Scientific contribution/Review

Molecular Grammar of RNA-binding Protein Interactions in Formation and Function of Ribonucleoprotein Complexes

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Abstract:
Ribonucleoproteins (RNPs) are macromolecular assemblies of proteins along RNA molecules to carry out specialized cellular processes. Understanding how RNA binding proteins (RBPs) and RNA sequences determine the interactions to form RNPs and ultimately steer biomolecular processes remains poorly understood. There is a mounting evidence that RNP assembly depends on the formation of a network of transient, multivalent RBP-RNA and RBP-RBP interactions, particularly between tyrosine residues from intrinsically disordered domains and arginine residues from RNA-binding domains of RBPs. Furthermore, RBPs, especially their intrinsically disordered regions, are hotspots for posttranslational modification (PTM) sites. Although PTMs have been well catalogued, little is known about how these modifications regulate RNP assembly and function. Some initial studies introduced the concept of the so-called phospho-switch, in which RBPs require phosphorylation for condensation of larger RNP complexes, but it remains unclear how this contributes to the protein function and the pattern of selective protein binding to RNA molecules. This short review will take a look at what is currently known in the field of RNPs, their interactions, and the phase-separated biomolecular condensates, which are intimately connected to RNPs and are important for several key cell processes.

Keywords: Ribonucleoproteins; RNA binding proteins; Multivalency; Intrinsically disordered proteins; Posttranslational modifications

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1. Introduction

The importance of RNA networks and interactions of RNA molecules is becoming more and more evident. RNA sequencing technologies showed us that much of the genome contains genes for RNA molecules that do not code for protein sequences. These RNA molecules play diverse roles many important cell processes. With the help of more than a thousand RNA binding proteins (RBPs), RNA molecules form various membraneless organelles that allow precise control of the listed processes (Somasundaram et al., 2022; Castello et al., 2016). This short review will give an overview of some basic concepts that are associated with RBP-RNA interactions: phase separation, multivalency and intrinsically disordered regions, and posttranslation modifications.

2. RNA networks

An important goal of studying RNA networks has been to elucidate the functions of RBPs as the core regulators of RNA processing. Proteins that act on RNA can be separated into two groups: 1) effectors, molecules that operate as executors of RNA processing or otherwise directly affect RNA processing, and 2) RBPs, proteins that bind RNA in a sequence- or structure-specific manner and are not basal or auxiliary components of effector assemblies (He et al., 2023).

RNA networks have certain traits as reviewed in He et al.(2023):

1. RNA sequence specificity: RBPs and other RNA molecules act only upon some transcripts, dependent on the transcripts' RNA sequence. This is the most basic regulatory level of RNA networks that determines which transcripts will be controlled by which factors.
2. Relayed RNA processing: Posttranscriptional processing events have to occur in a specific sequence or the cell homeostasis could be threatened. Specific cellular organization contributes to the correct sequence of RNA processing events.
3. Condensate formation: The formation of ribonucleoprotein (RNP) condensates increases local concentration of RBP–RNA modules along with their effector complexes. RNP condensation can stabilize RNA-RBP interactions, contributes to correct RNA folding for further processing, increases the rate of biochemical reactions, and/or assists in storage or transport of molecules.
4. Convergent molecular evolution: This type of evolution has an important role at different levels of RNA processing and contributes to the hierarchical structure of RNA networks. The end result is a unified fate of different transcripts by a common regulatory step.
5. Hierarchical organization: Hierarchically wired networks exhibit fewer connections, adapt faster to the environment, and show higher overall performance compared to non-hierarchical networks.

3. Phase separated ribonucleoparticle condensates

Eukaryotic cells are composed of several organelles, each one with specific functions. Membrane separated organelles provide spatiotemporal control over key cell processes. In addition to these types of organelles, cells also contain organelles that lack a lipid membrane separating them from their surroundings. These organelles are assemblies composed of proteins, nucleic acids, and other molecular components. Examples include assemblies in the nucleus such as the nucleolus, Cajal bodies, and nuclear speckles and also cytoplasmic structures such as stress granules, P-bodies, and germ granules. (Shin and Brangwynne, 2017). Many of such membraneless organelles have liquid-like properties and form by phase separation. This is a physical process that occurs when a saturated solution of components spontaneously separates into two phases: a dense phase and a diluted phase. These phases then coexist in equilibrium (Boeynaems et al., 2018). The molecular drivers of phase separation are so called "scaffolds" and molecules that preferentially partition into condensates created by scaffolds are so called "clients" (Posey et al., 2021). RNP condensates are an example of such phase separated systems. RNPs are macromolecular assemblies of proteins along RNA molecules to carry out specialized cellular processes (Shin and Brangwynne 2017; Banani et al., 2017).

