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RNA and liquid-liquid phase separation

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ABSTRACT

Liquid-Liquid Phase Separation (LLPS) is a biological phenomenon that refers to the components of similar properties form droplets condensate in cells. These droplets play an important role in maintaining the stability of order in cells. In the studies of phase separation, weak multivalent interactions between proteins have always been the focus of attentions. With the deepening research of phase separation, more and more evidences show that RNA, especially long noncoding RNA (lncRNA), also plays an important regulatory role in the phase separation. We summarized recent researches between phase separation and RNA, and focused on the function of non-coding RNA (ncRNA) in the process of phase separation. In fact, phase separation and RNA have a two-way regulation relationship. Noncoding RNA usually recruits proteins as molecular scaffolds to drive phase separation. On the other hand, phase separation is also involved in RNA transcription, transport, metabolism and other processes.

1. Liquid-liquid phase separation and RNA

1.1. Research progress of phase separation

Phase separation in cells is a biological phenomenon in which macromolecular substances form a unique liquid phase under a variety of interaction forces likely oil-water separation [1]. The droplets formed by the polymerization of macromolecules have fluidity and gradually tend to form spherical shapes, and can assemble and dissociate within a few seconds, thereby responding to signals quickly and accurately. It believes that the intracellular components can form reaction compartments by phase separation, thereby forming an environment in which multiple biological reactions occur simultaneously. As early as 1899, researchers proposed the hypothesis that the cytoplasm may contain suspended droplets of different chemical properties. Until the beginning of the 21st century, scientists accidently discovered that the P granules in C.elegans had the characteristics of droplets, which put forward the hypothesis that the phase separation causes specific molecules to gather, thereby forming an orderly compartment in the chaotic and crowded cells [2]. After 2009, phase separation has attracted enthusiastic attention in the biology. Scientists around the world have conducted a lot of research on the phase separation phenomenon in cells. In 2015, the development of phase separation reached a “blowout” period. In 2018, Science listed phase separation as one of the “Top Ten Scientific Annual Breakthroughs".

1.2. Research methods of phase separation

Phase-separated droplets have three distinct characteristics: fusion, reversibility and high dynamic [3,4]. Therefore, the phase-separated experiments mainly base on these three characteristics. Currently, fluorescence recovery after photobleaching (FRAP) is the most commonly method for phase separation studies *in vivo* [5,6]. First, label the phase separation of a certain RNA or protein in the droplet with a fluorescein, and then a high-intensity pulsed laser quench the fluorescence of a certain area in the droplet. Finally, the material exchange capacity of the phase-separated droplets can be inferred from the rate of fluorescence recovery. With the deepening of research, more and more precise methods are used in phase separation. For example, atomic force microscopy (AFM) can detect the shape of droplets through high-resolution imaging [7]. Biomembrane Force Probe (BFP) and optical tweezers (OT) can be used to detect droplet surface tension and elasticity, and OT can also be used to measure droplet fusion time [8–10].

In addition, the optogenetic has also been applied to the study of phase separation. The optoDroplet, Corelets, and Casdrop systems all use optogenetic methods for precision research of phase separation [11–13]. The optoDroplet system can control the conformation of the

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protein with IDRs structure through light to intervene the occurrence of phase transition process and simulate phase separation [11]. Corelets can be used to quantify the protein concentration of phase separation [12]. Casdrop can separate the protein in the designated area of the cell by light [13].

Since the interaction between ncRNA and the phase-separated structure is basically instantaneous and difficult to capture by conventional methods, the recently developed in situ single-molecule high-resolution localization and counting (iSHiRLoC) method has attracted widespread attention, because it can accurately measure the interaction between RNA and proteins of phase separated in living cells [14]. For example, using iSHiRLoC can accurately determine the location and interaction of miRNA, mRNA and process body (PB). And it can accurately study the dynamics of miRNA and RNA in the liquid phase [15].

2. RNA drivers the formation of phase separation droplet

2.1. RNA participates in the initiation of phase separation

The occurrence of phase separation is a complex biological process, which is generally considered to be the weak and multivalent interaction by intrinsically disordered regions (IDRs) or ligands of proteins [5,16–21]. However, more and more studies have found that ncRNA is also involved in regulating the occurrence of phase separation. The ncRNA can be used as a molecular scaffold to bind multiple RNA binding proteins (RBPs), so that RBPs are connected together to form a dynamic network, as known as phase separation droplets [18,22,23]. Major Satellite (MajSAT) has also been shown to form a complex with SAFB to promote phase separation to maintain the high-level structure of heterochromatin [24]. In addition, mRNA can also form phase-separated droplets through specific three-dimensional shapes and complementary base pairing [25]. For example, the RNA binding protein Whi3 can occur phase separation with the mRNA of cyclin CLN3 or the mRNA of actin BNI1 *in vitro*, but it is interesting that the two droplets cannot be fused with each other, while the droplets containing the same mRNA can fuse into a bigger droplet [25]. In summary, RNA can drive the occurrence of phase separation, and the structure of mRNA can work as a "passport" for different phase separation droplets.

2.2. LncRNAs participates in the driving process of phase separation

Previous studies have found that lncRNAs play an important role in the development of the central nervous system, tumorigenesis and development working as signal, guide, scaffold and decoy through binding proteins [26–29]. Now more and more studies have found that lncRNAs are involved in the regulation process of phase separation (Table 1, Fig. 1).

LncRNAs and the membraneless organelles in the cell are also inseparable. Normally, lncRNAs work as a molecular scaffold to initiate phase separation, thereby promoting the assembly of membraneless organelle. Paraspeckle is a typical membraneless organelle, which is rich in protein and RNA, and has obvious liquid phase characteristics [30,31]. It is worth noting that the formation mechanism of paraspeckle tends to be that lncRNA nuclear paraspeckle assembly transcript 1 (NEAT1) promotes the assembly of paraspeckles by driving phase separation. NEAT1 has two isoforms. The longer transcript, NEAT1_2 can be used as a molecular scaffold to provide multiple protein binding sites. These binding sites are used to bind RBPs, such as non-POU domain--containing octamer-binding protein (NONO) and splicing factor proline- and glutamine-rich (SFPQ), and increase the local concentration of RBPs to drive phase separation to form a highly ordered structure [30–32]. Interestingly, NEAT1 has a semi-extractability because of multivalent forces. This leads to the phenomenon that when using the Trizol method to extract RNA, most of NEAT1 remains in the phase-separated droplets, resulting in a very low expression level. However, after the phase separation structure is destroyed by heat treatment, NEAT1 is released into the solution, and the expression level can be about 20 times higher than the normal method [33]. This suggests that the scaffold RNA in the phase-separated droplets may all have the property of semi-extractability, and also proves the important position of NEAT1 as scaffold RNA in the formation of paraspeckles.

