Cell Chemical Biology

CellPress
OPEN ACCESS

Review

RNA and condensates: Disease implications and therapeutic opportunities

Tina W. Han,[1] Bede Portz,[1] Richard A. Young,[2,3] Ann Boija,[1,*] and Isaac A. Klein[1,*]
[1]Dewpoint Therapeutics, Boston, MA, USA
[2]Whitehead Institute for Biomedical Research, Cambridge, MA 02142, USA
[3]Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
*Correspondence: aboija@dewpointx.com (A.B.), iklein@dewpointx.com (I.A.K.)
https://doi.org/10.1016/j.chembiol.2024.08.009

SUMMARY

Biomolecular condensates are dynamic membraneless organelles that compartmentalize proteins and RNA molecules to regulate key cellular processes. Diverse RNA species exert their effects on the cell by their roles in condensate formation and function. RNA abnormalities such as overexpression, modification, and mislocalization can lead to pathological condensate behaviors that drive various diseases, including cancer, neurological disorders, and infections. Here, we review RNA's role in condensate biology, describe the mechanisms of RNA-induced condensate dysregulation, note the implications for disease pathogenesis, and discuss novel therapeutic strategies. Emerging approaches to targeting RNA within condensates, including small molecules and RNA-based therapies that leverage the unique properties of condensates, may revolutionize treatment for complex diseases.

INTRODUCTION

In the landscape of modern biology, the discovery that RNA and proteins condense into biomolecular condensates to regulate key cellular processes has fundamentally changed our understanding of the cell. This transformative era of cell biology was ushered in by the discovery in *C. elegans* that RNA and protein-rich P granules are liquid-like condensates formed via phase transition.[1] Since the discovery of biomolecular condensates, a new model of the cell has emerged in which spatiotemporal regulation of diverse cellular processes occurs by compartmentalization and concentration of molecules involved in shared processes into membraneless organelles.[2] These insights have motivated efforts throughout academia and industry to understand the composition and behavior of condensates, their molecular grammar, material properties, cellular functions, role in pathobiology, as well as their potential to facilitate new modes of drug discovery.[2,3]

Biomolecular condensates are mainly composed of proteins, DNA, and RNA, with the latter contributing to their formation, function, dysfunction, and therapeutic modulation. RNA is a biopolymer capable of dynamic, multivalent interactions with itself, small molecules, metabolites, other nucleic acids, and proteins.[4] In addition to these properties, its capacity to be modified, folded, and trafficked makes RNA ideal for structuring and regulating cellular condensates and their functions. Various RNA species regulate condensate formation and function, including long noncoding RNA (lncRNA), messenger RNA (mRNA), ribosomal RNA (rRNA), single-stranded RNA (ssRNA), and others.[5] Dysregulation of these RNAs, through mechanisms such as misexpression and post-transcriptional modifications commonly seen in diseases, influences biological processes by altering condensate behaviors.[5,6] Consequently, condensates serve as a crucial "missing link" between RNA and pathogenic effects. This understanding provides new insights into RNA's roles in disease and significant implications for drug discovery and development.

In this review, we present an integrated perspective on how the study of RNA and condensates is reshaping our understanding of disease and yielding novel therapeutic hypotheses. We begin with an overview of RNA's role in the formation and function of condensates and discuss how specific RNA species regulate diverse cellular processes through condensates. We then examine evidence suggesting that RNA-mediated condensate dysregulation contributes to disease pathogenesis across therapeutic areas such as oncology, neurology, and infectious diseases. Condensates, RNAs, as well as their modifiers and interactors, serve as targets for various therapeutic modalities; we explore how condensates impact the therapeutic effect of these molecules and provide several examples. Finally, we highlight areas for future research and speculate on how deeper insights into the interplay between RNA, condensates, and pathobiology may lead to novel therapeutic opportunities.

