Review

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Current status and perspectives of non-coding RNA and phase separation interactions

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SUMMARY Phase separation refers to a phenomenon in which different components of a cell collide and fuse with each other to form droplets such that some components are encapsulated within the droplet and some are blocked outside. It is prevalent in eukaryotic cells and is closely related to genome assembly and transcriptional regulation, enabling multiple biological functions. With the development of high-throughput sequencing technologies, several non-coding RNAs (ncRNAs) have been shown to play an important role in epigenetic regulation of gene expression in addition to their roles at the transcriptional and post-transcriptional levels. In addition, some ncRNAs are involved in the formation of membraneless organelles (MLOs), the regulation of genomic stability and stress response through phase separation. Notably, phase separation can also affect the biogenesis, processing and maturation of ncRNAs. This review summarizes recent discoveries related to the relationship between ncRNAs and phase separation, providing new perspectives to guide future interventions.

Keywords phase separation, non-coding RNA (ncRNA), membraneless organelle (MLO), genomic stability, stress response

1. Introduction

Phase separation between intracellular biomolecules usually refers to the separation of eukaryotic proteins or nucleic acids (mostly RNA) through protein-protein, protein-nucleic acid and nucleic acid-nucleic acid multivalent binding aggregates from the surrounding liquid (cytoplasm or nucleoplasm) to form a semi-liquid (*1*), thereby maintaining order in the crowded chaos of cells. Study of phase separation began in 2009 when Tony Hyman and Cliff Brangwynne stuck worm gonads filled with P particles between two thin glass plates and slid the plates against each other. Under the shear stress of the sliding plates, the solids should have fallen off, however, the particles merged like raindrops, dropping and beading together (*2*). In 2021, Michael Rosen and Steven McKnight discovered a weak force between RNA and protein molecules in test tubes, which came close to each other and formed droplet-like substances (*3*). In 2015, Julie Forman-Kay discovered that a protein that affects sperm function forms droplets within human cells; this study accelerated the research into phase separation (*4*).

Many intracellular biological processes involve phase separation (Figure 1). Accumulating evidence has revealed that multiple membraneless organelles (MLOs) within cells, such as paraspeckles, Cajal bodies and stress granules, are condensates formed *via* phase separation of specific proteins/RNAs. These dynamic MLOs, along with the normative membranous organelles, maintain the spatial order within the cell, thus ensuring that continuous or independent biochemical reactions and regulatory processes are performed in an orderly manner. In response to stimulation by cellular stressors such as heat shock, hypoxia, nutritional starvation and DNA damage, ncRNAs can control the stability of the intracellular environment by forming biological condensates through phase separation (*5*). SERRATE-mediated phase separation forms D-bodies, which are essential for miRNA production (*6*). In addition, phase separation can lead to the formation of physicochemical and mechanical filters, such as nuclear pores (*7*). Meanwhile, ncRNA-mediated phase separation is required for genomic stability (*8*). Phase separation can locally concentrate molecules in cohesive condensates to activate cytoskeletal structural responses, signaling processes and nucleation (*9*). The understanding that phase separation underlies the formation of membrane-

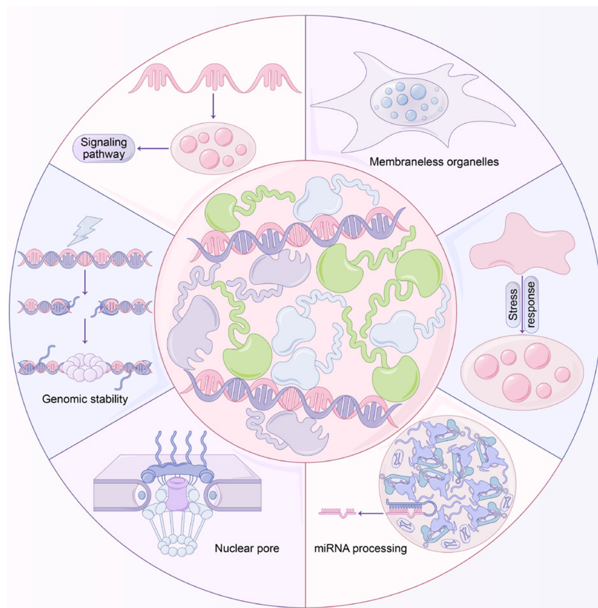

Figure 1. Summary of functions of phase separation. i. Formation of MLOs for spatial and temporal control. ii. Perceives external stimuli such as heat shock, hypoxia, nutritional starvation and DNA damage, and responds to them quickly. iii. Mediate miRNA production. iv. Formation of physicochemical and mechanical filters such as nuclear pore. v. Maintain genomic stability by repairing broken DNA duplexes. vi. Concentrate molecules in condensates to activate signaling process.

free compartments in cells has led to significant efforts to characterize the function of biomolecular condensates in neurodegenerative diseases and tumors (*7*).

The genomes of higher eukaryotes are commonly transcribed to produce large amounts of non-coding RNAs (ncRNAs) (*10*), which function in many ways and can interact with proteins, DNAs and RNAs to participate in various cellular activities, including but not limited to gene activation and silencing, RNA splicing, modification and editing and protein translation. Since the 1950s, various types of ncRNAs have been identified in eukaryotic cells, including the most abundant transfer RNA (tRNA), ribosomal RNA (rRNA), small nucleolar RNA (snoRNA), small nuclear RNA (snRNA), microRNA (miRNA), linear long ncRNA (lncRNA), circular RNA (circRNA), etc. ncRNAs can be classified according to their length: small (about 20 bp), intermediate (less than 200 bp) and long (longer than 200 bp). Small ncRNAs have attracted many studies, such as: piwi-interacting RNAs (piRNAs) and miRNAs [14]. Intermediate ncRNAs include snRNAs involved in splicing during protein synthesis, and the remaining ncRNAs larger than 200 bp are classified as lncRNAs, which are involved in epigenetic regulation of transcripts and inactivation of the X chromosome.

ncRNAs, especially miRNAs and lncRNAs, have been shown to play an important role in the pathogenesis of many diseases. miRNA-132/212 promotes axonal evolution, neural migration, plasticity, and has very promising applications in neurodegenerative diseases such as Alzheimer's, Parkinson's, and epilepsy (*11*). Zhixin Ling reported miR-193a inhibited prostate cancer cell growth, suppressed migration and invasion, and significantly reduced prostate cancer xenograft tumor growth (*12*). lncRNA ITGB8-AS1 is highly expressed in colon cancer cells and tissues, and promotes proliferation, colony formation and tumor growth of colon cancer cells through integrin-mediated focal adhesion signaling (*13*). lncRNA SNHG1 regulates Treg cell differentiation and thus immune escape from breast cancer by regulating the miR-448/IDO signaling axis (*14*). A growing body of research indicates that ncRNAs can not only serve as biomarkers for disease diagnosis and prognosis, but they are also expected to be targets for novel therapeutic strategies.

Although ncRNAs were initially defined as a class of RNA transcripts without coding capacity, it has been determined that some ncRNAs actually contain open reading frames that can to be translated into micropeptides or microproteins with regulatory functions in a variety of biological and oncological processes. ncRNA performs biological functions in the form of encoded peptides, suggesting it as a new tumor marker and anti-cancer drug target with high clinical translational value.

Recent studies have revealed different effects of RNA on the occurrence of phase separation in cells. Some RNAs can facilitate protein phase separation by decreasing the critical saturation concentration. For example, the RNA-binding protein PTB can bind to RNA in the absence of intrinsically disordered regions (IDRs) and undergo phase separation in vitro. In addition, RNA can influence the properties of intracellular phase separation products. For example, short poly(A) RNA (50 nt) has a minimal effect on the critical saturation concentration for phase separation of the P-granule protein LAF-1 but causes dramatic changes in the internal physical properties of LAF-1 phase separation droplets, thus reducing their viscosity (*15*). Furthermore, including lncRNAs, miRNAs, circRNAs and eRNAs, can be involved in the regulation of biological processes *via* phase separation. In this review, we have summarized recent studies on the regulatory functions of phase separation and discussed its research progress in various biological processes in recent years, beginning from the relationship between ncRNAs and phase separation (Figure 2).

2. Molecular characterization and environmental conditions of phase separation

Phase separation is a process in which large molecules are separated from a large volume of solvent into different liquid phases, which is affected by a variety of factors. First, it is driven by multivalent interactions and prion-like structural domains (PrLDs), and IDRs and low-complexity regions (LCRs) of the protein are essential for the formation of phase separation (*16*). IDRs

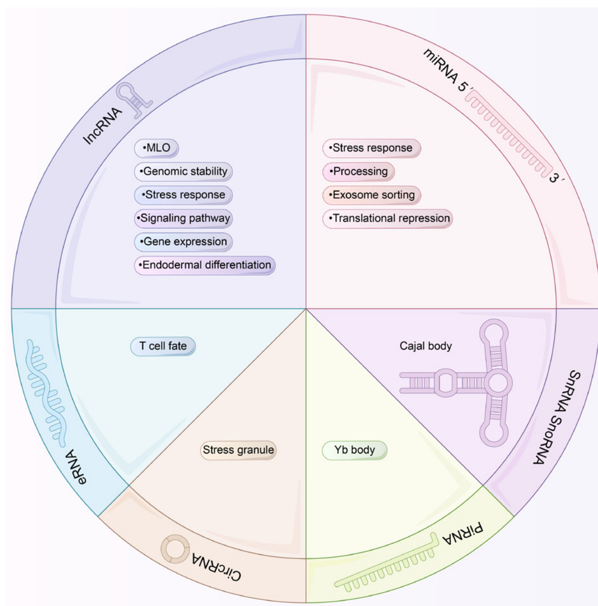

Figure 2. Interactions between ncRNAs and phase separation. i. lncRNAs mediate formation of MLOs, genomic stability, stress response, activation of signaling pathways, gene expression and endodermal differentiation via phase separation. ii. miRNAs regulate stress response by phase separation while phase separation contributes to miRNA processing, sorting miRNAs into exosomes and miRNA-mediated translational repression. iii. snRNA and snoRNA act as a scaffold for protein recruitment. iv. Yb bodies are multivalent condensates formed by phase separation and are responsible for piRNA biogenesis in Drosophila. v. circVAMP3 contributes to the formation of stress granules through phase separation. vi. ThymoD induces Bcl11b enhancer and promoter to form a loop that controls T cell development.

are usually free of the more aromatic and aliphatic amino acids that often construct the core of a folded structural domain. LCRs are regions with a higher proportion of a specific amino acid compared with other amino acids. In addition, the linear crosstalk of multiple identical/similar structural domains, such as SH2 and SH3 repeats of proteins such as NCK, and oligomerization of proteins can specifically mediate phase separation (*17*). During phase separation, solutions containing proteins and other biomolecules automatically form two phases, one enriched in proteins and one deficient in proteins. The dense phase formed is surrounded by a membrane that is selectively permeable to certain macromolecules and acts as a relative isolator, which ensures that various components of the cell are assembled at the right time and space to perform appropriate functions. The compartment formed *via* phase separation is similar to a liquid droplet and allows rapid exchange of substances with the surroundings (*18*).

