Review Article

Role of Epigenetic Modification of N^6-methyladenosine in Phase Separation

Si-Yu Liu[1], Yi Feng[2], Zi-Li Sun[1], Ming-Li Zou[1], Jun-Jie Wu[2], Zheng-Dong Yuan[2] and Feng-Lai Yuan[1,2,3*]

[1]*Wuxi Clinical Medicine School of Integrated Chinese and Western Medicine, Nanjing University of Chinese Medicine, Wuxi, Jiangsu, China;* [2]*Institute of Integrated Chinese and Western Medicine, The Hospital Affiliated to Jiangnan University, Wuxi, Jiangsu, China;* [3]*Department of Central Laboratory, The Third Hospital Affiliated to Nantong University, Wuxi, Jiangsu, China*

Abstract

In recent years, the development of biophysical analysis methods has crossed with macromolecular condensates in cells. Researchers are interested in membrane-less organelles assembling into biomolecule 'aggregates' with similar liquid-like properties of phase separation. Cell biologists now think that many of the membrane-less organelles observed in cells are formed by phase separation caused by interactions between proteins and nucleic acids. Phase separation, thus, becomes a major player in the control of a variety of biological functions. Nevertheless, the biophysical regulation of these cells is still poorly understood. Here, we reviewed the current literature that collectively reveals the roles of epigenetic modification of N^6-methyladenosine (m^{6}A) in phase separation.

Introduction

Phase transition/phase separation of biological macromolecules in cytoplasm has developed rapidly into a hot research area in recent years. Cells consist of many different organelles (or compartments) that separate cell functions. Organelles can be divided into compartments enveloped by lipid membranes (such as the nucleus, vacuoles, endoplasmic reticulum, and mitochondria) and organelles not enveloped by lipid membranes (such as P bodies, nucleoli, pericentric substances, stress granules, and reproductive particles). The distribution and assembly dynamics of non-membrane-bound compartments have aroused great interest over the last ten years and for good reason.[1–3] Accumulating evidence has indicated that these non-membrane-bound organelles, such as Cajal bodies, nucleoli, stress granules, synaptic cytoskeleton, and microRNA-induced silencing complex, behave as fluid droplets, which undergo phase separation phenomenon.[4,5]

Through the study of these non-membrane-bound organelles, scientists have gained a deep understanding of the molecular mechanism of different diseases. Earlier work in this area focused on stress granules involved in cell survival and its relationship with amyotrophic lateral sclerosis. Recently, several studies provided us with fresh insight into the dynamic of RNA modifications and RNA-protein interactions in contributing to phase separation and non-membrane-bound organelles' formation in cells.[6] However, it has never been clarified what triggers the aggregation of some macromolecules rather than others in the same droplet.

Thus, we reviewed the current literature on the roles of the modification of N^6-methyladenosine (m^{6}A) in phase separation, with a focus on recent research findings regarding m^{6}A regulation of phase separation.

Phase separation regulates m^{6}A-modified mRNAs

A recent report shows that the modification of m^{6}A could stimulate the formation of phase separation of mRNA and YTH N^6-methyladenosine RNA binding proteins (YTHDFs),[3] and subsequently affect the fate of those cells. m^{6}A modification occurs via a methyltransferase complex (referred to as dedicated writers) mainly consisting of methyltransferase like protein 3 (METTL3), methyltransferase like protein 14 (METTL14) and Wilms tumor 1-associated protein (WTAP). This modification can be reversed by the demethylases (referred to as dedicated erasers) like fat mass and obesity-associated protein (FTO) and AlkB homolog 5 (ALKBH5). Moreover, the YTH domain containing several m^{6}A-binding proteins, including YTHDF1, YTHDF2 and YTHDF3 in

Keywords: Methylation; Phase separation; m^{6}A; YTHDF protein.