RNA FEATURES REGULATING BIOMOLECULAR CONDENSATE FORMATION

Condensate formation and function is governed by the specific properties of, concentrations, and interactions between, biomolecules such as DNA, RNA, and protein (Figure 1). The role of proteins in condensate formation and function is well established, and the role of RNA is emerging as an equally critical component. Protein features such as intrinsic disorder, repeating modular domains, and specific motifs all determine the ability of proteins

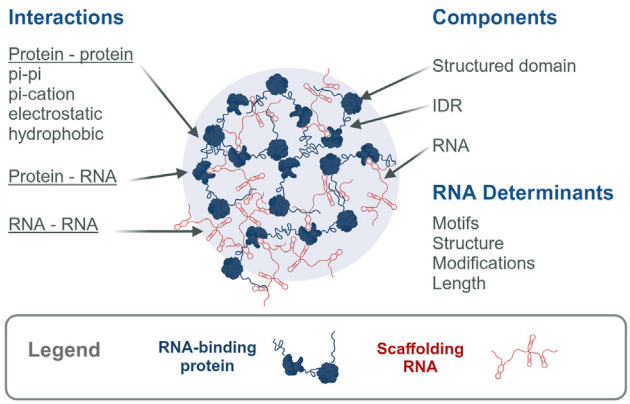

Figure 1. RNA features regulating biomolecular condensate formation

Schematic representation of homotypic and heterotypic interactions in a biomolecular condensate. RNA condensate assembly is governed by RNA-RNA, RNA-protein, and protein-protein interactions. RNA-binding proteins are concentrated spatially through binding to scaffolding RNAs and phase separation can be mediated by intrinsically disordered regions of the protein. Factors that influence RNA condensation include density of RBP binding motifs, secondary or tertiary structures, RNA modifications such as m^6A methylation which recruits phase separating readers and length which contributes to multivalency. For simplicity, a single RNA and protein type are illustrated. Figure created with BioRender.

to phase separate, as well as the emergent properties of the resulting condensate. Specific protein-protein interaction types are core determinants of condensate formation including pi-pi, pi-cation, electrostatic, and hydrophobic interactions. Analogous determinants of RNA phase separation include length, repetitive motifs, secondary structure, the presence and nature of counter-ions as well as epitranscriptomic modifications like methylation.[7–12]

General rules governing RNA phase separation are emerging from studies examining the condensate properties of RNAs in isolation, in the absence of proteins. RNAs undergo phase transitions at specific critical solution temperatures and ionic strengths, typical of protein phase transitions regulated by temperature, valency, and charge.[7,8] The impact of RNA length and sequence composition on RNA phase separation was directly explored in the context of repetitive sequences, such as those implicated in repeat expansion disorders such as amyotrophic laterals sclerosis (ALS), Huntington's disease (HD), and myotonic dystrophy type 1 (DM1).[7] Each disease is characterized by the expansion of distinct sequence repeats above a critical threshold, each of which undergoes a phase transition as a function of repeat length, consistent with a role for valency in RNA condensation.[7] Sequence composition also influences RNA condensate behavior with CAG (HD) and CUG (DM1) repeats forming spherical, gel-like condensates. In contrast, G_4C_2 repeats (ALS) form spherical condensates only for repeat lengths below the threshold associated with the disease while yielding mesh-like aggregates for repeat lengths above the disease threshold.[7]

In addition to valency and sequence, RNA condensation is influenced by the structure of RNA.[8,9] The condensation of RNAs in the absence of proteins depends on ionic strength and is influenced by metal ions that promote RNA folding. *In vitro* models of RNA condensates frequently employ temperature as a means of melting pre-existing RNA structures, and subsequent cooling in the presence of metal ions to promote re-folding that can drive condensation.[7–10] While these studies unfold RNA at temperatures not encountered in most organisms, they reveal a role for RNA structure that may be relevant to physiological condensates. In cells, RNAs can fold co-transcriptionally in the presence of metal ions, metabolites, DNA, proteins, and other RNAs, all of which can influence folding and possibly condensation. In bacteria, RNA structures known as riboswitches fold and recognize ions and metabolites, and these structures regulate gene expression.[9] Curiously, bacterial riboswitches form RNA condensates *in vitro* that depend partly on RNA structure.[9] The role of cognate ligands on riboswitch condensation has yet to be explored, but this work hints at the possibility of environmentally sensitive RNA condensates impacting gene expression in bacteria.[9] A similar model may exist in plants, where G-quadruplex structures adopted by the *SHR* mRNA leads to condensation *in vitro*. A range of physiological conditions promoting G-quadruplex formation drives *SHR* condensation, suggesting a model whereby environmentally tunable G-quadruplex formation drives RNA condensation impacting the pool of mRNA available for translation or decay.[10] In these models of *in vitro* RNA condensation, temperature serves as an energy input to remodel RNA structure. In cells, such rearrangements may instead be modulated by ATP-dependent RNA helicases which can locally unfold RNA-RNA interactions to modulate condensate formation or material properties.[13,14]