LncRNA driving phase separation can provide a new explanation mechanism for X chromosome silencing. Initially, researchers discovered that lncRNA Xist can recruit chromatin inhibitory proteins to form X-chromosome inactivation (XCI). The high resolution microscopy and RNA FISH show that each XCI contains about 100 Xist complexes. These complexes are spherical with a diameter of 300–400 nm, which has a high similarity to the phase separation droplets. So the hypothesis that X chromosome silence can be mediated by phase separation is proposed [34]. Xist contains 6 repetitive sequences A-F, of which the E repetitive sequence can be combined with RNA binding proteins, such as PTBP1, MATR3, LBR and TDP-43, to initiate the assembly of XCI [34,35]. Further studies have found that PTBP1 can multivalently bind to the E-repeat sequence of Xist to form phase-separated droplets, which drives the production of compartments in the nucleus and thus promotes

Table 1
LncRNA regulates the occurrence of phase separation.

lncRNA	Interacting protein	Functions	References
NEAT1	NONO SFPQ FUS	Acts as a molecular scaffold to promote paraspeckles assembly	[30–32]
Xist	PTBP1 MATR3 LBR TDP-43	Combine with RBP to drive X chromosome silencing	[34–37]
DIGIT	BRD3	Drives the phase separation of BRD3 to regulate the expression of genes related to endoderm differentiation	[39]
DilncRNA	53BP1	Promotes cell damage repair by initiating phase separation of DDR factor 53BP1	[22,40,41]
RIGSRNA	ACM proteins	Promotes A body to complete liquid-solid transformation, and finally forms amyloid-like protein and triggers cell dormancy	[43–45]

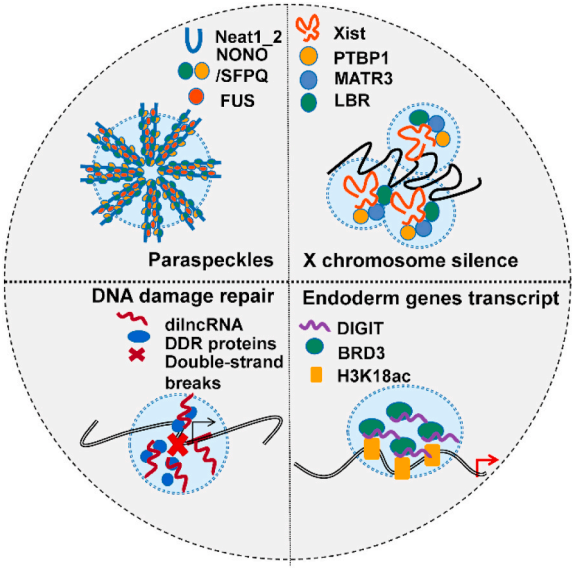

Fig. 1. LncRNAs are involved in phase separation in nucleus. LncRNAs always work as molecular scaffolds to recruit proteins and promote the occurrence of phase separation, including paraspeckles assembly, X-chromosome inactivation (XCI) assembly, DNA damage repair and regulation of endoderm genes transcript.

XCI-related gene silencing [36,37].

LncRNA DIGIT plays a role in the differentiation of endoderm cells [38]. DIGIT and BRD3 protein can combine with each other to form phase-separated droplets, and the addition of DIGIT reduce the threshold for phase separation of BRD3 [39]. These phenomena explain that DIGIT can promote phase separation of BRD3. Further research found that BRD3 interact strongly with H3K18ac, and DIGIT can also be enriched in the chromatin region labeled by H3K18ac [39]. It is speculated that DIGIT may promote the expression of genes related to endoderm differentiation by recruiting BRD3 to H3K18ac-enriched chromatin region to regulate endoderm differentiation.

LncRNAs also participate in the process of DNA damage repair through phase separation behavior. DNA damage response (DDR) focus is a globular and non-membrane nucleus, which can sense DNA damage, send damage signals and promote DNA repair. It is worth noting that the ncRNA transcribed at DNA double strand breaks (DSB) plays an important role in the formation of DDR [40,41]. Damage induced long noncoding RNA (dilncRNA) is transcribed by RNA polymerase II at DSB and promoted damage repair in corresponding to DSB [41]. The experiments *in vitro* have proved that a complete RNAPII pre-initiation complex (PIC) and dilncRNA can promote the phase separation of DDR factors 53BP1 at the DSB, which promotes cell damage repair [22].

In addition to promoting liquid-liquid phase separation, lncRNA can also occur in the process of promoting liquid-solid transition lncRNA. Studies have found that the ncRNA rIGSRNA produced by ribosomal gene spacers is involved in the maturation process of Amyloid bodies (A-bodies). Initially, A-bodies is a liquid-phase focus, similar to phase separation, but with the addition of rIGSRNA, it undergoes liquid-solid transformation, eventually forming amyloidogenic-like proteins and triggering cell dormancy. One hypothesis is that rIGSRNA recruits a large number of amyloid-converting motif (ACM) proteins through electrostatic interactions, exceeding the concentration threshold of liquid phase proteins, and ultimately leading to amyloid aggregates [42].

In summary, lncRNA generally acts as a molecular scaffold during the formation of phase separation, providing multivalent sites to bind one or more RBPs, thereby driving the occurrence of phase separation.

2.3. RNA drives the assembly of stress granules through phase separation

The formation mechanism of membraneless organelles has always been a research hotspot, but there are still some unclear problems. For example, how do membraneless organelles maintain their stability? What is the mechanism by which membraneless organelles respond to cell life activities in a highly dynamic manner? However, more and more studies have found that the phenomenon of phase separation occurs in the assembly process of membraneless organelles, and can explain the above problems well. Even some membraneless organelles have been confirmed to be phase separation droplets. For example, the occurrence of phase separation has been reported in membraneless organelles such as Stress granules (SGs), nucleolus, P bodies and Cajal bodies [1,46–48].

SGs are a protection mechanism for eukaryotic cells when they are under external pressure which is highly dynamic and reversible. When external pressure exists, SGs can be assembled quickly, and when the stress disappears, SGs will de-aggregate [49,50]. These characteristics are similar to phase separation, which has triggered an upsurge of research on phase separation in the formation of SGs. SGs are also rich in lncRNA NEAT1, which drives TDP-43 to assembly to form liquid-phase SGs [51]. In addition to lncRNA, other types of RNA also play an important role in driving phase separation to form SGs. SGs are rich in N6-methyladenosine (m6A) modified RNA and its binding proteins. These RNA can also drive phase separation. The m6A-modified RNA can be used as a scaffold to recruit and bind to the m6A reader YTHDF2, resulting in phase separation [52–54]. The study also found that the assembly of SGs depends on the core protein-RNA network, where G3BP1 is the central protein of the RBP-RNA interaction network related to SGs. And dimerization domain NTF2 and IDR1/2 of G3BP1 are necessary for the assembly of SGs [55,56]. G3BP1 can sense the increase of free RNA concentration in cells, and then its IDRs will undergo conformational changes after binding to RNA, thereby leading to RNA-dependent phase separation and ultimately driving the assembly of SGs [57,58]. It can be seen that the enrichment of RNA can drive phase separation in SGs, so that SGs with a liquid phase structure can dynamically and quickly respond to external pressure.