Second, phase separation of certain proteins can also be regulated by other related proteins, DNAs and RNAs. If a nucleic acid molecule has a strong and specific multivalent interaction with a protein molecule, the nucleic acid molecule promotes protein phase separation. If the strongly charged nature of the nucleic acid molecule acts mainly as a salt ion-like electrostatic shield, weakening protein interactions, addition of the nucleic acid molecule inhibits phase separation; if both nucleic acid-protein interaction and electrostatic shielding are present but weak, phase separation is usually promoted at low nucleic acid concentrations and inhibited at high nucleic acid concentrations. This phenomenon provides novel insights into the relationship between ncRNA and phase separation and its mode of regulation in biological processes.

Third, the occurrence of phase separation is highly dependent on the proteins and nucleic acids in solution, but also on the environment and physicochemical properties of the solution such as temperature, pH, salt ion concentration, salt ion type, and the presence of other biomolecules in solution (*19*). Once phase separation occurs, the biomolecule is present in two forms, one at a low concentration in the solution and one at a higher concentration in the 'droplet'. The two forms can be transformed into each other as the relevant conditions change. In other words, phase separation is a highly dynamic process.

3. Experimental approaches and vital roles of phase separation

Various experimental approaches continue to enhance our understanding of phase separation (Table 1). The existence of a phase separation mechanism was demonstrated by purifying the target substance expression and reconstituting phase separation in vitro (*20*). Microscopic imaging plays an indispensable role as the main technique for observing phase separation. Phase separation in vitro can be observed very simply with an ordinary light microscope (*21*). The occurrence of phase separation is characterized by a cloudy solution and the presence of droplets in the solution such as oil droplets in water. The use of PEG or lipid-coated slides allows for better observation and documentation of phase separation. Time-lapse imaging, fluorescence recovery after photobleaching (FRAP) and 1,6-hexanediol sensitivity assays have been used to verify the dynamics and reversibility of phase-separated droplets (*7*). In addition, phase separation can be detected using a method that detects turbidity of a solution, or *via* centrifugal precipitation (*17*). Electrophoretic mobility shift assay (EMSA) and thermal shift measurement are also used to verify protein and nucleic acid interactions.

The role of phase separation in various physiological processes confers its importance in clinical translation. YAP mediates the pro-tumor effects of IFN-γ *via* phase separation and can be used as a predictive biomarker and target for anti-PD-1 combination therapy (*22*). Wang Shuai *et al*. demonstrated that the phase separation activity of SARS2-NP protein can be a possible therapeutic target for neo-coronavirus infection, providing new possibilities for related drug development (*23*). Lu Bing *et al*. proposed a small-molecule compound called GSK-J4, which regulates

Table 1. A set of experiments used to verify phase separation

Event	EWebsite/Method	Description
Phase-separated	MetaDisorder (*http://iimcb.genesilico.pl/metadisorder/*)	▪ Integrating results from multiple individual predictors
Protein prediction	MobiDB (*http://mobidb.bio.unipd.it/about*) PLAAC (*http://plaac.wi.mit.edu/*) ZipperDB (*https://services.mbi.ucla.edu/zipperdb/*) FoldUnfold (*http://bioinfo.protres.ru/ogu/*) DISOPRED (*http://bioinf.cs.ucl.ac.uk/psipred/?disopred=1*) PONDR (*http://www.pondr.com/*) Iupred2a (*https://iupred2a.elte.hu/*) D2P2 (*http://d2p2.pro*)	▪ Combining annotations from external databases, indirect evidence from experimental data ▪ Predicting PrLDs ▪ Predicting fibril-forming segments ▪ Predicting IDRs ▪ Providing information on non-structural regions of protein ▪ Predicting IDRs ▪ Predicting disordered and binding sites ▪ Providing the specific location of IDRs
Function	Microscopic detection Turbidity measurements Centrifugal grading Inverse capillary velocity measurement FRAP Fluorescence correlation spectroscopy 1,6-hexanediol treatment	▪ Finding the presence of liquid droplets under the microscope (Incubation time and imaging parameters should be kept constant) ▪ Being detected by optical density measurement or direct static light scattering (Combined with microscope) ▪ Precipitating dense phase by centrifugation ▪ Using fluorescence or transmitted light microscopy to photograph a molten droplet and measuring the time it takes for two droplets to fully fuse into a single droplet ▪ Assessing changes in the state of matter over time ▪ Evaluating the diffusivity of individual molecules within the liquid state and the dilution of droplets ▪ Interfering with phase separation of many IDRs

phase separation of core regulatory circuitry factors, for therapeutic intervention in patients with chemoresistant and metastatic osteosarcoma (*24*). Ali Miserez *et al*. reported a coupled peptide that can form pH- and redox-responsive cohesive microdroplets through phase separation, thus facilitating delivery across cell membranes (*25*). In addition, stimulation of chaperone mechanisms to disassemble MLOs, induction of pathways that may inhibit aberrant phase separation, and development of antisense oligonucleotides to knock down RNAs can be evaluated as new strategies for the treatment of human diseases characterized by abnormal phase separation.

With the gradual increase in the study of phase separation, researchers have found that phase separation may lead to diseases such as neurodegenerative diseases, tumors, and aging. FUS aggregation is a pathological hallmark in patients with amyotrophic lateral sclerosis (ALS). FUS protein and hnRNPA1 form droplets in ALS, which become progressively more viscous and eventually form fibrous solids that are abnormally deposited in cells (*26*). Phosphorylation of Tau repeats under cellular protein conditions leads to phase separation of Tau and promotes its abnormal deposition in the brains of Alzheimer's disease patients (*27*). EWSR1 is a protein with a PrLD that is a fusion partner with transcription factors leading to oncogenicity. The abnormal 'phase separation' of EWSR1 promotes its accumulation near the genome associated with tumorigenesis, which is important for the oncogenicity of Ewing sarcoma (*28*). Super enhancers can induce the activation of classical oncogenes and other genes associated with tumorigenesis. Recent studies have found that transcription factors containing IDRs, transcriptional cofactors and RNA pol II form phase-separated condensates at super enhancers (*29*). APC phase separation was observed in colorectal cancer, resulting in excessive activation of the Wnt pathway, affecting cell differentiation and promoting rapid proliferation, playing a key role in the initiation of tumorigenesis (*30*). The tumor suppressor gene SPOP forms phase-separated membrane-free clusters in nuclear speckles, and these droplets play a central role in suppressing the development of a variety of human malignancies such as gastric, hepatocellular and prostate cancers (*31*). Biomolecular condensates are intracellular assemblies without membranes that are usually formed by phase separation. Its formation and dissolution can lead to protein misfolding and aggregation, which is often the cause of aging-related diseases (*32*).

4. Interaction between lncRNAs and phase separation

lncRNAs are ncRNAs that are > 200 nt in length. They play an important role in various life processes including a dosage compensation effect, epigenetic regulation, cell cycle regulation, and cell differentiation regulation. Like mRNAs, lncRNAs are transcribed from corresponding

genes with 5' caps and polyA tails, and are formed into mature lncRNAs by splicing. lncRNAs show a strong tissue-specific expression pattern, suggesting their integral role in cell type-specific processes. Abnormal expression or function of lncRNAs is closely related to the development of human diseases, including cancer and degenerative neurological diseases, and other serious human health risks (*33*). In recent years, it has been demonstrated that lncRNAs can mediate phase separation involved in various physiological processes such as the formation of membrane-free compartments and maintenance of genomic stability (Table 2).

4.1. lncRNA mediates the formation of MLOs through phase separation

The eukaryotic nucleus is not a homogeneous monospace organelle but a highly compartmentalized organelle separated by various types of membrane-free structures. In living organisms, cells ensure accurate spatial and temporal control of complex biochemical reactions through independent subcellular compartments. Although these compartments differ in composition, localization, and function, they have extremely similar morphological features, kinetic properties, and assembly (*34*). Understanding the mechanism and function of membrane-free compartments in detail has been a challenge because of their protein and RNA complexity. The interaction between proteins and RNAs leads to the formation of MLOs, which are involved in various biological functions including but not limited to response to environmental changes. The liquid condensates formed *via* lncRNA-related phase separation include nucleolus, paraspeckles, nuclear speckles, Cajal bodies, amyloid bodies (A-bodies), stress bodies and Omega speckles (Figure 3). These compartments are composed of specific lncRNAs, which play a role in their organization and function.

alluRNA

The nucleolus is the most evident structure in the interphase nucleus of eukaryotic cells, and its main function is to synthesize rRNA. It consists of three major components as follows: a fiber center (FC), dense fibrillar component (DFC) and granular component (GC). The lncRNA alluRNA, an RNA polymerase II transcript derived from the intron Alu element, plays an important role in the assembly and function of the nucleolus. Both NCL and NPM contain IDRs and LCRs that form protein structural domains. alluRNA acts as a scaffold to regulate nucleolus formation by promoting the self-organization of NCL/NPM-containing 'droplets' into domains that efficiently associate with rDNA and support Pol I transcriptional activity within the nucleolus, leading to nucleolus structure regulation and rRNA production (*35*).