Unlike proteins, the phosphodiester backbone of RNA imparts a uniform negative charge. This enables RNA to condense via charge balancing by positively charged counter-ions in a process known as complex coacervation.[11,15] In addition to positively charged RNA-binding proteins, counter-ions can include highly abundant, physiologically relevant polycations such as spermine and spermidine or potentially toxic dipeptide repeats translated from G_4C_2 hexanucleotide repeats in ALS.[16–18] Condensation serves as a model to explain the colocalization of dipeptide repeats and RNA in foci of G_4C_2 expanded C9orf72 ALS/FTD patients.[19] Despite the simple and non-specific nature of complex coacervation, the material properties of RNA coacervates can vary as a function of RNA sequence and length, and the nature of the counter-ion, even giving rise to droplets with layered architectures. This has led some to posit RNA condensates preceded cellular life in the RNA world.[18,20–22] In contrast to the polar phosphodiester backbone of RNA, nucleobases are planar, aromatic, and vary in hydrophobicity. It is thus possible that the partitioning of RNA into different condensates could vary as a function of folding, with double-stranded RNA being more polar and single-stranded RNA presenting bases for stacking with aromatic amino acids, other RNA, or DNA, or generally favoring more hydrophobic solvents.

Nuclear condensates, which are comprised of scaffolding RNA and associated proteins can be created *de novo* in cells by transcribing the scaffolding RNA. This phenomenon was first observed via ectopic expression of distinct ncRNAs associated with histone locus bodies, Cajal bodies, nuclear speckles, and nuclear stress bodies, and recruitment of those ncRNAs to repetitive genomic loci.[23] These RNA condensates in turn recruit

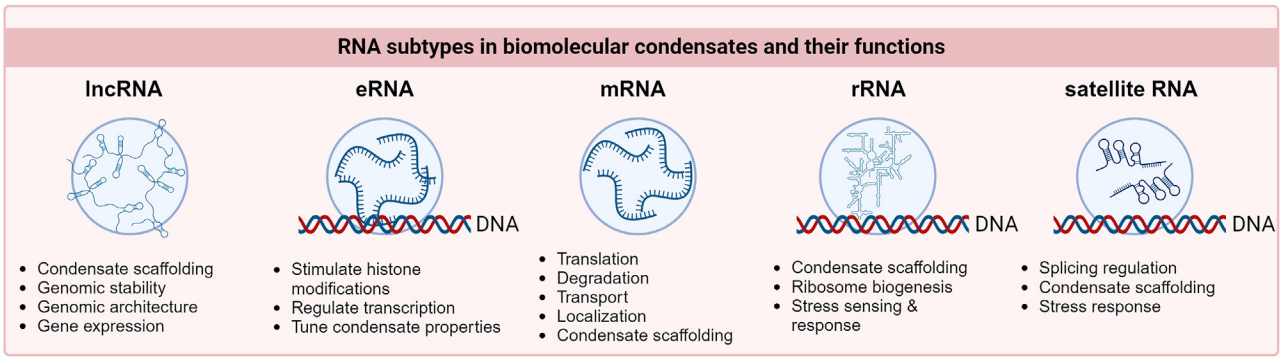

Figure 2. RNA subtypes in biomolecular condensates
Cartoon representation of lncRNA, eRNA, mRNA, rRNA and satellite RNA condensates and the recognized roles of the various RNA species in these condensates. RNA condensates in the nucleus frequently assemble around nascent transcripts of scaffolding RNAs and active transcription sites, which is depicted in the figure as condensates tethered to DNA. Figure created with BioRender.