Abbreviations: m^{6}A, N^6-methyladenosine; LLPS, liquid-liquid phase separation; YTHDF, YTH N^6-methyladenosine RNA binding protein; WTAP, Wilms tumor 1-associated protein; METTL, methyltransferase like protein; FTO, fat mass and obesity-associated protein; ALKBH5, AlkB homolog 5; YTHDC, YTH domain containing protein; HNRNPA2B1, heterogeneous nuclear ribonucleoprotein A2B1; IGF2BP1/2/3, insulin-like growth factor 2 mRNA binding protein 1/2/3; Prrc2a, proline-rich coiled-coil 2A; HNRNPC, heterogeneous nuclear ribonucleoprotein C; elF3, eukaryotic initiation factor 3; SRSF2, serine and arginine rich splicing factor 2.

Received: November 01, 2019; Revised: December 11, 2019; Accepted: February 07, 2020

[*]**Correspondence to:** Feng-Lai Yuan, Institute of Integrated Chinese and Western Medicine, The Hospital Affiliated to Jiangnan University, Wuxi, Jiangsu, 214041, China. Tel: +86-510-82603332, Fax: +86-510-82603332, E-mail: bjjq88@163.com

How to cite this article: Liu S-Y, Feng Y, Sun Z-L, Zou M-L, Wu J-J, Yuan Z-D, Yuan F-L. Role of Epigenetic Modification of N^6-methyladenosine in Phase Separation. *Exploratory Research and Hypothesis in Medicine* 2020;5(2):70–73. doi: 10.14218/ERHM.2019.00028.

Copyright: © 2020 Authors. This is an Open Access article distributed under the terms of the Creative Commons Attribution-Noncommercial 4.0 International License (CC BY-NC 4.0), permitting all non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

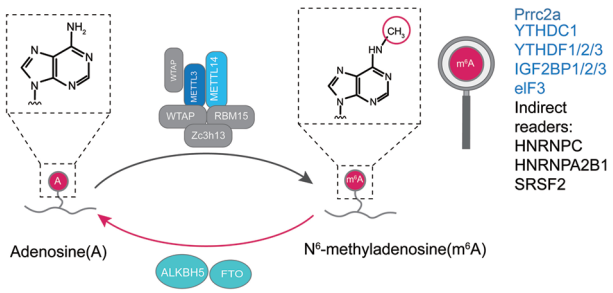

Fig. 1. m^{6}A modification is dynamically regulated by writer, eraser, and reader protein. Writer protein is a methyltransferase, like WTAP, METTL3, and METTL14. Eraser protein is a demethylase, like FTO and ALKBH5 localized primarily in the nucleus. Reader protein is a binding methylated protein, which is divided into in cytoplasm and in nucleus. In the nucleus, m^{6}A can be regulated by m^{6}A readers YTHDC1, HNRNPA2B1, and IGF2BP1/2/3, while in the cytoplasm, m^{6}A can be regulated by m^{6}A readers YTHDF1/2/3, YTHDC2, and eIF3. WTAP, Wilms tumor 1-associated protein; METTL, methyltransferase like protein; FTO, fat mass and obesity-associated protein; ALKBH5, AlkB homolog 5; YTHDC, YTH domain containing protein; HNRNPA2B1, heterogeneous nuclear ribonucleoprotein A2B1; IGF2BP1/2/3, insulin-like growth factor 2 mRNA binding protein 1/2/3; YTHDF1/2/3, YTH domain family proteins 1/2/3; Prrc2a, proline-rich coiled-coil 2A; HNRNPC, heterogeneous nuclear ribonucleoprotein C; eIF3, eukaryotic initiation factor 3; SRSF2, serine and arginine rich splicing factor 2.