Table 2. List of lncRNAs interacting with phase separation

lncRNA	Localization	Structure	Interaction	Function	References
alluRNA	Nucleus	Not known	NCL, NPM	Maintain nucleolar structure and function	*35*
NEAT1	Nucleus	tRNA-like small RNA at its 3' end	Paraspeckle components	Formation of paraspeckles	*36-39*
MALAT1	Nucleus	tRNA-like small RNA at its 3' end	SRSF1, SRSF2, SPOP	Formation of nuclear speckles	*42*
rIGSRNA	Nucleus	Not known	Short cationic peptidedomains	Formation of Amyloid bodies	*44*
HSATIII	Nucleus	Not known	SAFB, SR proteins, transcription factors	Formation of unclear stress bodies	*45-46*
hsr-omega	Nucleus	Not known	hnRNPs	Formation of omega speckles	*47*
TNBL	Nucleus	Not known	NPM1, SAM68	Formation of aggregates	*48*
dilncRNA	Nucleus	Not known	DNA damage responses RNAs and proteins	DNA damage responses	*52*
BGL3	Nucleus	Not known	BARD1	Homologous recombination	*53*
LINP1	Nucleus	Not known	Ku70/80	Non-homologous end joining	*54*
NORAD	Cytoplasm	Enriched with Pumilio response elements	PUM	Nucleation of PUM condensates	*8*
TERRA	Nucleus	Not known	TRF2	Telomere higher-ordered structure	*55*
Sme2	Nucleus	Not known	Smp	Recombination-independent pairing	*56-57*
SNHG8	Nucleus	Not known	Histone H1 proteins	Regulation of chromatin condensation	*58*
Xist	Nucleus	Repetitive RNA Subdomains	Repressive proteins complexes	Inactivation of X chromosome	*59-60*
GIRGL	Cytoplasm	Not known	CAPRIN1	Mediating glutamine deprivation stress	*63*
SNHG9	Cytoplasm	Six loops	LATS1	Progression of breast cancer	*64*
PNCTR	Nucleus	Not known	PTBP1	Inhibiting PTBP1 splicing activity	*65*
SLERT	Nucleus	Not known	DDX21	Facilitating Pol I transcription	*66*
DIGIT	Nucleus	Not known	BRD3	Regulating endoderm differentiation	*68*

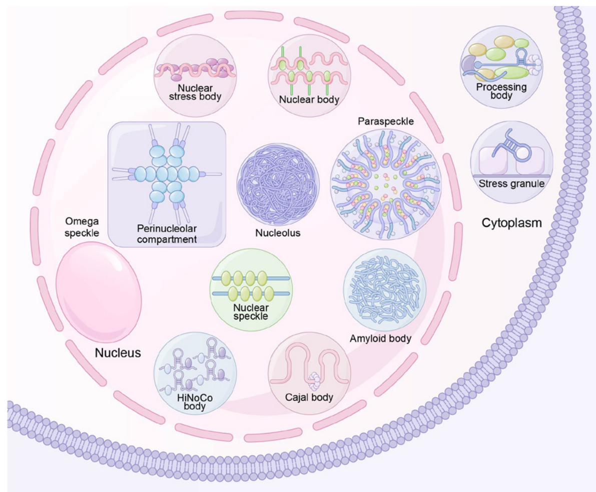

Figure 3. Biomolecular condensates formed by ncRNAs mediated phase separation. Nucleolus, Nuclear stress body, Perinucleolar compartment, Omega speckle, Nuclear body, Paraspeckle, Nuclear speckle, Amyloid body, HiNoCo body and Cajal body are found in the nucleus while processing body and stress granule are found in the cytoplasm. Not all compartments are present in each cell type, but are shown here for the sake of completeness. For example, Cajal bodies are only observed in a limited number of human cell types such as neurons and cancer cells.

NEAT1

Paraspeckles are composed of component proteins and RNA scaffolds that are essential for gene expression and intracellular homeostasis. They are subnucleosomes that are found near speckles, contain splicing factors and may regulate gene expression by sequestering mRNAs and proteins (*36*). The number of paraspeckles increases in many situations, such as when cells change from one state to another, are infected by viruses and bacteria, begin to degenerate and are in a state of inflammation, aging and cancer (*37*). Many paraspeckle proteins contain prion-like IDR structural domains that contribute to the formation of large phase-separated condensates in cell nuclei and hydrogels in vitro. Recent studies have shown that lncRNA NEAT1 can act as a scaffold to recruit specific component proteins to be enriched at high local concentrations. Subsequently, these component proteins recruit other proteins that mediate phase separation and the formation of paraspeckles through LCR. NEAT1 has three distinct RNA structural domains (A, B and C) that play a role in stabilization (A), isoform conversion (B) and scattering assembly (C) (*38*). NEAT1 has two transcripts, NEAT1-1 and NEAT1-2, both of which share the same 5' terminus. The longer NEAT1_2 isoform (22.7 kb in humans) is an important component of paraspeckles, whereas the shorter NEAT1_1 isoform (3.7 kb in humans) is dispensable. NEAT1-2 provides a structural scaffold for paraspeckles and has high-affinity constitutive sites for the core paraspeckle proteins NONO and SFPQ, which rapidly bind to NEAT1-2 ribonucleoproteins, which are intermediates in para-plaque formation. Subsequently, FUS and RBM14 are recruited and mediate the onset of phase separation, resulting in the formation of mature paraspeckles (*39*).

MALAT1

Nuclear speckles are prominent membrane-free compartments in the nucleus, which control different steps in gene expression, including transcription, splicing and mRNA export (*40*). An important function of nuclear speckles is to harbor spliceosomal small nuclear ribonucleoproteins (RNPs) and enable them to catalyze the removal of introns from transcriptionally active genes located at the periphery of the speckle. lncRNA MALAT1 is a major component of this droplet. It is localized in the periphery of nuclear speckles and in the centrally located pre-RNA splicing factor (*41*). Most proteins within nuclear speckles can directly bind to RNA, including SRSF1, SRSF2 and SPOP, leading to the recruitment of polyadenylated mRNA and uridine-rich small nuclear RNA within nuclear speckles and eventually phase separation to initiate droplet formation (*42*). m6A-modified MALAT1 acts as a scaffold to recruit YTHDC1 to nuclear speckles and regulate the expression of key oncogenes (*43*).

rIGSRNA

A-bodies are a prime example of the widespread use of architectural RNA in the construction of membrane-free compartments. They are non-dynamic MLOs, and their non-dynamic nature is attributed to proteins that adopt a reversible amyloid conformation. Assembly of A-bodies requires the expression of rIGSRNA derived from stimulus-specific sites of rDNA intergenic spacers. Low-complexity rIGSRNA sequences interact with short cationic peptides to induce a unique liquid-like phase in the nucleolus region. rIGSRNA is a determinant of A-body protein recruitment because its silencing reduces the number of liquid-like nucleolus foci and impairs the formation of mature A-bodies during heat shock (*44*).

HSATIII

HSATIII is transcriptionally repressed under physiological conditions, and its transcription is induced by HSF1 under heat shock stress conditions. Several repetitive sequences of HSATIII can bind to scaffold attachment factor B (SAFB), SR proteins and transcription factors to assemble nuclear stress bodies. Downregulation of HSATIII significantly affects the recruitment of RNA processing factors to nuclear stress bodies without altering the association of HSF-1 with these structures or the presence of acetylated histones in the nuclear stress bodies (*45*). Comprehensive HSATIII identification of RNA-binding proteins *via* mass spectrometry has helped to identify multiple splicing

factors in nuclear stress bodies, including SRSFs, whose phosphorylation status affects the splicing pattern. SRSFs rapidly dephosphorylate upon heat stress exposure. During stress recovery, CDC recruits substances such as CLK1 to nuclear stress bodies and accelerates the rephosphorylation of SRSF9, thereby promoting target intron retention (*46*).

hsr-omega

Omega speckles are scaffolded by hsr-omega and distributed in the interchromatin space, close to chromatin. In situ immunocytochemical staining using antibodies against heterogeneous nuclear RNA binding proteins (hnRNPs) such as HRB87F, Hrp40, Hrb57A and S5 has shown that all hnRNPs resulted in diffuse staining of chromatin regions in all cell types, in addition to the presence of a large number of spots. In addition, studies have revealed absolute co-localization of hnRNPs and omega speckles. Immunoprecipitation studies using hnRNP antibodies have demonstrated the physical association between hnRNPs and hsr omega. Therefore, hsr-omega plays an important structural and functional role in the organization and establishment of hnRNP-containing omega speckles, thereby regulating the transport and availability of hnRNPs and other associated RNA-binding proteins in the nucleus (*47*).

TNBL

Somatic global genomic hypomethylation is common in almost all cancer types, including colon cancer. Following DNA hypomethylation and histone acetylation, NBL2 repeat sequences are transcribed in colon cancer cell lines, exhibit promoter activity, and are contained in a novel non-polyA antisense lncRNA named TNBL. TNBL is stable throughout the mitotic cycle and forms perinuclear aggregates preferentially in the interphase nucleus near a subpopulation of NBL2 sites (*48*).

In addition, processing bodies (PBs) are RNPs formed *via* RNA-dependent phase separation, are enriched with various enzymes required for RNA processing and degradation and play a key role in the spatial regulation of gene expression. Super-resolution single-molecule fluorescence microscopy has shown that most miRNAs are stably anchored to the core or shell layer of PBs, whereas lncRNAs are temporarily bound to the PB shell, indicating that the localization of RNAs in PBs is closely related to the RNA species (*49*). Examining the effects of RNA assemblies on particle formation, and the effects of particle formation on RNA assemblies, may help to reconstruct at least some aspects of RNA particles in vitro.