2.4. RNA concentration affects the level of phase separation

Generally, the abnormal phase separation of RBP in the cytoplasm is an important factor in neurodegenerative diseases [59–61]. It is worth noting that there is often a very high concentration of RNA in the nucleus, while RNA in the cytoplasm is relatively low. Therefore, the RNA concentration may have a regulatory effect on the occurrence of abnormal phase separation.

A recent study found that RNA in the cell nucleus can dynamically regulate the phase separation behavior of transcription complexes using a non-equilibrium feedback control mechanism where the concentration of RNA changes with the transcription process [62]. Electrostatic interaction is the main driving force for phase separation and is usually mediated by amino acids, such as multivalent cation-pi, pi-pi [63,64]. In fact, many condensates in the cell nucleus are composed of protein with positive charges and RNA with negative charges. When RNA is at a low concentration, the interaction between the positive and negative charges will promote phase separation. But when the RNA concentration gradually increases, the negative charges is much higher than the positive charges, and the repulsion between the charges will cause the dissociation of the phase separation [62].

Associating the relationship between phase separation and RNA concentration, we can easily think of the abnormal phase separation behavior of Fused in sarcoma (FUS) in cytoplasm. FUS is a typical RNA binding protein, which can form a reversible phase separation structure through its amino-terminal prion-like domain (PrLD) [65]. The solid fiber FUS has been found in the cytoplasm of neurological degenerative diseases samples, indicating that excessive accumulation of cytoplasmic FUS might cause a series of diseases [65,66]. Interestingly, FUS rarely aggregates in the cell nucleus. Therefore, finding the cause of the abnormal phase separation in the FUS cytoplasm has become the focus of the treatment of this type of disease. *In vitro*, it was found that adding low concentration RNA can promote the formation of FUS phase separation droplets, while a system containing a higher RNA concentration inhibits the phase separation behavior of FUS [67,68]. This phenomenon suggests that the RNA concentration may be the reason of the different degrees of phase separation of FUS in the nucleus and cytoplasm.

3. Phase separation regulates RNA transcription , localization and metabolism

3.1. Phase separation regulates RNA transcription

Phase separation plays an important regulatory role in the process of mRNA transcription regulation. Richard A. Young first proposed the hypothesis that phase separation regulated RNA transcription in 2017 [69]. Nowadays, phase separation has become a new interpretation mechanism of transcription regulation. Proteins can influence transcription regulation by phase separation through enriching transcription factors, recruiting RNA polymerase II, assembling super enhancer regions and influencing the spatial structure of chromatin (Fig. 2).

3.1.1. Transcription factors regulate RNA transcription by phase separation

There is growing evidence that phase separation exists in transcription factors (TFs) of both plant and animal cells, and TFs could regulate transcription by its own phase separation to form dynamic transcription

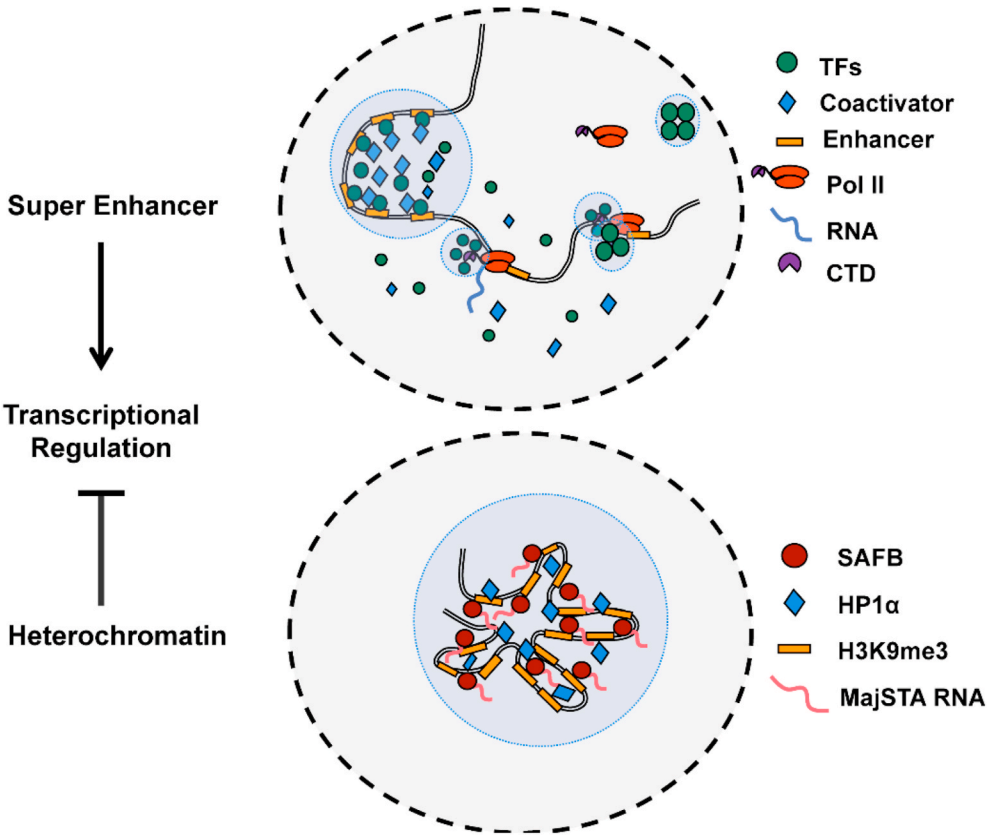

Fig. 2. Phase separation mediates RNA transcriptional regulation. The top figure shows that transcription factors and SEs all occur phase separation to promote the process of transcription regulation. The below figure displays that SAFB and MajSTA RNA can form phase-separated droplets to promote the transformation of chromosomes into heterochromatin.

regions [70,71]. For example, Yes-associated protein (YAP) is an important transcriptional co-activator, which can form phase-separated droplets in the nucleus, and the droplets isolate YAP from other transcription factors. Meanwhile, YAP enriches more transcription-related factors through its transcription activation domain (TAD) to activate the transcription process of YAP-specific downstream target genes [70]. Studies also proved that TFs can form phase-separated structures through the multivalent interaction between its low complexity domain (LCD) and carboxy-terminal domain (CTD) of RNA polymerase II [68]. In addition, the LCD domain of TFs can form high-density interaction centers near the transcription site using live cell single-molecule imaging technology, thereby TFs can fix their binding to the transcription site and recruit RNA polymerase II to activate transcription [72]. In summary, during the RNA transcription process, TFs can form transcriptional active compartments, compartmentalize and concentrate other transcription factors, enrich transcription-related proteins, and activate the transcription of downstream target genes through their own phase separation behavior.