Mechanisms that govern the formation and dissolution of RNP condensates include: membrane surfaces, protein chaperones, RNA helicases, and post-translational modifications of condensate components (He et al., 2023). The formation of membraneless RNP condensates was shown to execute specialized tasks such as DNA replication and repair, chromatin remodelling, transcription, and mRNA splicing (Somasundaram et al., 2022). In the following few paragraphs, there are several listed examples of such RNP condensates to illustrate their ubiquity and importance for key cell processes.

Transcription is thought to take place at discrete nuclear sites known as transcription factories, in the form of phase-separated condensates that allow compartmentalization and coupling of polymerases engaged at multiple genomic sites. It was shown by Shao et al. that RBPs constitute half of the chromatin proteome in embryonic stem cells. Based on their findings, researchers proposed that gene promoter-associated RNA molecules and their binding proteins enhance the phase separation of RNA polymerase condensates to promote active transcription (Shao et al., 2022).

Very recently, it was shown that MED1, an important part of the gene activation complex, called Mediator, selectively partitions RNA polymerase II into RNP condensates. This partitioning occurs together with partitioning of RNA polymerase II positive allosteric regulator while the negative regulators are excluded. Researchers showed that the IDRs of partitioned proteins are necessary and sufficient for selective compartmentalization and require alternating block of charged amino acids. By disrupting this charge pattern, researchers were able to prevent RNP partitioning (Lyons et al., 2023).

RNP condensation is not limited to eukaryotic cells. In bacteria, mRNA decay is controlled by megadalton scale macromolecular assemblies called RNA degradosomes, composed of nucleases and other RNA decay associated proteins. Research into bacterial cell biology has shown that RNA degradosomes can assemble into phase-separated structures, which were then termed bacterial ribonucleoprotein bodies. These bodies were shown to have many analogous properties to eukaryotic RNPs, such as processing bodies (these contain proteins involved in RNA turnover) and stress granules (these contain RNA molecules that are stalled in the pre-initiation complexes) (Muthunayake et al., 2020).

4. Multivalency and intrinsically disordered proteins

Our understanding how RBP and RNA sequences determine the interactions amongst themselves remains poorly understood. Research into this subject provided evidence that RNP assembly depends on the formation of a network of transient, multivalent RBP-RNA and RBP-RBP interactions (Brangwynne et al., 2015; Wang et al., 2018). This part of the review expands on the concept of multivalency: the presence of multiple sites that mediate interactions with other proteins. (Dasmeh et al., 2022).

RNP condensation is driven primarily by intrinsically disordered regions (IDRs) of different RBPs. These regions associate into multimeric complexes through weak, non-specific interactions, through transient secondary structures formed within IDRs, and through contributing interaction from the associated RNA molecules (He et al., 2023). Long noncoding RNAs with unique secondary structures are thought to be especially important in the phase separation process by binding to RBPs (Somasundaram et al., 2022). For some condensates in vitro, RNA molecules, not proteins, are required form maintenance of condensates (Cabral et al., 2022). Introducing unstructured regions into mRNA molecules can also affect processes such as translation initiation, pointing also to the importance of RNA structure in such condensates (Lai et al., 2022).

Multivalency in the form of bivalent binding is a common RNA binding strategy among RBPs, resulting in a higher binding affinity and sequence recognition specificity (Sohrabi-Jahromi and Söding, 2021). Multivalency can vary substantially between protein orthologs, however the length scale at which sequence motifs that enable such protein-protein interactions occur is conserved (Dasmeh et al., 2021).

