RNA and liquid-liquid phase separation

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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 like 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 accidentally 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 phase separation of a certain RNA or protein in the droplet with a fluorescence recovery after photobleaching (FRAP) is the most common method for phase separation 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 quenches 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
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 promote phase separation to maintain the high-level structure of heterochromatin [24].

Table 1

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

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 PTBPI, MATR3, LBR and TDP-43, to initiate the assembly of XCI [34,35]. Further studies have found that PTBPI 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 X chromosome inactivation (XCI) assembly, DNA damage repair and regulation of endoderm genes transcript.

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.
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, IncRNA can also occur in the process of promoting liquid-solid transition IncRNA. Studies have found that the ncRNA rGSRNA 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 rGSRNA, it undergoes liquid-solid transformation, eventually forming amyloidogenic-like proteins and triggering cell dormancy. One hypothesis is that rGSRNA 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, IncRNA 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 IncRNA NEAT1, which drives TDP-43 to form liquid-phase SGs [51]. In addition to IncRNA, 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 interaction will be weaker, 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 hypothesis that phase separation regulated RNA transcription in 2017 [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
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 IDR [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 IDRIs 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 ADC1P, 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 RNA 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 RNA 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, Argonau2e2 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
transcription process through phase separation. Phase separation also inseparable from RNA positioning, directional transport, and metabolic RNA. The transcription factor and Pol II can participate in the RNA for the phase separation of transcriptional regulation-related factors. The recent findings of research on RNA and phase separation, and focused on introducing the regulation mechanism of lncRNA on phase separation. Under normal circumstances, IncRNA acts as an organizer in the phase separation structure. That is to say, IncRNA 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 profoundness 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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Fig. 3. A model for phase separation to promote the transport of nascent RNA 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.

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