protein-binding partners, illustrating that the transcription of scaffolding RNAs nucleates condensate assembly.[23] The association of condensation-prone RNA-binding proteins (RBPs) with RNA can have direct effects on condensate formation and material properties. This interplay between RNA and protein species can give rise to reentrant behavior whereby RNA may nucleate condensate formation at lower RNA-protein ratios, dissolve condensates at higher RNA-protein, and modulate a spectrum of material properties at intermediate ratios.[24–27] As a result, RNA condensates can adopt layered topologies governed by the ratio of RNA-RBPs in each layer.[28–31] That RNA levels can govern the existence and material properties of condensates profoundly impacts biology in two ways. First, processes occurring in RNA condensates can be regulated spatiotemporally, localized to sites of RNA self-assembly, and timed by the duration of the RNA synthesis or decay. This has implications for bursts of gene expression in transcriptional condensates nucleated in part by the initial synthesis of RNA and limited in scale and time by RNA levels.[32,33] The partial immiscibility of layered RNA condensates also creates interfaces where the physiochemical environment created by components enriched in each layer may collaborate to execute functions.[34,35] The nucleolus is one example of a layered condensate where ribosomal RNAs are synthesized and processed in a stepwise trajectory though each layer.[36,37] As rRNAs are processed and folded they become more compact and less entangled with nucleolar scaffolds, and thus partition less favorably resulting in a flux of mature ribosomes from the center of the nucleolus where rRNAs are transcribed out into the nucleoplasm.[37]

Post-translational modifications of proteins regulate their condensation properties. Similarly, post-transcriptional modifications of RNA have been shown to regulate RNA condensation.[12,38–41] N6-methyladenosine (m^6A) methylation of RNA alters the recruitment of RNAs to cytoplasmic stress granules via association with RBP "readers" of this epitranscriptomic mark.[38] This may enable the preferential sorting of specific transcripts to stress granules, especially those with long exons that promote m^6A methylation and reader protein binding.[38] RNA methylation also regulates nuclear bodies. In myeloid leukemia, the m^6A methylation causes RNAs to co-condense with the reader protein, YTHDC1. The resulting nuclear bodies, dubbed nuclear YTHDC1-m^6A condensates or nYACs, protect recruited transcripts from RNA degradation and may confer a survival advantage.[41] The recruitment of RBP readers to methylated RNA impacts condensates in both the nucleus and cytoplasm. The impact of epitranscriptomic marks on the biophysical properties of RNA, and thus condensation, remains an open question.

The levels of scaffolding RNAs impact the material properties, composition, and duration of condensates can be tuned by RNA expression and decay. Structural and epitranscriptomic modifications further tune RNA condensates. Collectively, these rules govern emergent properties of RNA condensates with implications for physiology and disease. Thus, an increased understanding of the roles of RNA in condensates will aid in drug discovery; by revealing pathomechanisms, expanding target space, or deconvolving the mechanism of action of therapeutic compounds.[24,26,27,32,42,43]

RNA SUBTYPES IN BIOMOLECULAR CONDENSATES

RNA plays a critical role in condensate biology by scaffolding condensate structure, regulating condensate assembly, and modulating condensate properties. Additionally, RNA localization, transport, and function are regulated by condensates.[44] In this section, we will discuss the functions of different types of RNAs in condensates (Figure 2).

Long noncoding RNAs (lncRNAs) are longer than 200 nucleotides and are not predicted to encode proteins.[45] Despite more than 95,000 lncRNA genes having been annotated in various databases (GENCODE, NONCODE, etc.), fewer than 3,000 lncRNAs have been validated with biological functions.[45] Many lncRNAs remain associated with the locus where they are transcribed and assemble ribonucleoprotein condensates at those loci, and these compartments influence both local chromatin architecture and gene expression.[46] For example, pre-ribosomal RNAs (pre-rRNAs) provide structural scaffolding for ribosomal proteins and play critical roles in ribosome activities.[47] rRNAs are highly concentrated within the nucleolus where ribosome biogenesis predominantly takes place and nascent transcripts are required for seeding the nucleolar assembly.[48] A feature shared by many of these RNAs that nucleate condensate formation is that they commonly have a repetitive sequence, either

tandem repeats like the highly conserved Xist E-repeat domain[49] or discontinuous repetition of protein-binding sites like NEAT1[50] and NORAD,[51] enabling lncRNAs to scaffold multivalent interactions.