An important study for multivalency mechanisms in RBPs was performed by Wang et al. in 2018. Researchers showed for protein FUS (belongs to the FUS protein family, a class of intrinsically disordered scaffold proteins) that its phase transitioning is primarily governed by multivalent interactions among tyrosine residues from prion-like domains and arginine residues from RNA-binding domains, which are modulated by negatively

charged residues. Glycine residues enhanced the fluidity, whereas glutamine and serine residues promoted hardening into less dynamic structures. Based on their observations, researchers prepared a model to show that the measured saturation concentrations of phase separation are inversely proportional to the product of the numbers of arginine and tyrosine residues. Their results suggested a possibility to predict phase-separation properties based on protein amino acid sequences (Wang et al., 2018). Other research into this topic has also showed that positively charged amino acid residues in intrinsically disordered proteins (IDPs) could further enhance recruitment of other IDPs, possibly with cation–pi interactions. Poly-ethylene glycol (a crowding reagent used for in vitro phase separation) also increased IDP recruitment, which indicates the need for crowding conditions. Tyrosine residues of IDP proteins also strongly participated in recruitment of other IDPs (Jo et al., 2022).

5. Posttranslational modifications

In the final section, this review will touch upon the effect of posttranslational modifications (PTMs) for RNP condensations. IDPs frequently contain sites PTMs such as phosphorylation, and these modifications exhibit a high preference for IDR residues (Castello et al., 2016; Vieira-Vieira et al., 2022). Arginine methylation has also been noted to play a role in RBP regulation (Gayatri and Bedford, 2014). Generally, PTMs are implicated in regulating protein function by modulating the protein conformation, protein-protein interactions and the transition between ordered and disordered states of IDPs (Miao et al., 2018). However, despite PTMs being well known and catalogued, little is known about how these modifications regulate RNP assembly and function. Previous studies introduced the concept of the phospho-switch, in which RBPs require phosphorylation for condensation of larger RNP complexes (Monahan et al., 2017; Larson et al., 2017). Recently, it was shown that multi-phosphorylation of the C-terminus disordered segment of heteronuclear ribonucleoprotein A1, a key RNA-splicing factor, reduces its ability to locate to nuclear clusters. Similarly, phosphorylation of nucleophosmin 1, a nucleolar protein, was shown to be crucial for lowering its partitioning to the nucleolus and additional phosphorylation of distal sites enhanced its retention in the nucleoplasm (Sridharan et al., 2022). Studies such as these show a clear effect of PTMs on RNP formation, but it remains unclear how this contributes to the protein function and the pattern of selective protein binding to RNAs (Vieira-Vieira et al., 2022; Modic et al., 2021).

6. Conclusions

To conclude this short review, studying RNA networks, their regulation, interactions, and their constitutive elements is a challenging field, requiring multidisciplinary approaches, including approaches from cell biology, biophysics, bioinformatics, and others. Despite its challenges, this field of research is a "hot topic", as indicated by numerous research and review articles that were published in previous few years, especially in 2022 (see references). As compromised function of RBPs underlies the origin of many diseases (He et al., 2022), research into RNA networks will ultimately lead to development of novel therapies for many diseases.

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References

1. Banani SF, Lee O H, Hyman AA, Rosen MK. Biomolecular condensates: organizers of cellular biochemistry. Nature Reviews Molecular Cell Biology. 2017; 18: 285–298. DOI: 10.1038/nrm.2017.7
2. Boeynaems S, Alberti S, Fawzi NL, *et al.* Protein Phase Separation: A New Phase in Cell Biology. Trends in Cell Biology. 2018; 28: 420–435. DOI: 10.1016/j.tcb.2018.02.004