4.2. lncRNA affects genomic stability *via* phase separation

Although proteins and DNA were previously considered to be the major components of chromatin, scholars have recently recognized that RNA occupies a large amount of chromatin and acts as a regulator of nuclear structure (*50*). Many lncRNAs tend to remain in the nucleus and cooperate with protein complexes to regulate epigenetic regulation, which is essential for gene expression and genomic stability (*51*).

dilncRNA and BGL3

DNA damage can increase genomic instability, which can lead to cellular senescence and death. DNA damage response (DDR) identifies the sites of DNA damage and repairs them, which can be regulated by RNAs. In DDR, 53BP1 protein is recruited to form foci in DNA double-strand breaks (DSBs). These foci have recently been identified as biomolecular condensates with droplet-like behavior. Homologous recombination and non-homologous end-joining are two repair modes of DSBs. DDSR1, BGL3, PRLH1, and TERRA promote DNA break repair through homologous recombination, whereas HITT, LINP1 and SNHG promote DNA break repair through non-homologous end-joining.

Once a DSB has occurred, the MRE11-RAD50-NBS1 (MRN) complex recognizes the exposed DNA ends and recruits RNA polymerase II (RNAPII) to synthesize damage-induced long non-coding RNA (dilncRNA). dilncRNA promotes DDR proteins, such as 53BP1, to undergo phase separation, causing DDR lesions to exhibit fluid-like behavior, which in turn completes the process of DNA damage repair (*52*). LncRNA BGL3 acts as a molecular scaffold during homologous recombination by binding BARD1 and enhancing the binding of BARD1 to other repair proteins (*53*).

LINP1

Studies have confirmed that some RNAs can form phase-separated condensates through RNA-RNA interaction, such as the lncRNA LINP1. LINP1 is highly conserved among species and is involved in DNA repair in triple-negative breast cancer; its expression is closely associated with tumorigenesis. In the presence of 5% PEG400, full-length LINP1 is expressed as droplets ranging in size from 10 to 24 nm. Non-homologous end-joining does not depend on DNA homology but avoids the retention of DNA or chromosome breaks and the resulting DNA degradation or impact on viability by connecting two broken DNA ends to each other via Ku proteins. Ku is a heterodimer composed of 70 and 80 kDa polypeptides (Ku70/80) of heterodimers that bind to LINP1, which in turn multimerizes to stabilize the initial synaptic event, thus enabling efficient non-homologous end joining (*54*).

NORAD

NORAD is a highly conserved and cytoplasmically enriched lncRNA that is activated by DNA damage and is required for mammalian genomic stability. It drives efficient condensation and sequestration of PUM through multivalent PUM-NORAD RNA binding interactions and IDR-driven PUM-PUM interactions to form phase-separated PUM condensates that competitively repress other PUM-binding transcripts. NORAD deficiency leads to PUM hyperactivity, resulting in the suppression of PUM target mRNAs, which includes important regulators of mitosis, eventually leading to a significant genomic instability phenotype in NORAD-deficient cells (*8*).

TERRA

Telomeres are nuclear protein structures formed at the end of chromosomes, which are essential for chromosome integrity and stability. One of the hallmarks of pluripotent telomeres is high TERRA levels, and TERRA depletion induces telomere dysfunction. In humans, TERRA is transcribed from subtelomeric promoters at the ends of most chromosomes and is associated with telomere maintenance. In mice, TERRA originates primarily from the pseudoautosomal PAR locus; however, TERRA derived from chromosomes 18q, 2 and X has also been identified. TERRA is enriched in telomeric regions and scaffolds the nucleation of telomere-associated proteins. Phase separation provides an attractive model for coordinating various biochemical reactions occurring in genomic compartments and the nucleus and is fundamental for maintaining genomic integrity (*55*).

Sme2

Meiosis is the basic process of sexual reproduction in eukaryotes and plays an important role in the inheritance and variation of organisms. During this process, homologous chromosomes are selectively aligned and paired. Sme2 RNA is a meiosis-specific 1,500 nt lncRNA that accumulates at its locus, plays an active role in recombination-independent pairing, and is essential for recombination-independent pairing of homologous chromosomes in *Schizosaccharomyces pombe*. A portion of Sme2 RNA is present in the nucleus and co-localizes with Mei2p sites during the prophase of meiosis, forming Mei2p dots with droplet-like morphological features, which are smaller and clearer than the nucleolus and appear very compact (*56*). Smp proteins are conserved RNA-binding proteins that are associated with meiosis-specific lncRNAs that co-accumulate at sme2 and two other chromosomal loci. In addition, Smp proteins co-localize with Mei2-mCherry, a known protein located at the sme2 locus, and are required for robust pairing at the sme2 locus. Smp proteins contain IDRs necessary for phase separation. 1,6-hexanediol treatment reversibly disassembles these complexes and disrupts pairing at the relevant loci, suggesting that lncRNA-protein complexes mediate homologous chromosome recognition through phase separation, thereby mediating the pairing of homologous chromosomes (*57*).

SNHG8

The binding of histone H1 to ribosomes stabilizes the condensed state of chromatin and allows for the relative structural isolation of DNA. lncRNA SNHG8 is localized to chromatin and can interact with and promote the phase separation of histone H1. Overexpression of SNHG8 increases the amount of H1 bound to chromatin, promotes chromatin condensation, and induces gene expression patterns associated with epithelial differentiation (*58*).

lncRNA XIST mediates the silencing of gene transcription on the X chromosome during female mammalian development by recruiting repressive protein complexes (*59*). During early development, one of the two X chromosomes in female mammals is suppressed for dosage compensation. During this process, approximately 17 kb of XIST is produced from a region of the inactive X chromosome called the X inactivation center. It subsequently spreads along the entire chromosome, forming a cloud of XIST RNA on it, and compresses and inhibits it. In the nucleus, XIST RNA can act as a platform to create a microenvironment for repression of genes on inactive X chromosomes. For example, XIST recruits proteins such as HNRNPU (with a phase separation propensity of 2.5) and MATR3 (with a phase separation propensity of 1.5) to recruit the polycomb repression complex 2 (PRC2), which contributes to chromosome compaction and gene repression. Similarly, Andrea Cerase hypothesized that during X chromosome inactivation, XIST acts as a scaffold to recruit repressor proteins, which in turn recruit other IDR-containing proteins to bind to them, forming phase separation agglutination from which inactive X chromosomes are separated, and the peripheral localization of inactive X chromosomes helps to maintain phase separation. However, further experiments are warranted to support this hypothesis (*60*).

4.3. lncRNA mediates cellular stress response through phase separation

Stress is the adaptive response of an organism to environmental changes. Various factors such as hormones, neuroendocrine mediators, peptides and neurotransmitters are involved in maintaining the homeostasis of the central nervous system, digestive system, cardiovascular system and endocrine system. In recent years, it has been shown that membrane-free compartments formed via the phase separation of specific proteins are involved in the stress response. lncRNAs are

one of the important regulators of cellular stress. Some lncRNAs interact with proteins, resulting in the partition of molecules between dense cohesive and dilute liquid phases. Upregulation or translocation of lncRNAs may lead to changes in the local concentration of lncRNAs, which may trigger the nucleation of RNPs under stressful conditions (*5*).

MALAT1

MALAT1 is primarily localized to nuclear speckles and regulates selective splicing in nuclear speckles by interacting with serine- and arginine-rich family proteins. It is released from nuclear speckles shortly after heat shock via the PCBP-mediated pathway and localizes to HiNoCo bodies in an activity-dependent transcriptional manner. MALAT1 translocates from nuclear speckles to the nucleoplasm and is predominantly translocated to the nucleus after 10-15 min. HiNoCo bodies remain in the nucleus as long as 3 h after heat shock. They are disrupted after treatment with 1,6-hexanediol, indicating that they may be formed via phase separation. In addition, cell proliferation is reduced under heat shock stress after MALAT1 knockdown, suggesting that MALAT1/HiNoCo bodies are important for the heat shock response (*61*).

NEAT1

TDP-43 is a highly conserved RNP that is currently considered a pathological marker protein for neurodegenerative diseases such as ALS. The TDP-43 protein contains a nuclear localization signal, a nuclear export signal, and three potential Caspase-3 recognition sites. In recent years, TDP-43 has been shown to undergo phase separation in vitro. Nuclear bodies (NBs) are dynamic, membrane-free structures that contain specific nuclear proteins and RNAs to regulate nuclear function and homeostasis. Various cellular stresses trigger the dynamic, reversible formation of TDP-43 NBs, which are partially co-localized with paraspeckles, and their scaffold lncRNA. NEAT1 is dramatically upregulated in stressed neurons and mediates their nucleation. Because NEAT1 is > 20 kb in length and has a complex secondary structure, it can provide multiple binding sites for TDP-43 molecules. This provision may increase multivalent interactions, leading to the co-phase separation of TDP-43 and NEAT1, thus forming the first line of defense against stress and diseases (*62*).

GIRGL

Glutamine is the most abundant circulating amino acid, and glutaminase-1 (GLS1) is the rate-limiting enzyme for glutamine catabolism. Under glutamine-deficient conditions, GLS1 expression is inhibited. lncRNA GIRGL inhibits GLS1 translation via phase separation. Under physiological conditions, HuR affects the stability of GIRGL transcripts via an AGO2-mediated RNA-induced silencing complexes (RISCs) mechanism. In glutamine-deficient cells, GIRGL levels are upregulated owing to both a c-Jun-mediated increase in transcription and significantly longer half-lives, thus inducing the complex formation of CAPRIN1 and GLS1 dimers. This phenomenon helps to promote phase separation of CAPRIN1 and induces the formation of stress granules, which are produced by various factors, including heat shock, osmotic stress, oxidative stress, and nutrient starvation (*63*).

4.4. lncRNA regulates signaling pathways through phase separation

Some lncRNAs regulate signal transduction and cancer progression through phase separation. One of the core kinases of the Hippo pathway, LATS1, contains a PrLD in its N-terminal fragment, indicating that LATS1 can undergo phase separation. In addition, exogenously expressed LATS1-GFP forms puncta in the cytoplasm, whereas PrLD-deficient LATS1 mutants cannot form puncta. When the Hippo pathway is activated, MST, MAP4K, TAOK and other upstream regulators phosphorylate and activate LATS1. Activated LATS1 promotes YAP phosphorylation and isolates YAP in the cytoplasm, leading to YAP degradation. SNHG9, a conservative lncRNA in humans, rhesus monkeys and mice, is up-regulated in breast cancer and interacts directly with LATS1 to promote the formation of heavy particles or potential droplets containing LATS1. SNHG9 reduces LATS1 phosphorylation and its kinase activity, inhibiting the Hippo pathway and leading to the activation and translocation of YAP to the nucleus, thereby promoting breast cancer progression (*64*).