Examples of biomolecular condensates that are scaffolded by lncRNAs include paraspeckles, which are nuclear condensates that assemble around NEAT1[52] and regulate gene expression through sequestration of proteins such as NONO (non-POU domain containing, octamer-binding) and SFPQ (Splicing Factor Proline And Glutamine Rich),[53] and cytoplasmic NORAD-Pumilio bodies (NP bodies), which regulate genomic stability through sequestration of translational repressor Pumilio.[54] NEAT1 is additionally implicated in neurodegenerative diseases and dysregulated in multiple cancers,[52] and perturbation of NP bodies potentially may decrease cancer cell fitness or increase chemosensitivity.[55,56] In addition, X inactive specific transcript (XIST), one of the first lncRNA discovered, mediates X chromosome inactivation, a process important for dosage compensation in mammals,[57] through condensation around the silenced chromosome.[58] XIST is transcribed by the X chromosome destined to be silenced, and the nascent XIST transcript scaffolds the assembly of a condensate composed of phase-separating RNA-binding proteins such as PTBP1, MATR3, TDP-43, and CELF1. These proteins help to concentrate and localize XIST RNA around the inactivated chromosome,[58] recruiting chromatin modifiers and transcriptional repressors to establish gene silencing. Dysregulation of XIST RNA has been associated with multiple cancers[59] and a higher risk of autoimmune disease in women.[60]

A subset of lncRNAs called enhancer RNAs or eRNAs is bidirectionally transcribed from enhancers, *cis*-regulatory elements in the genome that exert spatiotemporal control over gene expression.[61] These short-lived transcripts regulate transcriptional activity by stimulating activating histone modifications, interacting with chromatin looping factors to promote enhancer-promoter interactions, and facilitating the transition of RNA polymerase II from transcriptional initiation to elongation.[62] eRNAs may also contribute to the formation of transcriptional condensates by trapping transcription factors and coactivators at enhancer sites, thus elevating local concentrations.[63,64] Some eRNAs show pervasive m^{6}A modifications that bind nuclear m^{6}A reader YTHDC1, a phase-separating protein, which recruits transcription factors to enhancers potentially through co-formation of condensates.[65] Depleting eRNAs through degradation or transcription inhibition, however, does not seem to perturb transcriptional condensate morphology, suggesting that, unlike the role NEAT1 plays in paraspeckles, eRNAs are not required as a structural component.[66] Furthermore, it has been proposed that eRNAs might modulate condensate properties in a non-equilibrium RNA feedback mechanism: low levels of RNA (i.e., short-lived eRNAs transcribed initially) promote and strengthen condensate formation, whereas high levels (i.e., transcriptional bursts during elongation phase) dissolves the condensate.[32,61]

Messenger RNAs (mRNAs) encode the instructions for making proteins and are the primary cargo for many biomolecular condensates that regulate translation, degradation, transport, and localization.[44] Not only are mRNA substrates acted on by condensates, the RNAs themselves can be essential for condensation through RNA-RNA or RNA-protein interactions.[5] mRNAs can scaffold the assembly of nuclear condensates, such as histone locus bodies (HLBs), which form adjacent to replication-dependent histone genes and are thought to regulate histone protein production as a function of the cell cycle.[67,68] Intriguingly, knock down of either RNA polymerase II or III subunits inhibits the assembly of stress granules (SGs) and P bodies,[69] suggesting that transcription products are required for these cytoplasmic condensates. Under stress, RNAs are released from polysomes and are thought to act as multivalent scaffolds for the assembly of phase-separating stress granule components.[70] Like eRNAs, mRNAs may also contribute to condensate formation through the recruitment of YTH domain-containing family proteins (YTHDFs) to m^{6}A methylated nucleotides which are found to be enriched in stress granules.[71] Conditions that induce stress granules also lead to the increase of m^{6}A methylation in mRNAs, typically in the 5′UTRs not normally modified by methylation.[71] Since knockdown of YTHDF impairs the formation of stress granules, which have been demonstrated to phase separate both *in vitro* and in cells, the recruitment of YTHDF proteins to stress granules may act to reinforce and stabilize stress granule assembly.[71]