the cytoplasm, and the nuclear YTH domain containing protein 1 (YTHDC1) have been identified to be the 'readers' of m^{6}A and to be able to regulate mRNA stability and translation, thereby mediating downstream effects (Fig. 1).[7–9] Recent studies indicated that mRNA metabolism needs the active involvement of the modification of m^{6}A, in particular modulating mRNA stability, determining cell fate, affecting transcriptional control of cell, and playing an important role in regulating the fate determination of various cell types,[10] lipid metabolism,[11] and immunity.[12] Scientists selected three of the major m^{6}A-binding YTH domain family proteins YTHDF1,2,3 as research subjects, trying to reveal the regulation mechanism of m^{6}A on mRNA stability and translation efficiency.[13] Primary protein sequence analysis has demonstrated that YTHDF protein consists of about 15 kDa of m^{6}A-binding YTH domain and a low complexity region of about 40 kDa.[14] It is speculated that the three genes may undergo liquid-liquid phase separation (LLPS) according to this structural arrangement (Fig. 2). YTHDF2 protein was the highest content of YTHDF paralogue purified from cells. Several studies have characterized that the factors regulating strength of intermolecular interactions, including temperature, pH and ionic strength, will affect the ultimate phase separation behavior.[15] The gene solution of YTHDF2 was transparent at the low-temperature of 4 °C; however, when the temperature raised to 37 °C, the YTHDF2 gene solution became turbid; the solution became clear again at the low-temperature of 4 °C. This finding served to confirm that the YTHDF2 phenomenon was LLPS. Meanwhile, Ries *et al.*[3] found that YTHDF2 protein at the physiological concentration (about 5 μM) increased sufficiently for phase separation occurring. Those authors additionally examined whether phase separation enhancement of YTHDF2 protein was regulated by m^{6}A modification *in vitro*. They confirmed that a 65 nucleotide-long transcriptome regions containing 10 modified m^{6}A caused a significant increase of the phase separation in YTHDF proteins. Consequently, the authors speculated that adjacent m^{6}A moieties on RNA promote 'multivalent interactions' between the intrinsically disordered regions of YTHDF proteins that were bound to m^{6}A through their YTH domain, thus facilitating phase separation.

To address whether m^{6}A can influence the phase separation of YTHDF2 gene, Ries *et al.*[3] confirmed that endogenous stress granules were formed after exposure to heat shock in m^{6}A methyltransferase14-knockout mouse embryonic stem cells; however, the localization of YTHDF2 genes in the stress granules was obviously reduced. Thus, the localization of YTHDF2 to stress granules under stress conditions relies on its modification with m^{6}A; that is, the subcellular localization of YTHDF2 gene is regulated by m^{6}A-modified mRNAs. The translation efficiency of m^{6}A-modified mRNA in methyltransferase14-knockout cells was obviously inhibited when cells are exposed to heat shock. Summarizing the above findings, Ries *et al.*[3] affirmed that the RNA modification by m^{6}A, especially polymethylated m^{6}A-mRNAs, could recruit multiple YTHDF2 genes, and obviously enhanced the phase separation of YTHDF gene. Conversely, the phase separation of YTHDF2 was found to regulate the translation efficiency of polymethylated mRNA. Ultimately, these findings proposed a novel theory for the precision regulation of m^{6}A modification on cellular biological processes.

In addition, Fu *et al.*[16] recently shown that when cells were under oxidative stress, a large number of m^{6}A-modified mRNAs were localized to stress granules. Additional studies indicated that YTHDF gene was very important for the formation of stress particles under oxidative stress. After knocking down of YTHDF by small interfering RNA, the level of stress granules decreased markedly. Interestingly, an article published in *Nature* proved that the knockout of METTL14 would not affect the stress granules' formation under heat shock conditions.[3] While the article from bioRxiv indicated that the binding of YTHDF and m^{6}A was hindered,[16] and