4.5. lncRNA regulates gene expression through phase separation

The function of many lncRNAs depends on their ability to interact with multiple copies of specific RNA binding proteins (RBPs). Such lncRNAs can mediate gene expression in various ways via phase separation.

PNCTR

lncRNA PNCTR is upregulated in multiple cancers and is associated with programmed cell death, which is encoded as a short tandem repeat in the rDNA intergenic spacer and contains many PTBP1-specific motifs. PTBP1 has been shown to stimulate the expression of several apoptosis activators by altering the splicing of its pre-mRNA or increasing its translation efficiency. It has recently been shown that PNCTR contains hundreds of PTBP1-specific motifs, acting as a scaffold structure to recruit PTBP1 and form relatively isolated perinuclear

compartments to control alternative splicing and promote cell survival (*65*).

SLERT

The nucleolus is a membrane-free nuclear condensate driven by phase separation, in which FC/DFC units are the site of RNA polymerase I (RNAPI)-mediated transcription of ribosomal DNA (rDNA) and pre-processing of rRNA. Rapid rRNA transcription occurs at the boundary of FC and DFC units, which is formed by the RNA helicase DDX21. lncRNA SLERT facilitates the transition of DDX21 from an open to a closed conformation through phase separation. DDX21 forms loose clusters in the closed conformation, providing the FC/DFC units with sufficient mobility and space required for RNAPI synthesis, thus regulating the multilayer nucleolus structure and enabling rapid RNAPI synthesis (*66*).

4.6. lncRNA regulates definitive endodermal differentiation through phase separation

The liver, lungs, pancreas and digestive tract originate from the endoderm, and the optimization and specification of endodermal differentiation are essential for generating the cell types of these organs. lncRNA DIGIT is induced during endodermal differentiation, and the loss of its expression results in defective endodermal differentiation (*67*). Mass spectrometry and immunoblotting have validated the interaction between DIGIT and the BRD3 protein, a member of the bromodomain and extra-terminal domain family of proteins. BRD3 and H3K18ac interact and occupy promoters and enhancers of genes in embryonic stem cells. Furthermore, DIGIT is enriched in H3K18ac-modified chromatin regions, and BRD3 occupancy decreases as DIGIT is depleted, suggesting that DIGIT recruits BRD3 to the H3K18ac locus via the bromodomain. DIGIT-induced BRD3 has the properties of a phase-separated condensate that regulates definitive endoderm differentiation (*68*).

5. Interaction between miRNAs and phase separation

Genes encoding miRNAs in the nucleus are transcribed by RNA polymerase to produce primary transcripts (pri-miRNAs) of several thousand bases in length. These pri-miRNAs are mainly processed via the protein complex of the micro-processor. This complex, which is approximately 400-500 kDa in size, mainly consists of two proteins, Drosha and Pasha. Drosha is an RNase III protein, whereas Pasha is a double-stranded RNA-binding protein involved in substrate recognition by Drosha. pri-miRNAs are further processed into precursor miRNAs (pre-miRNAs) containing 60-70 nt of stem-loop structure by the action of Drosha. pre-miRNAs are transported from the nucleus to the cytoplasm via the RanGTP/Exportin-5 transport protein (*69*). Nuclear pores responsible for transporting pre-miRNAs exhibit hydrogel characteristics (*70*). In the cytoplasm, pre-miRNAs are recognized by Dicer and form miRNA: miRNA* dimers through shearing and modification of the stem-loop structure. miRNA: miRNA* dimers are destabilized by the action of decapping enzymes and eventually generate mature, functional single-stranded miRNAs, which subsequently bind to the miRNP complex, whereas miRNA* is rapidly degraded. The mature miRNA, along with other proteins, forms the RISC, which degrades or inhibits the translation of the target mRNA (*71*). Both cleavage steps are catalyzed by the cleavage complex (D-body), which contains three core components, including the RNase III family protein Dicer-like 1 (DCL1), the double-stranded RBP, Hyponastic leaf 1 (HYL1) and the zinc finger protein Serrate (SE) (*6*).

5.1. miRNA regulates stress responses through phase separation

miRNAs can simultaneously regulate multiple targets and rapidly adapt to cellular metabolism in response to reversible stress. Drosophila miR-980 is one of the stress response factors and is associated with ovarian germ line differentiation. In a study, under stress conditions, miR-980 levels were significantly reduced, whereas Rbfox1 mRNA levels were increased, and a luciferase reporter gene assay verified that miR-980 directly targeted Rbfox1 in vitro and in the ovary. miR-980 also affects the protein levels of Rbfox1. Drosophila Rbfox1 protein contains multiple LCRs and can undergo phase separation under stress to form condensates, which can be disintegrated by 1,6-hexanediol. Therefore, miR-980-mediated Rbfox1 phase separation is a novel stress-responsive signaling cascade with profound effects on the survival, growth and differentiation of cells, and on the ability of organisms to survive under stress (Table 3) (*72*).

5.2. Phase separation regulates miRNA processing

The D-bodies are 0.2-0.8 μM in diameter and are essential for miRNA processing. They are central to miRNA biogenesis and represent phase-separated condensates of SE proteins, which contain IDRs (*73*). SE can form phase-separated droplets through weak intermolecular interactions generated by its N-terminal IDR and can subsequently recruit DCL1, HYL1 and pri/pre-miRNAs into the droplets to form D-bodies (*74*). As cleavage proceeds, pri-miRNAs are gradually consumed, intermolecular collisions are reduced, the interaction between HYL1 and SE is weakened and pri-miRNAs are released outside the cleavage vesicle. The release of miRNAs from D-bodies is achieved via co-transport.

Table 3. List of other ncRNAs (except lncRNAs) interacting with phase separation

ncRNA	Localization	Structure	Interaction	Function	References
miR-980	Cytoplasm	Not known	Rbfox1	Cell survival	*72*
miR-223	Cytoplasm	Not known	YBX1	Packaging RNAs into exosomes	*79*
snRNA snoRNA	Nucleus	Not known	Coilin	Forming Cajal bodies	*86-88*
piRNA	Cytoplasm	Uridine at the 5' end and 2'-O-methyl modification at the 3' end	Piwi proteins	Regulating gene silencing	*90-91* *96* *99*
circVAMP3	Cytoplasm	Closed loop	CAPRIN1	Inhibiting Hepatocellular Carcinoma	
ThymoD	Nucleus	Not known	Cohesin	Repositioning of the Bcl11b enhancer	

D-bodies are involved in gene transcription, RNA variable splicing and transposon silencing in addition to contributing to the maturation and release of miRNAs through phase separation. Therefore, phase separation may also play a role in these processes (*6*). Consistently, Seung Cho Lee and Robert A Martienssen reported that D-bodies are sites of miRNA biogenesis in plants and represent phase separated condensates of SE proteins containing IDRs.

FUS is one of the RNA-binding proteins containing an IDR and is associated with neurodegeneration. Mutations in its PrLD or nuclear localization signal enhance the conversion of FUS from liquid to solid deposits. FUS promotes Drosha recruitment on nascent pri-miRNAs, leading to miRNA maturation (*75*). As a pathological marker of ALS and frontotemporal dementia, TDP-43 fuses with FUS and is involved in the biogenesis and metabolism of coding RNAs and ncRNAs (*76*).

Stress-induced interactions between stress granules and miRNA-associated proteins (AGO2 and DICER) can regulate miRNA biogenesis by attenuating DICER catalytic activity (*70*). In a study, in a rat model of acute ischemic stroke with middle cerebral artery occlusion, miR-335 promoted the formation of stress granules by downregulating ROCK2 (*77*). Therefore, membraneless compartments and biomolecular condensates can regulate miRNA biogenesis. In addition, miRNAs can regulate the homeostasis of many biological and physiological processes such as transcription and apoptosis (*78*).

5.3. Phase separation specifically sorts miRNAs into exosomes

The RNA-binding protein YBX1 not only forms liquid biomolecular condensates in cells, but also undergoes phase separation in vitro via tyrosine- and arginine-rich motifs in the IDR. Two structural domains of YBX1, namely, CSD and IDR, are sorted together into cell-secreted exosomes, leading to the recruitment of miR-223 to droplets. Overexpression of YBX1 increases miR-223 levels in HEK293T and U2OS exosomes, and YBX1 phase separation is critical for the recruitment of miR-223 to exosomes (*79*). Point mutations in YBX1 inhibit its phase separation, prevent biomolecular condensation resulting from the admixture of YBX1 protein into cells and interfere with the sorting of miR-233 into exosomes. Increasing the RNA/YBX1 ratio initially promotes droplet size until the droplet is unstable or not produced.

5.4. Phase separation contributes to miRNA-mediated translational repression

The AGO family and miRNAs assemble into miRISCs that synergize with the GW182 protein to bind to target mRNAs with sequence complementarity to miRNAs, leading to their degradation (*80*). Nup358, a mutated protein present in patients with acute necrotizing encephalopathy, interacts with the C-terminal silencing domain of GW182 in its N-terminal region, affecting the inhibition of miRNA-mediated translation (*81*). miRISCs can assemble into phase-separated condensates through the interaction between AGO2 and GW182. Recent studies have also supported the phase-separating property of Nup358. Therefore, it can be speculated that Nup358 phase separation contributes to the stable binding of miRISCs to target mRNAs in the cytoplasm and hence plays a role in miRNA-mediated translational repression (*82*). In addition, AGO proteins undergo lipid-mediated phase separation to control de novo peptide ubiquitination (*83*).

FMRP is enriched in neuronal granules and can bind to RNA to regulate translation. It contains LCRs that form droplet-like condensates with 4E-BP2 and miR-125b, which coincides with in vitro inhibition of translation. Phosphorylation of the LCRs of FMRP increases its phase separation tendency and facilitates particle assembly, whereas their methylation decreases the phase separation tendency and facilitates particle disassembly (*84*).