Satellite RNAs transcribed from long tandem repeats in the centromeric and pericentromeric regions of chromosomes have been implicated in scaffolding several condensate types.[72] These regions are associated with heterochromatin and are usually transcriptionally silenced but produce highly repetitive lncRNAs in response to cell-cycle progression and cellular stresses.[73] Because of the inherent challenges of sequencing tandem repeats, satellite DNA, like many disease-associated repeat expansions, was not fully sequenced until recently with long-read sequencing technology.[74] AT-rich satellite RNAs vary in length and composition and fall into four major categories: αSat, which can be transcribed from the centromeres of every chromosome, and HSat1/2/3, which come from the pericentromeric sequences of select chromosomes.[73] αSat is an essential structural component of the centromere[75] where a subset of components have been shown to phase separate *in vitro* and in cells.[76] HSat1 is poorly understood, although recent findings suggest that these transcripts localize to the nucleolar periphery.[73] Nascent HSat2 RNAs nucleate CAST (cancer-associated satellite transcript) bodies and CAP (cancer-associated polycomb) bodies which sequester MeCP2, CTCF, polycomb repressive complex 1 (PRC1), all proteins associated with chromatin regulation, and epigenetic control.[73,77,78] Similarly, nascent HSat3 RNAs nucleate condensates called nuclear stress bodies (nSBs) upon induction by various cellular stresses including thermal, osmotic, UV irradiation, and chemical stress.[73] These stress-induced condensates recruit various RNA-binding proteins including splicing regulators SRSF9 and TDP-43 and are thought to promote unconventional intron retention in select pre-mRNAs.[79] Interestingly, the consensus repeat sequence for HSat3 is similar to the noncoding repeat expansion that causes spinocerebellar ataxia 31 (SCA31) which also accumulates in the nucleus, forms aberrant condensates, and sequesters similar RNA-binding proteins.[80]

MECHANISMS OF RNA DYSREGULATION CAUSING CONDENSATE PATHOLOGY

Diverse aberrations in RNA are seen in diseases, including overexpression, mislocalization, inappropriate post-translational

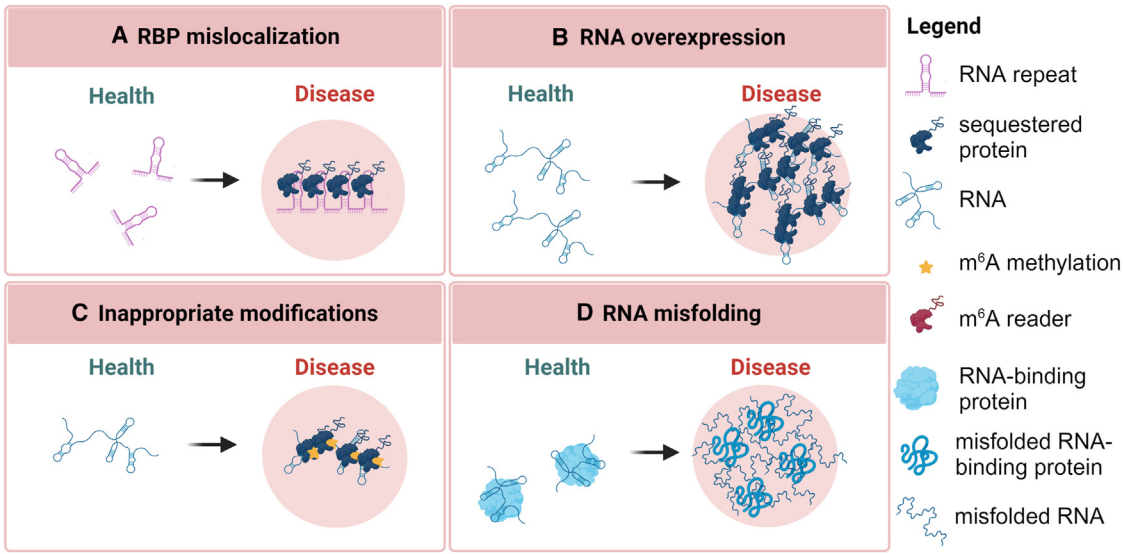

Figure 3. Mechanisms of RNA dysregulation causing condensate pathology

(A) Repeat expansions increase the propensity of RNAs to condense and for those condensates to mature into aggregates. This can be driven by structural features adopted by the repeats and sequester RNA-binding proteins (RBPs).

(B) Aberrant overexpression of RNA such as hSAT2 satellite repeats drives RNA condensate formation in cancer cells.

(C) Epitranscriptomic modifications, such as m^{6}A, can drive RNA co-condensation with reader proteins, such as YTHDC1.

(D) RNA-binding proteins have been proposed to function as RNA chaperones to resolve RNA condensates. Figure created with Biorender.

modification, and misfolding. These abnormalities are also known to modulate condensate structure and function. In this section, we review the evidence that RNA dysregulation causes condensate pathology (Figure 3).