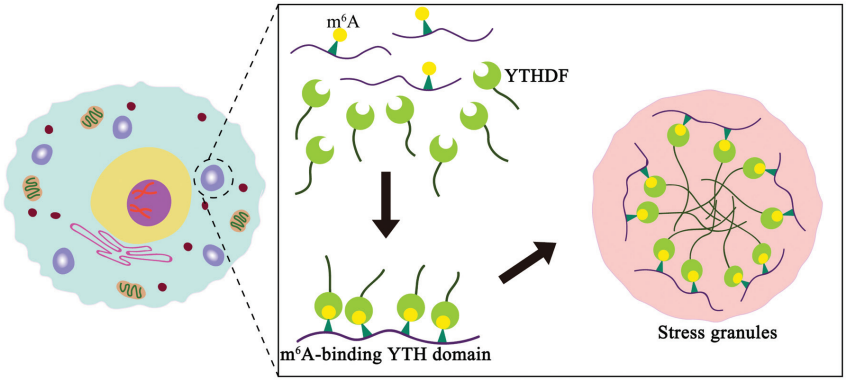

Fig. 2. m^{6}A is an intermediate linker between mRNA and YTHDF protein, which promotes YTHDF-mediated phase separation.

stress particle formation was markedly reduced. In other words, stress granules' formation under thermal and oxidative stress may be triggered by dissimilar genes; the underlying precise regulatory mechanisms require further analysis.[4] The two articles drew the reasonable conclusion from different perspectives; that is, m^{6}A modification can promote the phase separation process of specific RNA-binding proteins.

Recently, Gao *et al.*[17] found that multivalent m^{6}A-containing RNA can increase the phase separation YTHDF genes in HEK293 cells. Their data indicated that the m^{6}A promotion of phase separation of YTHDF proteins is related to the response of cells to stress. YTHDF1/2/3 comprise a family of m^{6}A readers. The researchers first analyzed the sequence of these three proteins and found a large number of low complexity domains. Then, they verified that both the low complexity domains and full-length proteins of YTHDF exhibited phase separation. m^{6}A has a key role in diverse biological processes by binding to the relevant YTH domain-containing genes, as well as binding to other translation initiation factors. Multivalent m^{6}A-driven phase separation of YTHDF genes may contribute to the physiology of a cell in the stress response.

These studies provide an experimental basis for the regulation of m^{6}A phase separation and gene expression regulation and also provide an understanding for the regulation of key gene expression during cell fate determination and disease development.[18–20] At present, the structures of membrane-less organelles of phase separation are very clear, and a subsequent major objective is to investigate the distinct properties of phase separation. Concentrated states of DNA, protein, and RNA are a primary aspect of intracellular compartments.[21] It has become commonly accepted that large amounts of these condensates form via LLPS, resulting in liquid-like states of intracellular matter. Thus, intracellular liquid condensates can drive richer structural assembly than uniform droplets from multilayer structures, such as nucleoli and stress particles, to liquid crystal assemblies, such as spindle-shaped and actin condensates.[21,22]

Physical and chemical properties of phase separation will be an increasingly important subject for future challenges. Employing m^{6}A-modified mRNAs governed by phase separation as a target to regulate various cellular and physiological processes, such as transcriptional control, stress exposure, signal transduction, *etc.* will also provide a new insight for m^{6}A-regulated phase separation in different research fields. Research on the relationship between phase separation and disease is progressing rapidly. The current research is primarily focusing on the molecular pathogenesis of neurodegenerative diseases.[23] Some gene point mutations, such as FUS protein, can increase the transition from a solid phase to a liquid solvent and may be the reason for the formation of insoluble protein accumulation which is commonly found in neurodegenerative disorders.[24] Moreover, Nuclear transport has been shown to decrease nucleic acid binding protein concentration in the cytoplasm, dissolve the LLPS structure, and prevent neurotoxicity formation attributed to solid aggregation.[25]

Hypothesis

Recent studies have indicated that the modification of m^{6}A is involved in the regulation process of mRNA metabolism, in particular in modulating mRNA stability, determining cell fate, affecting different cellular biological systems, and taking a responsible role in cell fate determination, immunity, and lipid metabolism. Scientists have tried to explain the regulation mechanisms of m^{6}A to the translation efficiency and stability of mRNA based on three of the major m^{6}A-binding proteins. Primary gene sequence analysis showed that the YTHDF genes consisted of an m^{6}A-binding YTH location of about 15 kDa and a low complexity location of about 40 kDa. Thus, the authors speculated that the YTHDF genes may undergo LLPS based on this structural arrangement (Fig. 2).