3.1.2. RNA polymerase II participates in multiple regulatory transcription processes through phase separation

Carboxy terminal domain (CTD) that extend from the core of RNA polymerase II (Pol II) is a tandem repeat sequence. Pol II-CTD is a typical unstructured disordered region structure and participates in multiple transcription processes. The transcription process of eukaryotes includes three main stages: initiation, elongation and termination. Pol II-CTD plays a key transcriptional regulatory role in the entire transcription process. Pol II-CTD can bind to other proteins with IDR to drive phase separation through weak multivalent interactions [68,73]. What is important is that at different transcription stages, CTD binds to different proteins by regulating its own phosphorylation level to form different phase-separated droplets, thereby coupling multiple transcription processes [74–76].

In the transcription initiation, the phosphorylation level of CTD is low, which can form phase-separated droplets by interacting with the Mediator complex, thereby anchoring Pol II to the transcription initiation sites [75]. In the process of transcription elongation, the liquid droplet of CycT1 can bind to the CTD of Pol II, so that Pol II is recruited into the droplet to continue to participate in transcription elongation. At the same time, the Cyclin Box of CycT1 can bind to CDK9 of the transcription elongation factor p-TEFb. Then CDK9 highly phosphorylate CTD to efficiently activate the transcription elongation of Pol II [76]. However, when Pol II-CTD is phosphorylated by CDK9, it mainly interacts with SRSF2 and forms an RNA splicing complex through phase separation, completing the transition from transcription to splicing [74,75]. In summary, Pol II-CTD participates in different stages of transcriptional regulation through its own different phosphorylation modifications, which also makes Pol II participate in different stages of transcription.

3.1.3. Phase separation mediates super enhancer regulation of transcription

In 2013, Richard A. Young proposed the concept of Super-enhancers (SEs), which is the enriched regions of Oct4, Sox and Nanog. In subsequent studies, SEs are defined as an enhancer cluster formed by consecutively arranged enhancers [77,78]. In 2017, Richard A. Young discovered that SEs can bind to high-density transcription-related proteins and RNAs, and proposed that the phase separation theory can be used to explain how supermolecules participate in gene expression regulation for the first time [69]. Later studies have found that TFs and mediator complexes can form a droplet-like condensate structure at the SEs through its IDRs [79,80]. In 2018, Richard A. Young established a model between phase separation and SEs to study regulation of gene

transcription. They found that there are two transcription coactivators BRD4 and MED1 that can form phase separation droplets at SEs and recruit transcription complexes to achieve compartmentalization of the transcription process, thereby improving transcription efficiency and maintaining relative order within the cell [78]. Recent studies have also found that the IDRs of signal factors related to the WNT, TGF-β and JAK/STAT pathways can form phase-separated structures at specific SEs, thereby promoting cell fate determination [78]. These studies fill the gaps of SEs between phase separation and RNA transcription regulation, and provide experimental evidence for the model of phase separation and super enhancer in regulating gene expression.

3.1.4. Phase separation affects the spatial organization of chromatin structure

Chromatin is a linear structure formed by the highly ordered folding of genomic DNA and nuclear proteins in the nucleus of eukaryotic cells [81]. Nucleosomes that are composed of DNA and histones are the basic building blocks of chromosome. Nucleosomes are connected by a piece of linker DNA, which folds into a highly compressed chromatin structure under a series of precise regulation [81,82]. Studies have found that proteins responsible for regulating the structure and compartmentalization of chromatin can lead to phase separation in both animal and plant cells [13,83]. Histones with different acetylation levels can undergo different phase separation, thus forming different reaction compartments. In particular, bromodomain-containing proteins, such as BRD4, can promote the high acetylation of histones to drive phase separation. And the phase separation droplets with high acetylation levels cannot fuse with unacetylated histone droplets [84]. This study proves that eukaryotic cells can modulate the chromatin in different ways to regulate the phase separation behavior of histones, thereby affecting the spatial structure of chromatin and transcription behavior.

The heterochromatin is another important part of the eukaryotic genome and plays an important role in nucleation, DNA repair, genome stabilization, transposon and gene silencing. The general view on the formation of heterochromatin is that DNA compresses sequences to isolate related enzymes and molecules, thereby silencing sequences. However, some protein complexes with large molecular weight can bind to heterochromatin domains, while some smaller proteins cannot. So this mechanism is unclear. In fact, many chromatin-related proteins can occur phase separation with DNA, such as ADCP1, BRD4, HP1 and CBX2 [85–89]. Therefore, another hypothesis of heterochromatin formation has emerged: phase separation can regulate the formation of heterochromatin structure [24,88]. Heterochromatin protein 1 (HP1) is a key protein for the formation of heterochromatin. It contains an N-terminal chromodomain (CD) domain which can recognize H3K9me3 and a C-terminal chromo shadow domain (CSD) for binding to other proteins [90]. The multivalent interactions between CD domain of HP1 and H3K9me3 lead to phase separation. At the same time, HP1 binds SUV39H1 and RIM28 through its CSD to form a complex with more CD domains, and further binds more H3K9me3, finally achieving chromatin compression and forming heterochromatin [91]. In neural precursor cells, Pros can occur phase separation in the heterochromatin region of the centromere. When the cell enters the end of mitosis, Pros combining to HP1a transforms the phase-separated droplets into a low-fluidity gel state, which drives the formation and expansion of heterochromatin regions in neurons and inhibits gene transcription [92].

Proteins are modified to produce phase-separated compartments with similar structures but different properties. These immiscible droplets ensure the efficient and orderly process of transcription in the nucleus. At the same time, the discovery of phase separation provides a reasonable explanation for the formation of heterochromatin. These findings provide a new direction for the study of chromatin spatial organization and the function of the genome.

3.2. Two-way positioning regulatory between RNA and phase separation

The distribution of RNA in cells is asymmetric in time and space. This asymmetric distribution allows RNA to guide the synthesis of local proteins, leading to the generation of cell polarity and developmental polarity [93–95]. A new mechanism for RNA localization in different subcellular structures is that phase-separated droplets can enrich RNA to determine RNA localization [5]. For example, many RNP structures are rich in RNA and RBPs. RBPs form higher-order protein aggregates through phase separation, and provide multiple RNA binding sites to recruit RNA, so that RNA can be recruited into corresponding phase-separated droplets [18]. This process increases the local concentration of RNA in the cell and promotes the localization of RNA. In addition, ANXA11 can also dynamically couple RNA to lysosomes through phase separation to mediate the process of RNA transport on neurons [96].

