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Commentary

Significance of RNA-Binding Proteins and its *In vitro* and *In vivo* RNA-Centric Approaches

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DESCRIPTION

RNA-Binding Proteins (RBPs) control the life and fate of all Ribonucleic Acid (RNAs), performing tasks ranging from transcription factors to single-stranded RNA binding proteins to assembly in Ribonucleoprotein Complexes (RNPs) like the spliceosome. RNAs are also starting to control how many cognate binding proteins function. Small Nuclear Rnas (snRNAs) are essential parts of the spliceosome, while Long Non-Coding Rnas (lncRNAs) have been shown to act as scaffolds in the organisation of RNP assembly, attract transcription factors or chromatin modifying complexes. By forming weak associations with RNAs, chromatin, or RBPs, lncRNAs may indirectly promote the regulation of gene expression. As a result, the fate of RBPs and RNAs is influenced by one another. However, new evidence suggests that non-classical RBPs have intrinsically disordered regions that can interact with RNA, proteins, or chromatin. Small polar amino acid interactions to RNA in conserved Arginine–Glycine–Glycine (RGG) are less specific and are thought to be in charge of causing the moonlighting activities of metabolic enzymes like Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) in addition to their function in the formation of membrane-less organelles like stress granules through liquid-liquid phase separation.

The identification of RNA-binding proteins necessitates optimised detection methods, particularly for lcnRNAs, which are characterised by low expression and instability.

They are separated into RNA- and protein-centric methods. In RNA-centric and protein-centric approaches, RNA or protein is employed as a bait, respectively, in Immunoprecipitation (IP) techniques to explore RNA-protein interactions. The protein-centric strategy discovers RNAs and binding sites that are targeted by a known RBP, whereas the RNA-centric method looks for cognate and novel RBPs coupled to a specific RNA of interest. *In vitro* RNA-Centric Approaches include IP isolation of an RNA of interest and immunoblotting or mass spectrometry detection of the bound proteins. The *in vitro* method is best suited for characterising known interactions, such as mutating the nucleotides responsible for protein binding or validating RBP binding to the target RNA under different cellular conditions such as growth, oxidative stress, protein depletion, or drug treatment. The majority of *in vitro* pulldown techniques concentrate on an *In vitro* -Transcribed (IVT) RNA of interest that can be immunoprecipitated in a number of ways, including by first adding affinity tags or 3′ extensions to the RNA or by hybridising the RNA to target-specific antisense probes. Additionally, poly(A) mRNA-associated RBPs have been enhanced using poly(dT) oligonucleotide capture.

An IVT RNA can be 5′-tagged with biotin or an aptamer sequence to serve as an affinity handle. Proteins are frequently marked with a mass tag to determine quantification in order to detect RNA-interacting proteins in MS. Chemical labelling of proteolyzed peptides, metabolic labelling in Stable Isotope Labeling by/with Amino acids in Cell culture (SILAC), or spike-in of peptide standards are all methods of labelling. Utilising label-free direct protein measurement in contrast to a control condition. On the other hand, *in vivo* methods often capture an endogenous RBP after cross-linking it to its corresponding RNA in the cell. Before purifying the RNA under denaturing conditions, cells are exposed to Ultraviolet (UV) light or formaldehyde to preserve the covalent RNA-protein complexes and eliminate weakly linked proteins. Antisense oligonucleotide probes are employed in this stage to hybridise with and draw down the target RNA. Target-specific probes were used in a tandem purification method after mRNA was poly(A)-enriched. Tiled 5′-biotinylated probes were applied to the Long Non-Coding RNA Xist Inactive Specific Transcript (lncRNA)XIST to recover 81 Xist-interacting proteins from HeLa cells, including Spen and Heterogenous Nuclear Ribonucleoprotein K (hnRNPK), as a result of a cell lysis-induced RNA fragmentation phase. To identify new RBPs for the short Small Nuclear RNA (snRNAs) U1 and U2, Complete Identification of RNA-Binding Proteins By Mass Spectrometry (ChIRP-MS) was employed. By effectively cross-linking 4-thiouridine (4sU)-containing

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transcripts to RBPs, RNA-Binding Regions (RBR-ID) proteomic identification of RNA-binding regions could map the RNA-binding areas of RBPs from embryonic stem cell nuclei.

The Messenger RNA (mRNA)/Peptide Nucleic Acid (PNA) complex is in direct contact with proteins, which the UV radiation enables the activated PNA to cross-link with. Following pulldown with an antisense biotinylated probe, mRNA-interacting proteins are subsequently identified by MS. For lncRNAs, the cognate RBPs of the lncRNAs Nuclear Enriched Abundant Transcript 1 (*NEAT1*) and Metastasis Associated Lung Adenocarcinoma Transcript 1 (*MALAT1*) in human cells and of roX2 in flies were validated by Western blot. Desthiobiotin probes for RNA pulldown from cross-linked chromatin extracts and sequencing of the recovered DNA. The type of reagent used determines the degree, effectiveness, and specificity of cross-linking; for RNA-protein interactions, UV light typically outperforms formaldehyde because it does not cause protein-protein bonding, captures only direct contacts ('zero distance,'), and is irreversible. Formaldehyde, on the other hand, may be preferable due to better cross-linking efficiency because the recovery of RNA-protein complexes is frequently low and necessitates large cell input. Both chemicals' cross-linking effectiveness is biassed towards particular nucleotide sequences.

CONCLUSION

Additionally, on a DNA-sequencing platform, there are techniques like RNA compete and RNA Massively Parallel Array (RNAMaP) that quantitatively evaluate the kinetics of a particular RNA's binding to an RNA-binding protein through its mutational library. These methods break out the contributions of RNA primary and secondary structure to binding affinities and identify RBPs' preferred sequence motifs.