Optimization of a method to study protein-RNA interactions

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Interactions between proteins and RNA are crucial for the proper functioning and regulation of various cellular processes, significantly influencing the processing of RNA molecules. These interactions take place at multiple stages, such as transcription, splicing, transport, localization, and degradation, ensuring that RNA is effectively processed and utilized within the cell. RNA-binding proteins (RBPs) are key components in these interactions, and gaining insights into them can enhance our understanding of gene expression and regulation within cellular physiology. The impact of protein-RNA interactions extends beyond a specific disease or cellular environment, as they play an essential role in many pathological states, including viral infections, where viruses often exploit host RBPs to facilitate their own replication. This demonstrates the flexibility and adaptability of RNA-binding mechanisms in both health and disease contexts. As scientists dig deeper into the intricate relationships between RNAs and proteins, the potential development of innovative therapeutic approaches targeting these interactions could significantly change treatment methodologies for a range of diseases, underscoring the necessity for continued research in this vital field of molecular biology. In our study, we aim to optimize the Orthogonal Organic Phase Separation (OOPS) technique. This method, designed to examine RNA-protein interactions, addresses several shortcomings of earlier techniques by minimizing the required starting material and reducing bias towards specific RNA types. OOPS operates on the principle of sampling the interface during a standard TRIzol extraction to enrich RNA-binding proteins (RBPs) along with their associated RNA. A notable benefit of OOPS is its capability to recover RNA-binding proteins that interact with both coding and non-coding RNAs, in contrast to methods that depend solely on poly-A selection. This technique has been effectively employed to isolate RBPomes from diverse cell types, such as embryonic cells, tumor-derived cells, non-tumor-derived human cell lines, and bacteria. Thus, OOPS serves as a robust tool for exploring RNA-protein interactions across various biological contexts and dynamic conditions. We investigated RNA-protein interactions in human cells infected with *T. cruzi* at various time points to understand how these interactions evolve as the disease progresses. The protocol is based on the UV irradiation of cells to create protein-RNA adducts, followed by cell lysis and multiple rounds of phase separation to enrich these adducts. The specificity of OOPS is enhanced in its final stages, where enriched interfaces are digested with RNases or proteases to release either the RBPs or the RNA linked to proteins. This method will allow us to recovery both free (non-cross-linked) RNA and proteins, as well as UV cross-linked RNA-protein adducts from a single sample.

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