For a long time, how to quickly transport a large amount of primary rRNA to the dense fibril component (DFC) for subsequent processing has been an unsolved mystery. Recent studies have found that the phase separation mechanism can explain the problem of rRNA directed transmission. Fibrin (FBL) is an important processing factor in the processing of rRNA precursors and contains the IDR domain [97]. The phase separation formed by FBL in the DFC through its IDR self-polymerization can bind to the pre-rRNA and guide the pre-rRNA to complete the directional transport process from the transcription site to the DFC. The phase separation-mediated directional transport process also promotes the nucleolus assembly of DFC [98] (Fig. 3). This discovery provides new ideas for pre-rRNA sorting and transport mechanism, and promotes the studies between phase separation and RNA directed transcription.

The above conclusions indicate that phase separation can help complete RNA positioning, but in fact, RNA positioning can also be used as a regulatory factor for phase separation, providing an explanation for the formation of phase separation in space and time [99,100]. Studies proved that the asymmetric localization of mRNA leads to the polarity of protein distribution. Proteins synthesized at the far end usually contain inherent disordered regions [101]. These IDR regions can promote protein post-translational modification and high-order assembly [101]. In short, the localization of RNA can guide the local translation of proteins to produce IDR-rich proteins, which provides the necessary conditions for phase separation.

To sum up, the phase separation and RNA localization are polar in both time and space, and there is a close relationship between them. Phase separation can recruit RNA by enriching the protein which can bind to RNA, and realize the directed transcription of RNA. The asymmetric distribution of RNA can guide the local translation of proteins, achieve a high local concentration of specific proteins, and provide conditions for the occurrence of phase separation.

3.3. Phase separation regulates RNA metabolism

The metabolic phase of RNA is also accompanied by phase separation. MicroRNA-induced silencing complex (miRISC) is a multi-protein complex that can recognize and bind target mRNA through its microRNA, thereby inducing gene silencing [102]. The two core proteins of miRISC, Argonaute2 and TNRC6B, can generate LLPS, thereby enriching target mRNA and recruiting deadenylation factors, accelerating the demethylation of target mRNA [103]. The mechanism of phase separation mediated miRISC to promote target gene silencing proves that protein phase separation plays an important role in RNA metabolism, which indicates that phase separation may play an important role in post-transcriptional gene regulation.

4. Discussion

More and more evidences show that phase separation plays an important biological function in organisms, which can be summarized as

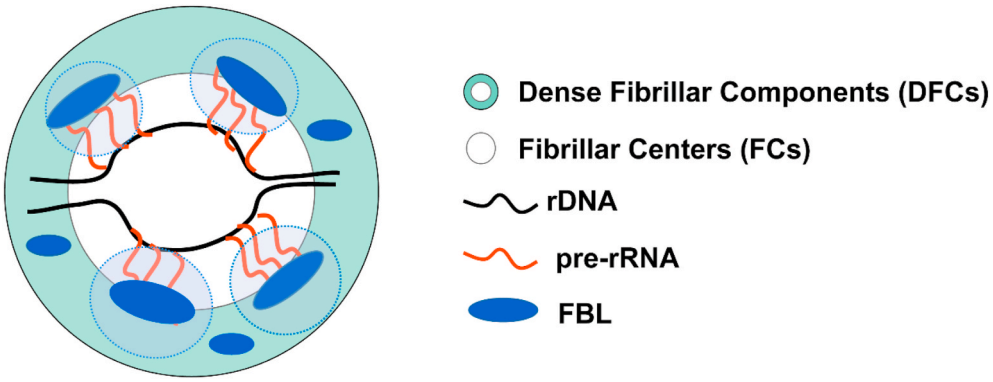

Fig. 3. A model for phase separation to promote the transport of nascent rRNA precursors. The FBL can bind to the pre-rRNA to form phase separation in the DFC, which guides the pre-rRNA to finish directional transport process.

(1) Enrich and concentrate of reactants component to improve the degree of reaction; (2) Isolate the reaction from the outside to ensure order and efficiency of the reaction; (3) Form membraneless and reversible organelles, which quickly respond to reaction signals. With the advancement of phase separation research technology, more and more RNAs are found in the phase separation structure, indicating that RNA also participates in the phase separation process. We summarized recent research on RNA and phase separation, and focused on introducing the regulation mechanism of lncRNA on phase separation. Under normal circumstances, lncRNA acts as an organizer in the phase separation structure. That is to say, lncRNA provides multiple binding sites to bind proteins, which can be assembled in a certain order, drive phase separation. In addition, the type and higher structure of RNA can also give specificity to the phase-separated droplets. The emerging evidence also proves that RNA plays a concentration-dependent feedback regulation for the phase separation of transcriptional regulation-related factors.

We also introduced the regulation mechanism of phase separation on RNA. The transcription factor and Pol II can participate in the RNA transcription process through phase separation. Phase separation also regulates the RNA transcription process by organizing the spatial structure of SEs and chromatin regions. In addition, phase separation is inseparable from RNA positioning, directional transport, and metabolic degradation.

Accumulating evidences have proved that RNA-mediated phase separation is closely related to human diseases. The recent findings of phase separation mainly focus on the relationship between the assembly process of phase separation and neurodegenerative diseases. For instance, the abnormal accumulation of FUS in the cytoplasm is one of the pathogenic mechanisms for neurodegenerative disease. Recently, studies have shown that the aggregation of FUS in the cytoplasm is linked to FUS phase separation. It is worth noting that the FUS phase separation is regulated by RNA concentration [67,68]. Therefore, the regulation of RNA-associated FUS phase separation may be an important factor in neurodegenerative disease. However, the RNA-mediated phase separation in diseases, either in the respect of profundity or extensiveness, is in the preliminary stage at present. There is no doubt that the molecular mechanism of RNA-mediated phase separation in diseases need to be further investigated. We daringly predicted that RNA-mediated phase separation may involve in more diseases. RNA-mediated phase separation can also be new aspect to apply to disease diagnostic criteria and treatment target.