RNA overexpression can drive pathological condensate formation. For example, LINE-1 (L1) retrotransposon propagation requires condensation of L1 protein ORF1p with L1 RNA which is normally attenuated via host defense mechanisms such as epigenetic silencing.[81] In cancers, however, L1 RNA is overexpressed and retrotransposition is dysregulated, leading to DNA damage and genomic instability.[82] Similarly, satellite RNAs, transcribed from the normally silenced pericentromeric regions of chromosomes, aberrantly activate in disease and promote the formation of condensates that sequester regulatory proteins such as chromatin-modifying proteins and splicing factors that lead to loss of function.[73,78,83] In addition, the GIRGL (glutamine insufficiency regulator of glutaminase lncRNA) is upregulated by metabolic stress, scaffolds a condensate that translationally represses GLS1 mRNA, and allows cancer cells to survive glutamine deprivation.[84] DilncRNA, a lncRNA induced by DNA damage, forms condensates that recruit DNA repair factors, allowing cancer cells to rapidly repair DNA damage before apoptosis is triggered.[85]

RNA-scaffolded condensates are involved in mRNA transcription with implications for driving cancer gene expression programs. A recent report describes the formation of mesh-like networks of RNA and RNA-binding proteins at sites of active transcription in the nucleus.[86] This observation is intriguing given that diverse DNA-binding transcription factors (TFs) moonlight as RNA-binding proteins in transcriptional condensates. This RNA binding enables a feedback loop in which transcripts condense with the TF responsible for their production.[87] This allows for self-regulated transcriptional output, governed by local RNA levels and condensate formation.[32,86] Dysregulation of transcriptional condensates could amplify gene expression in multiple ways: (1) overexpression of transcription factors could overcome inherent feedback mechanisms governed by protein-RNA ratios, and (2) regulation of the material state of transcriptional condensates could allow for more prolonged bursts of expression. Notably, MYC has been identified as both a transcription factor capable of RNA-binding and a protein capable of acting as a surfactant to alter the material state of condensates.[32,88]

Repeat expansion disorders are typified by genomically unstable repeat sequences that when transcribed, can trigger pathological nuclear condensates that sequester and mislocalize RNA-binding proteins.[89] For example, in myotonic dystrophy type 1 (DM1), a triplet CTG repeat tract in the 3′UTR of the DMPK gene can aberrantly expand from under 50 to over thousands of copies. The transcripts containing the expanded repeats scaffold aberrant condensates that sequester the splicing factor MBNL, leading to splicing dysregulation.[90] The length of the CTG repeat expansion in DM1 patients strongly correlates with the severity of symptoms and age of onset.[91] Furthermore, CTG repeats phase separate *in vitro* around a similar critical copy number threshold that correlates with DM1 patients,[7] suggesting that the repeat RNA condensate may underlie the disease mechanism. In cases where the repeat expansion is located in coding regions, repeat-containing transcripts can be further translated to give rise to toxic polypeptide tracts. In HD, CAG repeats in the first exon of the huntingtin (HTT) gene are translated into polyglutamine (polyQ) tracts that lead to aggregation of misfolded HTT protein in bodies that resemble solidified condensates.[92] Lastly, noncoding repeat expansions can also produce toxic peptide products through repeat-associated non-AUG (RAN) translation, which has been observed for