6. Interaction between other ncRNAs and phase separation

snRNA and snoRNA

Cajal bodies are organelles present in the nucleus of proliferating cells. They are often close to the nucleolus, which is the most prominent nucleolar structure (*85*). Cajal bodies are structures that coordinate, facilitate and investigate the nuclear phase of spliceosomal snRNP biogenesis. They undergo fusion and fission events

and are sensitive to 1,6-hexanediol treatment, which is a key feature of the droplet organelle. Cajal body-associated regions are enriched with highly expressed histone genes and U sn/snoRNA loci that form intra- and inter-chromosomal clusters (*86*). snRNAs and snoRNAs act as scaffolds for local recruitment through RNA-protein interactions at high protein concentrations. The Cajal body structural protein, coilin, is both highly phosphorylated and methylated, which is essential for nucleosome assembly (*87*). Coilin, along with another essential Cajal body protein called SMN1, can oligomerize and interact with various key effector proteins and snRNPs. It forms a shell around the periphery of Cajal bodies, whereas SMN1 forms the inner core. Notably, Logan *et al*. demonstrated that disruption of Cajal bodies is associated with altered levels of primary and mature miRNAs and the let-7a mRNA target HMGA2 and suggested that Cajal bodies and miRNA processing mechanisms functionally interact to promote the biogenesis of miRNAs and snRNPs (88).

piRNA

piRNAs are small ncRNAs (24-31 nt) that can form complexes with Piwi proteins of the AGO family (*89*). piRNAs are characterized by uridine at the 5' end and 2'-O-methyl modification at the 3' end (*90*). They are mainly found in mammalian germ cells and stem cells and form piRNA complexes by binding to Piwi proteins. They regulate gene silencing pathways by binding to piRNA complexes.

In *Drosophila*, Yb bodies are liquid-like multivalent condensates that are considered the site of piRNA biogenesis in ovarian somatic cells. Yb is the major component of Yb bodies and consists of the C-terminus of helicase, RNA helicase and the extended Tudor structural domain; among which, the structural domain of RNA helicase is required for Yb-RNA interactions, Yb body formation and piRNA biogenesis (*91*).

AGO2 and TNRC6B are core protein components of RISCs, and multivalent interactions between the glycine/tryptophan-rich structural domain of TNRC6B and the tryptophan-binding pocket in the PIWI structural domain of AGO2 facilitate the formation of phase-separated droplets (*92*). Phase separation can isolate miRNA targets and accelerate their deadenylation, thereby regulating the rate of mRNA translation and decay. In a study on *Caenorhabditis elegans*, an IP assay verified that DEPS-1 binds to the PIWI protein PRG-1 through its PIWI binding site and forms elongated cohesions *in vivo* that mediate piRNA-dependent silencing (93).

circRNA

circRNAs are a unique class of RNA molecules. Unlike traditional linear RNAs, circRNA molecules have a closed-loop structure, are not affected by RNA exonucleases and are more stably expressed and less susceptible to degradation (*94*). Recent studies have shown that circRNAs are rich in miRNA binding sites, which act as miRNA sponges in cells, thereby relieving the repressive effect of miRNAs on their target genes and elevating their expression levels. This mechanism of action is known as the competitive endogenous RNA (ceRNA) mechanism (*95*). Several studies have shown that circRNAs play an important regulatory role in diseases.

circVAMP3, formed by reverse splicing of exons 3 and 4 of the VAMP3 gene, is lowly expressed in hepatocellular carcinoma tissues and correlates negatively with patient prognosis. In a study, an immunoprecipitation assay showed that antibodies to the RNA-binding protein CAPRIN1 or G3BP1 significantly enriched circVAMP3 relative to IgG antibodies. In addition, circVAMP3 promoted CAPRIN1 and G3BP1 phase separation in a concentration-dependent manner, leading to the formation of a stress granule, inhibition of c-Myc translation to downregulate protein levels of the Myc proto-oncogene protein and impairment of HCC cell proliferation and metastasis in vitro and *in vivo* (*96*).

eRNA

The ncRNA transcribed from enhancers is defined as 'enhancer RNA' (eRNA) (*97*). Studies have shown that eRNA transcription can stabilize or enhance phase separation. Super enhancer RNA promotes phase separation of super enhancer through RNA-RNA interactions and successfully forms local liquid condensates (*98*).

T cells are the primary performers of human immune function, and their developmental progression is regulated by a combination of transcriptional regulators. During T cell development, the intergenic region of Bcl11b containing enhancers is relocated from the lamina to the nuclear interior to direct the Bcl11b enhancer to the Bcl11b promoter. Enhancer RNA ThymoD mediates the repositioning of Bcl11b enhancers. ThymoD transcription promotes the demethylation of CTCF binding sites, recruiting the cohesin complex to the transcribed region to activate the cohesin-dependent loop that induces loop formation of Bcl11b enhancers and promoters, allowing epigenetic labeling of the activated deposited loop domain to facilitate phase separation (*99*).

7. Conclusion

Protein phase separation has emerged as a potential mechanism for regulating biological function. RNA-protein condensates are essentially hydrogel-like droplets rich in RNA and RNA-associated proteins. Stress-induced phase separation via RNA-binding proteins may be an evolutionarily conserved mechanism for cellular adaptation to and survival against environmental

stress (*76*). Notably, the sequence-specific base-pairing properties of RNAs can lead to their phase separation and are responsible for many neurological disorders such as Huntington's disease, muscular dystrophy, and ALS (*100*). The genomes of higher eukaryotes are commonly transcribed to produce large amounts of ncRNAs, which are key regulators of embryonic development, DNA damage response, and human diseases such as neuronal diseases, heart dysfunction and cancer.

The extensive involvement of ncRNAs and phase separation in the regulation of physiological processes suggests that their functional significance remains to be discovered. Therefore, specific regulatory mechanisms of ncRNAs associated with phase separation should be assessed to enhance the understanding of ncRNA regulation and function.

The association between ncRNAs and phase separation offers novel insights into the biology of enigmatic ncRNAs. Although the properties that allow ncRNAs to act as a scaffold structure remain uncertain, the underlying mechanisms can be explained with further investigation. Several questions remain to be addressed: What are the physicochemical mechanisms underlying the assembly of MLOs? How can precise IDRs and drug target regions be predicted? Can specific kinetics of lncRNA-driven phase separation be used as markers of disease? Can specific MLOs be targeted by targeting the corresponding RNAs? How can phase separation be accurately controlled? More research into phase separation in diseases is required to derive medical benefits from therapeutic strategies, early and accurate diagnosis and disease prevention.

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References

1. Zhang H, Ji X, Li P, Liu C, Lou J, Wang Z, Wen W, Xiao Y, Zhang M,Zhu X. Liquid-liquid phase separation in biology: mechanisms, physiological functions and human diseases. Sci China Life Sci. 2020; 63:953-985.
2. Dolgin E. What lava lamps and vinaigrette can teach us about cell biology. Nature. 2018; 555:300-302.
3. Li P, Banjade S, Cheng H C, Kim S, Chen B, Guo L, Llaguno M, Hollingsworth J V, King D S, Banani S F, Russo P S, Jiang Q X, Nixon B T,Rosen M K. Phase transitions in the assembly of multivalent signalling proteins. Nature. 2012; 483:336-40.
4. Nott T J, Petsalaki E, Farber P, Jervis D, Fussner E, Plochowietz A, Craggs T D, Bazett-Jones D P, Pawson T, Forman-Kay J D,Baldwin A J. Phase transition of a disordered nuage protein generates environmentally responsive membraneless organelles. Mol Cell. 2015; 57:936-947.
5. Onoguchi-Mizutani R,Akimitsu N. Long noncoding RNA and phase separation in cellular stress response. J Biochem. 2022; 171:269-276.
6. Xie D, Chen M, Niu J, Wang L, Li Y, Fang X, Li P,Qi Y. Phase separation of SERRATE drives dicing body assembly and promotes miRNA processing in Arabidopsis. Nat Cell Biol. 2021; 23:32-39.
7. Alberti S, Gladfelter A,Mittag T. Considerations and Challenges in Studying Liquid-Liquid Phase Separation and Biomolecular Condensates. Cell. 2019; 176:419-434.
8. Elguindy M M,Mendell J T. NORAD-induced Pumilio phase separation is required for genome stability. Nature. 2021; 595:303-308.
9. Feng Z, Chen X, Zeng M,Zhang M. Phase separation as a mechanism for assembling dynamic postsynaptic density signalling complexes. Curr Opin Neurobiol. 2019; 57:1-8.
10. Nakagawa S, Yamazaki T,Hirose T. Molecular dissection of nuclear paraspeckles: towards understanding the emerging world of the RNP milieu. Open Biol. 2018; 8:180150
11. El Fatimy R, Boulaassafre S, Bouchmaa N, El Khayari A, Vergely C, Malka G,Rochette L. The emerging role of miRNA-132/212 cluster in neurologic and cardiovascular diseases: Neuroprotective role in cells with prolonged longevity. Mech Ageing Dev. 2021; 199:111566.
12. Ling Z, Wang X, Tao T, Zhang L, Guan H, You Z, Lu K, Zhang G, Chen S, Wu J, Qian J, Liu H, Xu B,Chen M. Involvement of aberrantly activated HOTAIR/EZH2/miR-193a feedback loop in progression of prostate cancer. J Exp Clin Cancer Res. 2017; 36:159.
13. Lin X, Zhuang S, Chen X, Du J, Zhong L, Ding J, Wang L, Yi J, Hu G, Tang G, Luo X, Liu W,Ye F. lncRNA ITGB8-AS1 functions as a ceRNA to promote colorectal cancer growth and migration through integrin-mediated focal adhesion signaling. Mol Ther. 2022; 30:688-702.
14. Pei X, Wang X,Li H. LncRNA SNHG1 regulates the differentiation of Treg cells and affects the immune escape of breast cancer via regulating miR-448/IDO. Int J Biol Macromol. 2018; 118:24-30.
15. Elbaum-Garfinkle S, Kim Y, Szczepaniak K, Chen C C, Eckmann C R, Myong S,Brangwynne C P. The disordered P granule protein LAF-1 drives phase separation into droplets with tunable viscosity and dynamics. Proc Natl Acad Sci U S A. 2015; 112:7189-94.
16. Tsang B, Pritisanac I, Scherer S W, Moses A M,Forman-Kay J D. Phase Separation as a Missing Mechanism for Interpretation of Disease Mutations. Cell. 2020; 183:1742-1756.
17. Zeng M, Shang Y, Araki Y, Guo T, Huganir R L,Zhang M. Phase Transition in Postsynaptic Densities Underlies Formation of Synaptic Complexes and Synaptic Plasticity. Cell. 2016; 166:1163-1175 e12.
18. Bergeron-Sandoval L P, Safaee N,Michnick S W. Mechanisms and Consequences of Macromolecular Phase Separation. Cell. 2016; 165: 1067-1079.
19. McSwiggen D T, Mir M, Darzacq X,Tjian R. Evaluating phase separation in live cells: diagnosis, caveats, and functional consequences. Genes Dev. 2019; 33:1619-1634.
20. Ray S, Singh N, Kumar R, *et al*. alpha-Synuclein aggregation nucleates through liquid-liquid phase