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References

[1] Y. Shin, C.P. Brangwynne, Liquid phase condensation in cell physiology and disease, Science 357 (2017).
[2] C.P. Brangwynne, C.R. Eckmann, D.S. Courson, et al., Germline P granules are liquid droplets that localize by controlled dissolution/condensation, Science 324 (2009) 1729–1732.
[3] F. Gasset-Rosa, S. Lu, H. Yu, et al., Cytoplasmic TDP-43 de-mixing independent of stress granules drives inhibition of nuclear import, loss of nuclear TDP-43, and cell death, Neuron 102 (2019) 339–357 e337.
[4] P.P. Gopal, J.J. Nirschl, E. Klinman, et al., Amyotrophic lateral sclerosis-linked mutations increase the viscosity of liquid-like TDP-43 RNP granules in neurons, Proc. Natl. Acad. Sci. U. S. A. 114 (2017) E2466–E2475.
[5] S.F. Banani, H.O. Lee, A.A. Hyman, et al., Biomolecular condensates: organizers of cellular biochemistry, Nat. Rev. Mol. Cell Biol. 18 (2017) 285–298.
[6] A.A. Hyman, C.A. Weber, F. Julicher, Liquid-liquid phase separation in biology, Annu. Rev. Cell Dev. Biol. 30 (2014) 39–58.
[7] J. Zemła, J. Danilkiewicz, B. Orzechowska, et al., Atomic force microscopy as a tool for assessing the cellular elasticity and adhesiveness to identify cancer cells and tissues, in: Seminars in Cell & Developmental Biology, Elsevier, 2018.
[8] W. Chen, V.I. Zarnitsyna, K.K. Sarangapani, et al., Measuring receptor–ligand binding kinetics on cell surfaces: from adhesion frequency to thermal fluctuation methods, Cell. Mol. Bioeng. 1 (2008) 276.
[9] L.M. Jawerth, M. Ijavi, M. Ruer, et al., Salt-dependent rheology and surface tension of protein condensates using optical traps, Phys. Rev. Lett. 121 (2018), 258101.
[10] I. Alshareedah, T. Kaur, J. Ngo, et al., Interplay between short-range attraction and long-range repulsion controls reentrant liquid condensation of ribonucleoprotein–RNA complexes, J. Am. Chem. Soc. 141 (2019) 14593–14602.
[11] Y. Shin, J. Berry, N. Pannucci, et al., Spatiotemporal control of intracellular phase transitions using light-activated optoDroplets, Cell 168 (2017) 159–171, e114.
[12] D. Bracha, M.T. Walls, M.T. Wei, et al., Mapping local and global liquid phase behavior in living cells using photo-oligomerizable seeds, Cell 175 (2018) 1467–1480, e1413.
[13] Y. Shin, Y.C. Chang, D.S.W. Lee, et al., Liquid nuclear condensates mechanically sense and restructure the genome, Cell 175 (2018) 1481–1491, e1413.
[14] S. Pitchiaya, V. Krishnan, T.C. Custer, et al., Dissecting non-coding RNA mechanisms in cellulo by single-molecule high-resolution localization and counting, Methods 63 (2013) 188–199.
[15] S. Pitchiaya, M.D.A. Mourao, A.P. Jalihal, et al., Dynamic recruitment of single RNAs to processing bodies depends on RNA functionality, Mol. Cell. 74 (2019) 521–533, e526.
[16] J.B. Woodruff, B. Ferreira Gomes, P.O. Widlund, et al., The centrosome is a selective condensate that nucleates microtubules by concentrating tubulin, Cell 169 (2017) 1066–1077, e1010.
[17] V.N. Uversky, Intrinsically disordered proteins in overcrowded milieu: membrane-less organelles, phase separation, and intrinsic disorder, Curr. Opin. Struct. Biol. 44 (2017) 18–30.
[18] Y. Lin, D.S. Protter, M.K. Rosen, et al., formation and maturation of phase-separated liquid droplets by RNA-binding proteins, Mol. Cell. 60 (2015) 208–219.
[19] L. Malinovska, S. Kroschwald, S. Alberti, Protein disorder, prion propensities, and self-organizing macromolecular collectives, Biochim. Biophys. Acta 1834 (2013) 918–931.
[20] D.T. Murray, M. Kato, Y. Lin, et al., Structure of FUS protein fibrils and its relevance to self-assembly and phase separation of low-complexity domains, Cell 171 (2017) 615–627, e616.
[21] S.F. Banani, A.M. Rice, W.B. Peeples, et al., Compositional control of phase-separated cellular bodies, Cell 166 (2016) 651–663.
[22] F. Pessina, F. Giavazzi, Y. Yin, et al., Functional transcription promoters at DNA double-strand breaks mediate RNA-driven phase separation of damage-response factors, Nat. Cell Biol. 21 (2019) 1286–1299.