Conclusions

Phase separation is increasingly recognized as key regulatory biological macromolecules in cytoplasm. It is time to expand the research focus to phase separation for numerous biological functions. Studying the m^{6}A-mediated phase separation mechanism, especially finding or screening key proteins will provide a theoretical basis for verifying the effectiveness of target macromolecules.

Acknowledgments

None.

Funding

Funding for this study was provided by the Natural Science Foundation of China (81770876).

Conflict of interest

The authors have no conflicts of interest related to this publication.

Author contributions

Designing, reviewing and revising the article (YFL), drafting of the manuscript (LSY), reviewing and drawing (FY, WJJ), collecting materials and revising the manuscript (YZD, ZML, SZL), approved the final version submitted (all the authors).

References

[1] Brangwynne CP, Eckmann CR, Courson DS, Rybarska A, Hoege C, Gharakhani J, *et al*. Germline P granules are liquid droplets that localize by controlled dissolution/condensation. Science 2009;324(5935):1729–1732. doi:10.1126/science.1172046.
[2] Marnik EA, Updike DL. Membraneless organelles: P granules in Caenorhabditis elegans. Traffic 2019;20(6):373–379. doi:10.1111/tra.12644.
[3] Ries RJ, Zaccara S, Klein P, Olarerin-George A, Namkoong S, Pickering BF, *et al*. m(6)A enhances the phase separation potential of mRNA. Nature 2019;571:424–428. doi:10.1038/s41586-019-1374-1.
[4] Wang JH, Wang LY, Diao JB, Shi YG, Shi Y, Ma HH, *et al*. Binding to m(6)A RNA promotes YTHDF2-mediated phase separation. Protein Cell 2019. doi:10.1007/s13238-019-00660-2.
[5] Shin Y, Chang YC, Lee DSW, Berry J, Sanders DW, Ronceray P, *et al*. Liquid nuclear condensates mechanically sense and restructure the genome. Cell 2019;176(6):1518. doi:10.1016/j.cell.2019.02.025.
[6] Alberti S, Gladfelter A, Mittag T. Considerations and challenges in studying liquid-liquid phase separation and biomolecular condensates. Cell 2019;176(3):419–434. doi:10.1016/j.cell.2018.12.035.
[7] Liu SY, Feng Y, Wu JJ, Zou ML, Sun ZL, Li X, *et al*. m(6)A facilitates YTHDF-independent phase separation. J Cell Mol Med 2019;24(2):2070–

