANFORD *et al.* 2008; ULE *et al.* 2003). Khd1-TAP has a predicted molecular weight of approximately 63 kilodaltons (kDa), but Western blot analysis shows the protein migrates at slightly less than 75 kDa during SDS-PAGE (data not shown). When a high concentration of RNAse A is used, Khd1-RNA complexes migrate slightly higher than 75 kDa (Figure S2). With lower RNAse A concentrations, longer RNA molecules are maintained leading to an increase in the molecular weight of the complexes (Figure S2).

Immunoprecipitation of Khd1-TAP from un-crosslinked cells was used to determine whether pure samples of Khd1 were obtained. Mass spectrometry of the band at about 75 kDa revealed no major protein species co-migrating with Khd1-TAP (data not shown), suggesting the signal on the autoradiogram derives specifically from Khd1-RNA complexes.

Immunoprecipitation of other RNA-binding proteins following crosslinking resulted in the formation of protein-RNA complexes of an expected size based on the molecular weight of the protein, but no complexes were seen when proteins without RNA-binding domains were used (data not shown).

Based on the above results, we conclude the sequences we obtained derive specifically from interactions between Khd1 and its endogenous RNA targets.

LITERATURE CITED

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