[23] W.M. Aumiller Jr., F. Pir Cakmak, B.W. Davis, et al., RNA-based coacervates as a model for membraneless organelles: formation, properties, and interfacial liposome assembly, Langmuir 32 (2016) 10042–10053.
[24] X. Huo, L. Ji, Y. Zhang, et al., The nuclear matrix protein SAFB cooperates with major satellite RNAs to stabilize heterochromatin architecture partially through phase separation, Mol. Cell 77 (2020) 368–383, e367.
[25] E.M. Langdon, Y. Qiu, A. Ghanbari Niaki, et al., mRNA structure determines specificity of a polyQ-driven phase separation, Science 360 (2018) 922–927.
[26] Y. Huang, Q. Guo, X.P. Ding, et al., Mechanism of long noncoding RNAs as transcriptional regulators in cancer, RNA Biol. 17 (2020) 1680–1692.
[27] K.C. Wang, H.Y. Chang, Molecular mechanisms of long noncoding RNAs, Mol. Cell. 43 (2011) 904–914.
[28] A.D. Ramos, R.E. Andersen, S.J. Liu, et al., The long noncoding RNA Pnky regulates neuronal differentiation of embryonic and postnatal neural stem cells, Cell Stem Cell 16 (2015) 439–447.
[29] N. Lin, K.Y. Chang, Z. Li, et al., An evolutionarily conserved long noncoding RNA TUNA controls pluripotency and neural lineage commitment, Mol. Cell. 53 (2014) 1005–1019.
[30] T. Hirose, T. Yamazaki, S. Nakagawa, Molecular anatomy of the architectural NEAT1 noncoding RNA: the domains, interactors, and biogenesis pathway required to build phase-separated nuclear paraspeckles, Wiley Interdisciplinary Reviews: RNA. 10 (2019) e1545.
[31] A.H. Fox, S. Nakagawa, T. Hirose, et al., Paraspeckles: where long noncoding RNA meets phase separation, Trends Biochem. Sci. 43 (2018) 124–135.
[32] T. Yamazaki, S. Souquere, T. Chujo, et al., Functional domains of NEAT1 architectural lncRNA induce paraspeckle assembly through phase separation, Mol. Cell. 70 (2018) 1038–1053, e1037.
[33] T. Chujo, T. Yamazaki, T. Kawaguchi, et al., Unusual semi-extractability as a hallmark of nuclear body-associated architectural noncoding, RNA s 36 (2017) 1447–1462.
[34] A. Cerase, A. Armaos, C. Neumayer, et al., Phase separation drives X-chromosome inactivation: a hypothesis, Nat. Struct. Mol. Biol. 26 (2019) 331–334.
[35] D. Cirillo, M. Blanco, A. Armaos, et al., Quantitative predictions of protein interactions with long noncoding RNAs 14 (2017) 5–6.
[36] P. Li, S. Banjade, H.C. Cheng, et al., Phase transitions in the assembly of multivalent signalling proteins, Nature 483 (2012) 336–340.
[37] A. Pandya-Jones, Y. Markaki, J. Serizay, et al., A protein assembly mediates Xist localization and gene silencing, Nature 587 (2020) 145–151.
[38] K. Daneshvar, J.V. Pondick, B.M. Kim, et al., DIGIT is a conserved long noncoding RNA that regulates GSC expression to control definitive endoderm differentiation of embryonic stem cells, Cell Rep. 17 (2016) 353–365.
[39] K. Daneshvar, M.B. Ardehali, I.A. Klein, et al., lncRNA DIGIT and BRD3 protein form phase-separated condensates to regulate endoderm differentiation, Nat. Cell Biol. 22 (2020) 1211–1222.
[40] S. Francia, F. Michelini, A. Saxena, et al., Site-specific DICER and DROSHA RNA products control the DNA-damage response, Nature 488 (2012) 231–235.
[41] F. Michelini, S. Pitchiaya, V. Vitelli, et al., Damage-induced lncRNAs control the DNA damage response through interaction with DDRNAs at individual double-strand breaks, Nat. Cell Biol. 19 (2017) 1400–1411.
[42] T.P. Knowles, M. Vendruscolo, C.M. Dobson, The amyloid state and its association with protein misfolding diseases, Nat. Rev. Mol. Cell Biol. 15 (2014) 384–396.
[43] M. Wang, M. Bokros, P.R. Theodoridis, et al., Nucleolar sequestration: remodeling nucleoli into amyloid bodies 10 (2019) 1179.
[44] M. Wang, X. Tao, M.D. Jacob, et al., Stress-induced low complexity RNA activates physiological amyloidogenesis, Cell Rep. 24 (2018) 1713–1721, e1714.
[45] T.E. Audas, D.E. Audas, M.D. Jacob, et al., Adaptation to stressors by systemic protein amyloidogenesis, Dev. Cell 39 (2016) 155–168.
[46] M. Feric, N. Vaidya, T.S. Harmon, et al., Coexisting liquid phases underlie nucleolar subcompartments, Cell 165 (2016) 1686–1697.
[47] A. Molliex, J. Temirov, J. Lee, et al., Phase separation by low complexity domains promotes stress granule assembly and drives pathological fibrillization, Cell 163 (2015) 123–133.
[48] K.M. Neugebauer, Special focus on the cajal body, RNA Biol. 14 (2017) 669–670.
[49] P. Ivanov, N. Kedersha, P. Anderson, Stress granules and processing bodies in translational control, Cold Spring Harb Perspect Biol 11 (2019), a032813.
[50] D.S.W. Protter, R. Parker, Principles and properties of stress granules, Trends Cell Biol. 26 (2016) 668–679.
[51] C. Wang, Y. Duan, G. Duan, et al., Stress induces dynamic, cytotoxicity-antagonizing TDP-43 nuclear bodies via paraspeckle LncRNA NEAT1-mediated liquid-liquid phase separation, Mol. Cell. 79 (2020) 443–458, e447.
[52] Y. Gao, G. Pei, D. Li, et al., Multivalent m(6)A motifs promote phase separation of YTHDF proteins, Cell Res. 29 (2019) 767–769.
[53] R.J. Ries, S. Zaccara, P. Klein, et al., m(6)A enhances the phase separation potential of mRNA, Nature 571 (2019) 424–428.
[54] J. Wang, L. Wang, J. Diao, et al., Binding to m(6)A RNA promotes YTHDF2-mediated phase separation, Protein Cell 11 (2020) 304–307.
[55] D.W. Sanders, N. Kedersha, D.S.W. Lee, et al., Competing protein-RNA interaction networks control multiphase intracellular organization, Cell 181 (2020) 306–324, e328.
[56] J. Guillen-Boixet, A. Kopach, A.S. Holehouse, et al., RNA-induced conformational switching and clustering of G3BP drive stress granule assembly by condensation, Cell 181 (2020) 346–361, e317.
[57] P. Yang, C. Mathieu, R.M. Kolaitis, et al., G3BP1 is a tunable switch that triggers phase separation to assemble stress granules, Cell 181 (2020) 325–345, e328.
[58] Y. Fu, X. Zhuang, m6A-binding YTHDF proteins promote stress granule formation by modulating phase separation of stress granule proteins, bioRxiv (2019), 694455.
[59] J.R. Mann, A.M. Gleixner, J.C. Mauna, et al., RNA binding antagonizes neurotoxic phase transitions of TDP-43, Neuron 102 (2019) 321–338, e328.
[60] I.R. Mackenzie, A.M. Nicholson, M. Sarkar, et al., TIA1 mutations in amyotrophic lateral sclerosis and frontotemporal dementia promote phase separation and alter stress granule dynamics, Neuron 95 (2017) 808–816, e809.
[61] J.J. Bouchard, J.H. Otero, D.C. Scott, et al., Cancer mutations of the tumor suppressor SPOP disrupt the formation of active, phase-separated compartments, Mol. Cell. 72 (2018) 19–36, e18.
[62] J.E. Henninger, O. Oksuz, K. Shrinivas, et al., RNA-mediated feedback control of transcriptional condensates, Cell 184 (2021) 207–225, e224.
[63] R.M. Vernon, P.A. Chong, B. Tsang, et al., Pi-Pi contacts are an overlooked protein feature relevant to phase separation, Elife 7 (2018), e31486.
[64] J. Wang, J.M. Choi, A.S. Holehouse, et al., A molecular grammar governing the driving forces for phase separation of prion-like RNA binding proteins, Cell 174 (2018) 688–699, e616.
[65] S.N. Rhoads, Z.T. Monahan, D.S. Yee, et al., The role of post-translational modifications on prion-like aggregation and liquid-phase separation of FUS, Int. J. Mol. Sci. 19 (2018) 886.
[66] T. Murakami, S. Qamar, J.Q. Lin, et al., ALS/FTD mutation-induced phase transition of FUS liquid droplets and reversible hydrogels into irreversible hydrogels impairs RNP granule function, Neuron 88 (2015) 678–690.
[67] S. Maharana, J. Wang, D.K. Papadopoulos, et al., RNA buffers the phase separation behavior of prion-like RNA binding proteins, Science 360 (2018) 918–921.
[68] K.A. Burke, A.M. Janke, C.L. Rhine, et al., Residue-by-Residue view of in vitro FUS granules that bind the C-terminal domain of RNA polymerase II, Mol. Cell. 60 (2015) 231–241.
[69] D. Hnisz, K. Shrinivas, R.A. Young, et al., A phase separation model for transcriptional control, Cell 169 (2017) 13–23.
[70] D. Cai, D. Feliciano, P. Dong, et al., Phase separation of YAP reorganizes genome topology for long-term YAP target gene expression, Nat. Cell Biol. 21 (2019) 1578–1589.
[71] H. Zhou, Z. Song, S. Zhong, et al., Mechanism of DNA-induced phase separation for transcriptional repressor VRN1, Angew. Chem. 131 (2019) 4912–4916.
[72] S. Chong, C. Dugast-Darzacq, Z. Liu, et al., Imaging dynamic and selective low-complexity domain interactions that control gene transcription, Science 361 (2018).
[73] I. Kwon, M. Kato, S. Xiang, et al., Phosphorylation-regulated binding of RNA polymerase II to fibrous polymers of low-complexity domains, Cell 155 (2013) 1049–1060.
[74] K.M. Harlen, L.S. Churchman, The code and beyond: transcription regulation by the RNA polymerase II carboxy-terminal domain, Nat. Rev. Mol. Cell Biol. 18 (2017) 263–273.
[75] Y.E. Guo, J.C. Manteiga, J.E. Henninger, et al., Pol II phosphorylation regulates a switch between transcriptional and splicing condensates, Nature 572 (2019) 543–548.
[76] H. Lu, D. Yu, A.S. Hansen, et al., Phase-separation mechanism for C-terminal hyperphosphorylation of RNA polymerase II, Nature 558 (2018) 318–323.
[77] D. Hnisz, B.J. Abraham, T.I. Lee, et al., Super-enhancers in the control of cell identity and disease, Cell 155 (2013) 934–947.
[78] W.K. Cho, J.H. Spille, M. Hecht, et al., Mediator and RNA polymerase II clusters associate in transcription-dependent condensates, Science 361 (2018) 412–415.
[79] A. Boija, I.A. Klein, B.R. Sabari, et al., Transcription factors activate genes through the phase-separation capacity of their activation domains, Cell 175 (2018) 1842–1855, e1816.
[80] A. Gurumurthy, Y. Shen, E.M. Gunn, et al., Phase separation and transcription regulation: are super-enhancers and locus control regions primary sites of transcription complex assembly? Bioessays 41 (2019), 1800164.
[81] R.D. Kornberg, Chromatin structure: a repeating unit of histones and DNA, Science 184 (1974) 868–871.
[82] D.E. Olins, A.L. Olins, Chromatin History: Our View from the Bridge, vol. 4, 2003, pp. 809–814.
[83] N. Wang, C. Liu, Implications of liquid–liquid phase separation in plant chromatin organization and transcriptional control, and development, Current opinion in genetics 55 (2019) 59–65.
[84] B.A. Gibson, L.K. Doolittle, M.W.G. Schneider, et al., Organization of chromatin by intrinsic and regulated phase separation, Cell 179 (2019) 470–484, e421.
[85] S. Zhao, L. Cheng, Y. Gao, et al., Plant HP1 protein ADCP1 links multivalent H3K9 methylation readout to heterochromatin formation, Cell Res. 29 (2019) 54–66.
[86] B.R. Sabari, A. Dall'Agnese, A. Boija, et al., Coactivator condensation at super-enhancers links phase separation and gene control, Science 361 (2018).
[87] A.G. Larson, D. Elnatan, M.M. Keenen, et al., Liquid droplet formation by HP1α suggests a role for phase separation in heterochromatin, Nature 547 (2017) 236–240.
[88] A.R. Strom, A.V. Emelyanov, M. Mir, et al., Phase separation drives heterochromatin domain formation, Nature 547 (2017) 241–245.
[89] R. Tatavosian, S. Kent, K. Brown, et al., Nuclear condensates of the Polycomb protein chromobox 2 (CBX2) assemble through phase separation, J. Biol. Chem. 294 (2019) 1451–1463.
[90] G. Lomberk, L. Wallrath, R. Urrutia, The heterochromatin protein 1 family, Genome Biol. 7 (2006) 228.