separation. Nat Chem. 2020; 12:705-716.
21. Gussmann F, Hansen-Goos H, Dietrich S,Roth R. Liquid-liquid phase separation in an inhomogeneous ternary colloid-polymer mixture. J Chem Phys. 2021; 154:224504.
22. Yu M, Peng Z, Qin M, *et al*. Interferon-gamma induces tumor resistance to anti-PD-1 immunotherapy by promoting YAP phase separation. Mol Cell. 2021; 81: 1216-1230e9.
23. Wang S, Dai T, Qin Z, Pan T, Chu F, Lou L, Zhang L, Yang B, Huang H, Lu H,Zhou F. Targeting liquid-liquid phase separation of SARS-CoV-2 nucleocapsid protein promotes innate antiviral immunity by elevating MAVS activity. Nat Cell Biol. 2021; 23:718-732.
24. Lu B, Zou C, Yang M, He Y, He J, Zhang C, Chen S, Yu J, Liu K Y, Cao Q,Zhao W. Pharmacological Inhibition of Core Regulatory Circuitry Liquid-liquid Phase Separation Suppresses Metastasis and Chemoresistance in Osteosarcoma. Adv Sci (Weinh). 2021; 8:e2101895.
25. Sun Y, Lau S Y, Lim Z W, Chang S C, Ghadessy F, Partridge A,Miserez A. Phase-separating peptides for direct cytosolic delivery and redox-activated release of macromolecular therapeutics. Nat Chem. 2022; 14:274-283.
26. Hofweber M, Hutten S, Bourgeois B, Spreitzer E, Niedner-Boblenz A, Schifferer M, Ruepp M D, Simons M, Niessing D, Madl T,Dormann D. Phase Separation of FUS Is Suppressed by Its Nuclear Import Receptor and Arginine Methylation. Cell. 2018; 173:706-719e13.
27. 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.
28. Boulay G, Sandoval G J, Riggi N, Iyer S, Buisson R, Naigles B, Awad M E, Rengarajan S, Volorio A, McBride M J, Broye L C, Zou L, Stamenkovic I, Kadoch C,Rivera M N. Cancer-Specific Retargeting of BAF Complexes by a Prion-like Domain. Cell. 2017; 171:163-178e19.
29. Hnisz D, Abraham B J, Lee T I, Lau A, Saint-Andre V, Sigova A A, Hoke H A,Young R A. Super-enhancers in the control of cell identity and disease. Cell. 2013; 155:934-47.
30. Schepers A,Clevers H. Wnt signaling, stem cells, and cancer of the gastrointestinal tract. Cold Spring Harb Perspect Biol. 2012; 4:a007989.
31. Cai D, Liu Z,Lippincott-Schwartz J. Biomolecular Condensates and Their Links to Cancer Progression. Trends Biochem Sci. 2021; 46:535-549.
32. Alberti S,Hyman A A. Biomolecular condensates at the nexus of cellular stress, protein aggregation disease and ageing. Nat Rev Mol Cell Biol. 2021; 22:196-213.
33. Peng W X, Koirala P,Mo Y Y. LncRNA-mediated regulation of cell signaling in cancer. Oncogene. 2017; 36:5661-5667.
34. Soltys K,Ozyhar A. Transcription Regulators and Membraneless Organelles Challenges to Investigate Them. Int J Mol Sci. 2021; 22:12758.
35. Caudron-Herger M, Pankert T, Seiler J, Nemeth A, Voit R, Grummt I,Rippe K. Alu element-containing RNAs maintain nucleolar structure and function. EMBO J. 2015; 34:2758-74.
36. Imamura K, Imamachi N, Akizuki G, *et al*. Long noncoding RNA NEAT1-dependent SFPQ relocation from promoter region to paraspeckle mediates IL8 expression upon immune stimuli. Mol Cell. 2014; 53:393-406.
37. McCluggage F,Fox A H. Paraspeckle nuclear condensates: Global sensors of cell stress? Bioessays. 2021; 43:e2000245.
38. Hirose T, Yamazaki T,Nakagawa S. Molecular anatomy of the architectural NEAT1 noncoding RNA: The domains, interactors, and biogenesis pathway required to build phase-separated nuclear paraspeckles. Wiley Interdiscip Rev RNA. 2019; 10:e1545.
39. Fox A H, Nakagawa S, Hirose T,Bond C S. Paraspeckles: Where Long Noncoding RNA Meets Phase Separation. Trends Biochem Sci. 2018; 43:124-135.
40. Fei J, Jadaliha M, Harmon T S, Li I T S, Hua B, Hao Q, Holehouse A S, Reyer M, Sun Q, Freier S M, Pappu R V, Prasanth K V,Ha T. Quantitative analysis of multilayer organization of proteins and RNA in nuclear speckles at super resolution. J Cell Sci. 2017; 130:4180-4192.
41. Goyal B, Yadav S R M, Awasthee N, Gupta S, Kunnumakkara A B,Gupta S C. Diagnostic, prognostic, and therapeutic significance of long non-coding RNA MALAT1 in cancer. Biochim Biophys Acta Rev Cancer. 2021; 1875:188502.
42. Sawyer I A, Sturgill D,Dundr M. Membraneless nuclear organelles and the search for phases within phases. Wiley Interdiscip Rev RNA. 2019; 10:e1514.
43. Wang X, Liu C, Zhang S, Yan H, Zhang L, Jiang A, Liu Y, Feng Y, Li D, Guo Y, Hu X, Lin Y, Bu P,Li D. N(6)-methyladenosine modification of MALAT1 promotes metastasis via reshaping nuclear speckles. Dev Cell. 2021; 56:702-715e8.
44. Wang M, Tao X, Jacob M D, Bennett C A, Ho J J D, Gonzalgo M L, Audas T E,Lee S. Stress-Induced Low Complexity RNA Activates Physiological Amyloidogenesis. Cell Rep. 2018; 24:1713-1721e4.
45. Valgardsdottir R, Chiodi I, Giordano M, Cobianchi F, Riva S,Biamonti G. Structural and functional characterization of noncoding repetitive RNAs transcribed in stressed human cells. Mol Biol Cell. 2005; 16:2597-604.
46. Ninomiya K, Adachi S, Natsume T, Iwakiri J, Terai G, Asai K,Hirose T. LncRNA-dependent nuclear stress bodies promote intron retention through SR protein phosphorylation. EMBO J. 2020; 39:e102729.
47. Prasanth K V, Rajendra T K, Lal A K,Lakhotia S C. Omega speckles - a novel class of nuclear speckles containing hnRNPs associated with noncoding hsr-omega RNA in Drosophila. J Cell Sci. 2000; 113 Pt 19:3485-97.
48. Dumbovic G, Biayna J, Banus J, Samuelsson J, Roth A, Diederichs S, Alonso S, Buschbeck M, Perucho M,Forcales S V. A novel long non-coding RNA from NBL2 pericentromeric macrosatellite forms a perinucleolar aggregate structure in colon cancer. Nucleic Acids Res. 2018; 46:5504-5524.
49. Pitchiaya S, Mourao M D A, Jalihal A P, Xiao L, Jiang X, Chinnaiyan A M, Schnell S,Walter N G. Dynamic Recruitment of Single RNAs to Processing Bodies Depends on RNA Functionality. Mol Cell. 2019; 74:521-533 e6.
50. Thakur J,Henikoff S. Architectural RNA in chromatin organization. Biochem Soc Trans. 2020; 48:1967-1978.
51. Guh C Y, Hsieh Y H,Chu H P. Functions and properties of nuclear lncRNAs-from systematically mapping the interactomes of lncRNAs. J Biomed Sci. 2020; 27:44.
52. Pessina F, Giavazzi F, Yin Y, Gioia U, Vitelli V, Galbiati A, Barozzi S, Garre M, Oldani A, Flaus A, Cerbino R, Parazzoli D, Rothenberg E,d'Adda di Fagagna F. Functional transcription promoters at DNA double-strand breaks mediate RNA-driven phase separation of damage-