2072. doi:10.1111/jcmm.14847.
[8] Wang Y, Mao J, Wang X, Lin Y, Hou G, Zhu J, *et al*. Genome-wide screening of altered m6A-tagged transcript profiles in the hippocampus after traumatic brain injury in mice. Epigenomics 2019;11(7):805–819. doi:10.2217/epi-2019-0002.
[9] Luo GZ, MacQueen A, Zheng G, Duan H, Dore LC, Lu Z, *et al*. Unique features of the m6A methylome in Arabidopsis thaliana. Nat Commun 2014;5:5630. doi:10.1038/ncomms6630.
[10] Wu Y, Xie L, Wang M, Xiong Q, Guo Y, Liang Y, *et al*. Mettl3-mediated m(6)A RNA methylation regulates the fate of bone marrow mesenchymal stem cells and osteoporosis. Nat Commun 2018;9:4772. doi:10.1038/s41467-018-06898-4.
[11] Zhong X, Yu J, Frazier K, Weng X, Li Y, Cham CM, *et al*. Circadian clock regulation of hepatic lipid metabolism by modulation of m(6)A mRNA methylation. Cell Rep 2018;25(7):1816–1828.e4. doi:10.1016/j.celrep.2018.10.068.
[12] Han D, Liu J, Chen C, Dong L, Liu Y, Chang R, *et al*. Anti-tumour immunity controlled through mRNA m(6)A methylation and YTHDF1 in dendritic cells. Nature 2019;566:270–274. doi:10.1038/s41586-019-0916-x.
[13] Lu W, Tirumuru N, St Gelais C, Koneru PC, Liu C, Kvaratskhelia M, *et al*. N(6)-Methyladenosine-binding proteins suppress HIV-1 infectivity and viral production. J Biol Chem 2018;293:12992–13005. doi:10.1074/jbc.RA118.004215.
[14] Zhuang M, Li X, Zhu J, Zhang J, Niu F, Liang F, *et al*. The m6A reader YTHDF1 regulates axon guidance through translational control of Robo3.1 expression. Nucleic Acids Res 2019;47(9):4765–4777. doi:10.1093/nar/gkz157.
[15] Ceballos AV, McDonald CJ, Elbaum-Garfinkle S. Methods and strategies to quantify phase separation of disordered proteins. Methods Enzymol 2018;611:31–50. doi:10.1016/bs.mie.2018.09.037.
[16] Fu Y, Zhuang X. m6A-binding YTHDF proteins promote stress granule formation by modulating phase separation of stress granule proteins. bioRxiv 2019:694455. doi:10.1101/694455.
[17] Gao Y, Pei G, Li D, Li R, Shao Y, Zhang QC, *et al*. Multivalent m6A motifs promote phase separation of YTHDF proteins. Cell Res 2019;29(9):767–769. doi:10.1038/s41422-019-0210-3.
[18] Ji P, Wang X, Xie N, Li Y. N6-methyladenosine in RNA and DNA: an epitranscriptomic and epigenetic player implicated in determination of stem cell fate. Stem Cells Int 2018;2018:3256524. doi:10.1155/2018/3256524.
[19] Wang H, Xu B, Shi J. N6-methyladenosine METTL3 promotes the breast cancer progression via targeting Bcl-2. Gene 2020;722:144076. doi:10.1016/j.gene.2019.144076.
[20] Li T, Hu PS, Zuo Z, Lin JF, Li X, Wu QN, *et al*. METTL3 facilitates tumor progression via an m(6)A-IGF2BP2-dependent mechanism in colorectal carcinoma. Mol Cancer 2019;18:112. doi:10.1186/s12943-019-1038-7.
[21] Shin Y, Brangwynne CP. Liquid phase condensation in cell physiology and disease. Science 2017;357(6357):eaaf4382. doi:10.1126/science.aaf4382.
[22] Weirich KL, Banerjee S, Dasbiswas K, Witten TA, Vaikuntanathan S, Gardel ML. Liquid behavior of cross-linked actin bundles. Proc Natl Acad Sci 2017;114(9):2131–2136. doi:10.1073/pnas.1616133114.
[23] Ambadipudi S, Biernat J, Riedel D, Mandelkow E, Zweckstetter M. Liquid-liquid phase separation of the microtubule-binding repeats of the Alzheimer-related protein Tau. Nat Commun 2017;8:275. doi:10.1038/s41467-017-00480-0.
[24] Qamar S, Wang G, Randle SJ, Ruggeri FS, Varela JA, Lin JQ, *et al*. FUS phase separation is modulated by a molecular chaperone and methylation of arginine cation-pi interactions. Cell 2018;173:720–734.e15. doi:10.1016/j.cell.2018.03.056.
[25] Duan Y, Du A, Gu J, Duan G, Wang C, Gui X, *et al*. PARylation regulates stress granule dynamics, phase separation, and neurotoxicity of disease-related RNA-binding proteins. Cell Res 2019;29:233–247. doi:10.1038/s41422-019-0141-z.