[91] L. Wang, Y. Gao, X. Zheng, et al., Histone modifications regulate chromatin compartmentalization by contributing to a phase separation mechanism, Mol. Cell. 76 (2019) 646–659, e646.
[92] X. Liu, J. Shen, L. Xie, et al., Mitotic implantation of the transcription factor prospero via phase separation drives terminal neuronal differentiation, Dev. Cell 52 (2020) 277–293, e278.
[93] S.V. Puthanveettil, RNA transport and long-term memory storage, RNA Biol. 10 (2013) 1765–1770.
[94] D.W. Houston, Regulation of cell polarity and RNA localization in vertebrate oocytes, in: International Review of Cell and Molecular Biology, Elsevier, 2013, pp. 127–185.
[95] A.R. Buxbaum, G. Haimovich, R.H. Singer, The right place at the right time: visualizing and understanding mRNA localization 16 (2015) 95–109.
[96] Y.-C. Liao, M.S. Fernandopulle, G. Wang, et al., RNA granules hitchhike on lysosomes for long-distance transport, using annexin A11 as a molecular tether, Cell 179 (2019) 147–164, e120.
[97] F.M. Boisvert, S. van Koningsbruggen, J. Navascues, et al., The multifunctional nucleolus, Nat. Rev. Mol. Cell Biol. 8 (2007) 574–585.
[98] R.W. Yao, G. Xu, Y. Wang, et al., Nascent pre-rRNA sorting via phase separation drives the assembly of dense fibrillar components in the human nucleolus, Mol. Cell. 76 (2019) 767–783, e711.
[99] S.E. Cabral, K.L. Mowry, Organizing the oocyte: RNA localization meets phase separation, Curr. Top. Dev. Biol. 140 (2020) 87–118.
[100] E.M. Langdon, A.S. Gladfelter, A new lens for RNA localization: liquid-liquid phase separation, Annu. Rev. Microbiol. 72 (2018) 255–271.
[101] R.J. Weatheritt, T.J. Gibson, M.M. Babu, Asymmetric mRNA localization contributes to fidelity and sensitivity of spatially localized systems, Nat. Struct. Mol. Biol. 21 (2014) 833–839.
[102] M. Zeng, Y. Shang, Y. Araki, et al., Phase transition in postsynaptic densities underlies formation of synaptic complexes and synaptic plasticity, Cell 166 (2016) 1163–1175, e1112.
[103] L.B. Case, X. Zhang, J.A. Ditlev, et al., Stoichiometry controls activity of phase-separated clusters of actin signaling proteins, Science 363 (2019) 1093–1097.