3. Brangwynne CP, Tompa P, Pappu RV. Polymer physics of intracellular phase transitions. Nature Physics. 2015; 11: 899–904. DOI: 10.1038/nphys3532
4. Cabral SE, Otis JP, Mowry KL. Multivalent interactions with RNA drive recruitment and dynamics in biomolecular condensates in *Xenopus* oocytes. iScience. 2022; 25: 104811. DOI: 10.1016/j.isci.2022.104811
5. Castello A, Fischer B, Frese CK, et al. Comprehensive Identification of RNA-Binding Domains in Human Cells. Molecular Cell. 2016; 63: 696–710. DOI: 10.1016/j.molcel.2016.06.029
6. Dasmeh P, Doronin R, Wagner A. The length scale of multivalent interactions is evolutionarily conserved in fungal and vertebrate phase-separating proteins. Genetics. 2022; 220: 1–7. DOI: 10.1093/genetics/iyab184
7. Gayatri S, Bedford M T. Readers of histone methylarginine marks. Biochimica et Biophysica Acta. 2014; 1839: 702–710. DOI: 10.1016/j.bbagrm.2014.02.015
8. He S, Valkov E, Cheloufi S, Murn J. The nexus between RNA-binding proteins and their effectors. Nat Rev Genet. 2023. 24: 276-294. DOI: 10.1038/s41576-022-00550-0
9. Jo Y, Jang J, Song D, *et al.* Determinants for intrinsically disordered protein recruitment into phase-separated protein condensates. Chemical Science. 2022; 13: 522–530. DOI: 10.1039/D1SC05672G
10. Lai W-JC, Zhu M, Belinite M, et al. Intrinsically Unstructured Sequences in the mRNA 3' UTR Reduce the Ability of Poly(A) Tail to Enhance Translation. Journal of Molecular Biology. 2022; 434: 167877. DOI: 10.1016/j.jmb.2022.167877
11. Larson AG, Elnatan D, Keenen MM, et al. Liquid droplet formation by HP1α suggests a role for phase separation in heterochromatin. Nature. 2017; 547: 236–240. DOI: 10.1038/nature22822
12. Lyons H, Veettil R T, Pradhan P, et al. Functional partitioning of transcriptional regulators by patterned charge blocks. Cell. 2023; 186: 1–19. DOI: 10.1016/j.cell.2022.12.013
13. Miao Y, Tipakornsaowapak T, Zheng L, et al. Phospho-regulation of intrinsically disordered proteins for actin assembly and endocytosis. The FEBS Journal. 2018; 285: 2762–2784. DOI: 10.1111/febs.14493
14. Modic M, de Los Mozos IR, Steinhauser S, et al. Epiblast morphogenesis is controlled by selective mRNA decay triggered by LIN28A relocation. bioRxiv preprint. 2021. DOI: 10.1101/2021.03.15.433780
15. Monahan Z, Ryan VH, Janke AM, *et al.* Phosphorylation of the FUS low-complexity domain disrupts phase separation, aggregation, and toxicity. The EMBO journal. 2017; 36: 2951–2967. DOI: 10.15252/EMBJ.201696394
16. Muthunayake NS, Tomares DT, Childers WS, Schrader JM. Phase-separated bacterial ribonucleoprotein bodies organize mRNA decay. WIREs RNA. 2020; 11: e1599. DOI: 10.1002/wrna.1599
17. Posey AE, Holehouse AS, Pappu RV. Phase Separation of Intrinsically Disordered Proteins. In: Methods in Enzymology Volume 611: Intrinsically Disordered Proteins; 2018; pp. 1–30.
18. Shao W, Bi X; Pan Y, et al. Phase separation of RNA-binding protein promotes polymerase binding and transcription. Nature Chemical Biology. 2022; 18: 70–80. DOI: 10.1038/s41589-021-00904-5
19. Shin Y, Brangwynne C P. Liquid phase condensation in cell physiology and disease. Science. 2017; 357: eaaf4382. DOI: 10.1126/science.aaf4382
20. Sohrabi-Jahromi S, Söding J. Thermodynamic modeling reveals widespread multivalent binding by RNA-binding proteins. Bioinformatics. 2021; 37: i308–i316. DOI: 10.1093/bioinformatics/btab300
21. Somasundaram K, Gupta K, Jain N, Jana S. LncRNAs divide and rule: The master regulators of phase separation. Frontiers in Genetics. 2022; 13: 930792. DOI: 10.3389/fgene.2022.930792
22. Sridharan S, Hernandez-Armendariz A, Kurzawa N, et al. Systematic discovery of biomolecular condensate-specific protein phosphorylation. Nature Chemical Biology. 2022; 18: 1104–1114. DOI: 10.1038/s41589-022-01062-y
23. Vieira-Vieira C H, Dauksaite V, Sporbert A, et al. Proteome-wide quantitative RNA-interactome capture identifies phosphorylation sites with regulatory potential in RBM20. Molecular Cell. 2022; 82: 2069–2083.e1–e8. DOI: 10.1016/j.molcel.2022.03.024
24. Wang J, Choi J-M, Holehouse AS, et al. A Molecular Grammar Governing the Driving Forces for Phase Separation of Prion-like RNA Binding Proteins. Cell. 2018; 174: 688–699. DOI: 10.1016/j.cell.2018.06.006