response factors. Nat Cell Biol. 2019; 21:1286-1299.
53. Fijen C,Rothenberg E. The evolving complexity of DNA damage foci: RNA, condensates and chromatin in DNA double-strand break repair. DNA Repair (Amst). 2021; 105:103170.
54. Thapar R, Wang J L, Hammel M, *et al*. Mechanism of efficient double-strand break repair by a long non-coding RNA. Nucleic Acids Res. 2020; 48:10953-10972.
55. Novo C L. A Tale of Two States: Pluripotency Regulation of Telomeres. Front Cell Dev Biol. 2021; 9:703466.
56. Yamashita A, Watanabe Y, Nukina N,Yamamoto M. RNA-assisted nuclear transport of the meiotic regulator Mei2p in fission yeast. Cell. 1998; 95:115-23.
57. Ding D Q, Okamasa K, Katou Y, Oya E, Nakayama J I, Chikashige Y, Shirahige K, Haraguchi T,Hiraoka Y. Chromosome-associated RNA-protein complexes promote pairing of homologous chromosomes during meiosis in *Schizosaccharomyces pombe*. Nat Commun. 2019; 10:5598.
58. He P, Zhang C, Ji Y, Ge M K, Yu Y, Zhang N, Yang S, Yu J X, Shen S M,Chen G Q. Epithelial cells-enriched lncRNA SNHG8 regulates chromatin condensation by binding to Histone H1s. Cell Death Differ. 2022; 29:1569-1581
59. Patil D P, Chen C K, Pickering B F, Chow A, Jackson C, Guttman M,Jaffrey S R. m(6)A RNA methylation promotes XIST-mediated transcriptional repression. Nature. 2016; 537:369-373.
60. Cerase A, Armaos A, Neumayer C, Avner P, Guttman M,Tartaglia G G. Phase separation drives X-chromosome inactivation: a hypothesis. Nat Struct Mol Biol. 2019; 26:331-334.
61. Onoguchi-Mizutani R, Kirikae Y, Ogura Y, Gutschner T, Diederichs S,Akimitsu N. Identification of a heat-inducible novel nuclear body containing the long noncoding RNA MALAT1. J Cell Sci. 2021; 134:jcs253559.
62. Wang C, Duan Y, Duan G, Wang Q, Zhang K, Deng X, Qian B, Gu J, Ma Z, Zhang S, Guo L, Liu C,Fang Y. Stress Induces Dynamic, Cytotoxicity-Antagonizing TDP-43 Nuclear Bodies via Paraspeckle LncRNA NEAT1-Mediated Liquid-Liquid Phase Separation. Mol Cell. 2020; 79:443-458e7.
63. Wang R, Cao L, Thorne R F, Zhang X D, Li J, Shao F, Zhang L,Wu M. LncRNA GIRGL drives CAPRIN1-mediated phase separation to suppress glutaminase-1 translation under glutamine deprivation. Sci Adv. 2021; 7:eabe5708
64. Li R H, Tian T, Ge Q W, *et al*. A phosphatidic acid-binding lncRNA SNHG9 facilitates LATS1 liquid-liquid phase separation to promote oncogenic YAP signaling. Cell Res. 2021; 31:1088-1105.
65. Yap K, Mukhina S, Zhang G, Tan J S C, Ong H S,Makeyev E V. A Short Tandem Repeat-Enriched RNA Assembles a Nuclear Compartment to Control Alternative Splicing and Promotes Cell Survival. Mol Cell. 2018; 72:525-540e13.
66. Wu M, Xu G, Han C, Luan P F, Xing Y H, Nan F, Yang L Z, Huang Y, Yang Z H, Shan L, Yang L, Liu J,Chen L L. lncRNA SLERT controls phase separation of FC/DFCs to facilitate Pol I transcription. Science. 2021; 373:547-555.
67. Daneshvar K, Pondick J V, Kim B M, Zhou C, York S R, Macklin J A, Abualteen A, Tan B, Sigova A A, Marcho C, Tremblay K D, Mager J, Choi M Y,Mullen A C. DIGIT Is a Conserved Long Noncoding RNA that Regulates GSC Expression to Control Definitive Endoderm Differentiation of Embryonic Stem Cells. Cell Rep. 2016; 17:353-365.
68. Daneshvar K, Ardehali M B, Klein I A, *et al*. lncRNA DIGIT and BRD3 protein form phase-separated condensates to regulate endoderm differentiation. Nat Cell Biol. 2020; 22:1211-1222.
69. Correia de Sousa M, Gjorgjieva M, Dolicka D, Sobolewski C,Foti M. Deciphering miRNAs' Action through miRNA Editing. Int J Mol Sci. 2019; 20:6249.
70. Kucherenko M M,Shcherbata H R. miRNA targeting and alternative splicing in the stress response - events hosted by membrane-less compartments. J Cell Sci. 2018; 131:jcs202002.
71. Lee S C,Martienssen R A. Phase separation in plant miRNA processing. Nat Cell Biol. 2021; 23:5-6.
72. .Kucherenko M M,Shcherbata H R. Stress-dependent miR-980 regulation of Rbfox1/A2bp1 promotes ribonucleoprotein granule formation and cell survival. Nat Commun. 2018; 9:312.
73. Gao S, Wang J, Jiang N, *et al*. Hyponastic Leaves 1 protects pri-miRNAs from nuclear exosome attack. Proc Natl Acad Sci U S A. 2020; 117:17429-17437.
74. Wu X, Shi Y, Li J, Xu L, Fang Y, Li X,Qi Y. A role for the RNA-binding protein MOS2 in microRNA maturation in Arabidopsis. Cell Res. 2013; 23:645-57.
75. Pessina F, Gioia U, Brandi O, Farina S, Ceccon M, Francia S,d'Adda di Fagagna F. DNA Damage Triggers a New Phase in Neurodegeneration. Trends Genet. 2021; 37:337-354.
76. Ling S C. Synaptic Paths to Neurodegeneration: The Emerging Role of TDP-43 and FUS in Synaptic Functions. Neural Plast. 2018; 2018:8413496.
77. Si W, Ye S, Ren Z, Liu X, Wu Z, Li Y, Zhou J, Zhang S, Li Y, Deng R,Chen D. miR335 promotes stress granule formation to inhibit apoptosis by targeting ROCK2 in acute ischemic stroke. Int J Mol Med. 2019; 43:1452-1466.
78. Jie M, Feng T, Huang W, Zhang M, Feng Y, Jiang H,Wen Z. Subcellular Localization of miRNAs and Implications in Cellular Homeostasis. Genes (Basel). 2021; 12:856.
79. Liu X M, Ma L, Schekman R. Selective sorting of microRNAs into exosomes by phase-separated YBX1 condensates. Elife. 2021; 10:e71982
80. Niaz S,Hussain M U. Role of GW182 protein in the cell. Int J Biochem Cell Biol. 2018; 101:29-38.
81. Sahoo M R, Gaikwad S, Khuperkar D, *et al*. Nup358 binds to AGO proteins through its SUMO-interacting motifs and promotes the association of target mRNA with miRISC. EMBO Rep. 2017; 18:241-263.
82. Deshmukh P, Singh A, Khuperkar D,Joseph J. Acute necrotizing encephalopathy-linked mutations in Nup358 impair interaction of Nup358 with TNRC6/GW182 and miRNA function. Biochem Biophys Res Commun. 2021; 559:230-237.
83. Gao Y, Zhu Y, Wang H, Cheng Y, Zhao D, Sun Q,Chen D. Lipid-mediated phase separation of AGO proteins on the ER controls nascent-peptide ubiquitination. Mol Cell. 2022; 82:1313-1328e8.
84. Tsang B, Arsenault J, Vernon R M, Lin H, Sonenberg N, Wang L Y, Bah A,Forman-Kay J D. Phosphoregulated FMRP phase separation models activity-dependent translation through bidirectional control of mRNA granule formation. Proc Natl Acad Sci U S A. 2019; 116:4218-4227.
85. Trinkle-Mulcahy L,Sleeman J E. The Cajal body and the nucleolus: "In a relationship" or "It's complicated"? RNA Biol. 2017; 14:739-751.

86. Meier U T. RNA modification in Cajal bodies. RNA Biol. 2017; 14:693-700.
87. Ogg S C,Lamond A I. Cajal bodies and coilin--moving towards function. J Cell Biol. 2002; 159:17-21.
88. Logan M K, McLaurin D M,Hebert M D. Synergistic interactions between Cajal bodies and the miRNA processing machinery. Mol Biol Cell. 2020; 31:1561-1569.
89. Seto A G, Kingston R E,Lau N C. The coming of age for Piwi proteins. Mol Cell. 2007; 26:603-609.
90. Shigematsu M, Kawamura T, Morichika K, Izumi N, Kiuchi T, Honda S, Pliatsika V, Matsubara R, Rigoutsos I, Katsuma S, Tomari Y,Kirino Y. RNase kappa promotes robust piRNA production by generating 2',3'-cyclic phosphate-containing precursors. Nat Commun. 2021; 12:4498.
91. Hirakata S, Ishizu H, Fujita A, Tomoe Y,Siomi M C. Requirements for multivalent Yb body assembly in transposon silencing in Drosophila. EMBO Rep. 2019; 20:e47708.
92. Sheu-Gruttadauria J,MacRae I J. Phase Transitions in the Assembly and Function of Human miRISC. Cell. 2018; 173:946-957e16.
93. Suen K M, Braukmann F, Butler R, Bensaddek D, Akay A, Lin C C, Milonaityte D, Doshi N, Sapetschnig A, Lamond A, Ladbury J E,Miska E A. DEPS-1 is required for piRNA-dependent silencing and PIWI condensate organisation in Caenorhabditis elegans. Nat Commun. 2020; 11:4242.
94. Salzman J. Circular RNA Expression: Its Potential Regulation and Function. Trends Genet. 2016; 32:309-316.
95. Zang J, Lu D,Xu A. The interaction of circRNAs and RNA binding proteins: An important part of circRNA maintenance and function. J Neurosci Res. 2020; 98:87-97.
96. Chen S, Cao X, Zhang J, Wu W, Zhang B, Zhao F. circVAMP3 Drives CAPRIN1 Phase Separation and Inhibits Hepatocellular Carcinoma by Suppressing c-Myc Translation. Adv Sci (Weinh). 2022; 9:e2103817.
97. Ye R, Cao C, Xue Y. Enhancer RNA: biogenesis, function, and regulation. Essays Biochem. 2020; 64:883-894.
98. Debek S, Juszczynski P. Super enhancers as master gene regulators in the pathogenesis of hematologic malignancies. Biochim Biophys Acta Rev Cancer. 2022; 1877:188697.
99. Isoda T, Moore A J, He Z, Chandra V, Aida M, Denholtz M, Piet van Hamburg J, Fisch K M, Chang A N, Fahl S P, Wiest D L,Murre C. Non-coding Transcription Instructs Chromatin Folding and Compartmentalization to Dictate Enhancer-Promoter Communication and T Cell Fate. Cell. 2017; 171:103-119e18.
100. Fujino Y, Nagai Y. The molecular pathogenesis of repeat expansion diseases. Biochem Soc Trans. 2022; 50:119-134.

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