Reversible cross-linking combined with immunoprecipitation to study RNA-protein interactions in vivo. [1]

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Abstract	Protein-RNA interactions play indispensable structural, catalytic, and regulatory roles within the cell. Understanding their physical association in vivo provides valuable insight into their assembly, function, and regulation in the cellular milieu. Inspired by the chromatin immunoprecipitation assay, we have developed a ribonucleoprotein (RNP) immunoprecipitation assay to study RNA-protein interactions in vivo. This method takes advantage of the highly reactive, reversible crosslinker formaldehyde, combined with high-stringency immunoprecipitation to identify specific RNAs associated with a given protein. The RNP immunoprecipitation (RIP) assay was developed using RNA-protein interactions of hepatitis delta virus (HDV) as a model system. HDV is an RNA virus with a single-stranded circular RNA genome that encodes one viral protein, hepatitis delta antigen (HDAg). The high affinity of HDAg for the HDV RNA genome, combined with the well-characterized anti-HDAg antibodies, made this system a logical starting point for the development of the RIP assay. Cells with replicating HDV were crosslinked with formaldehyde and the HDV RNPs were immunoprecipitated using anti-HDAg antibodies. The crosslinks were then reversed by heat treatment, and the immunoprecipitated HDV RNAs were identified by reverse transcription polymerase chain reaction (RT-PCR). The specificity of this assay was tested using HDV mutants and heterologous antibodies for immunoprecipiation followed by RT-PCR with HDV-specific primers. This experiment showed no nonspecific immunoprecipitation of the HDV RNPs. The method was tested further using protein-RNA interactions known to exist in the U1 snRNP. The results indicate that the RIP assay is a powerful tool to identify RNA-protein interactions in vivo and has the potential to unravel the cellular network of RNP complexes in their native setting.
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