Table 1. Disease-associated condensate lncRNAs

lncRNA	Condensate	Function	Disease	Reference
NEAT1	Paraspeckles	Sequesters proteins	Cancers, neuro-degenerative disease	Fox et al.[52]
MALAT1	Nuclear speckles	Sequesters proteins	Cancers	Hou et al.[101]
NORAD	NORAD Pumilio bodies	Sequesters proteins	Cancers	Elguindy et al.[54]
GIRGL	Stress granules	Sequesters RNA	Cancers	Wang et al.[84]
EPH41L4A-AS1	Nucleoli	Sequesters protein	Cancers	Liao et al.[102]
DilncRNA	DNA repair foci	DNA repair	Cancers	Michelini et al.[85]
LINE-1 RNA	LINE-1 RNPs	Retrotransposition	Cancers	Sil et al.[81]
XIST	Barr Bodies	X chromosome inactivation	Autoimmune disorders	Dou et al.[60]
Viral RNA	Inclusion bodies, virus factories, Negri bodies	Viral replication	HIV, RSV, IAV	Risso-Ballester et al.,[103] Monette et al.,[104] Etibor et al.,[105] Nevers et al.[106]
Viral RNA	Nucleocapsid bodies	Evade host immune response	SARS-CoV-2	Iserman et al.[107]
C9orf72 intron $(G_4C_2)_n$	Repeat expansion foci	Sequesters proteins; toxic translation product	ALS/FTD	Česnik et al.[108]
DMPK 3′UTR $(CUG)_n$	Repeat expansion foci	Sequesters proteins	DM1	Taneja et al.[109]
CNBP intron $(CCUG)_n$	Repeat expansion foci	Sequesters proteins	DM2	Margolis et al.[110]
TCF4 intron $(CUG)_n$	Repeat expansion foci	Sequesters proteins	FECD	Mootha et al.[111]
FMR1 5′UTR $(CGG)_n$	Repeat expansion foci	Sequesters proteins	FXTAS	Sellier et al.[112]
ATXN10 intron $(ATTCC)_n$	Repeat expansion foci	Sequesters proteins	SCA10	Yang et al.[113]
BEAN1/TK2 intron $(TGGAA)_n$	Repeat expansion foci	Sequesters proteins	SCA31	Ishiguro et al.[100]
HSat2	CAST, CAP	Sequesters proteins	Cancers, FSHD, ICF, Herpes virus infection	Arends et al.,[114] Ninomiya et al.[73]
HSat3	Nuclear stress bodies	Sequesters proteins, intron retention	Cancers	Ninomiya et al.[73]

C9orf72 hexanucleotide expansions giving rise to dipeptide repeats that localize to inclusion bodies in C9-FTD/ALS patient brains[93] RAN translation has also been observed in other repeat expansion disorders such as FXTAS,[94] SCA8,[95] and DM1.[96]

Post-transcriptional modifications may also drive pathogenic condensate formation. eRNAs, which are enriched at enhancers and can regulate gene expression across long genomic distances[62,64,97] are regulated by m^6A methylation. Methylation promotes condensate formation and plays diverse gene regulatory roles in gene expression in cancer.[65] m^6A-mRNA also scaffolds YTHDC1-dependent nuclear bodies that protect RNAs from degradation by the PAXT-exosome complex. These nuclear bodies are upregulated in acute myeloid leukemia (AML) cells and promote high expression levels of oncogenic factors like MYC.[41]

The propensity of RNA to condense may be balanced by appropriate RNA folding, association with RBPs, and the action of ATP-dependent RNA helicase chaperones.[13,98] Tauber et al. have shown that through ATP-dependent RNA binding, the DEAD-box helicase eIF4A can both inhibit RNA phase separation *in vitro* as well as reduce stress granule formation in cells.[13] This adaptive equilibrium is also perturbed in repeat expansion disease.[99] In these diseases, naturally occurring repeats grow by thousands of copies, creating aggregation-prone RNAs that contribute to the loss-of-function of their associated mRNA transcripts or toxic gain-of-function via the recruitment of aggregation-prone RBPs.[99] For example, Ishiguro et al. have demonstrated in *Drosophila* that the RNA-binding protein TDP-43 acts as a chaperone for the UGGAA expansion found in SCA31 and suppresses aggregation of repeat RNA and associated toxicity.[100] In addition, these toxic RNAs may cause misfolding of aggregation-prone RBPs, thus further contributing to loss of function in the disease state.[100]

DISEASES WHERE RNA CONDENSATES PLAY A ROLE

In this section, we discuss how RNA-mediated condensate dysregulation contributes to disease with examples from neurology, oncology, infectious disease, and aging-related diseases (Table 1).

Mislocalization and aggregation of TDP-43, a broadly expressed RNA/DNA-binding protein that regulates RNA processing, is a pathological hallmark of ALS and frontotemporal dementia (FTD), even in patients without TDP-43 mutations.[115] Under

stress, TDP-43 is transiently recruited to cytoplasmic SGs, but persistent cytoplasmic accumulation of TDP-43 leads to irreversible, toxic aggregates which have also been observed in Alzheimer's, other dementias, chronic traumatic encephalopathy, stroke, multiple sclerosis, limbic-predominant age-related TDP-43 encephalopathy (LATE), cerebral age-related TDP-43 with sclerosis (CARTS), inclusion body myositis, and other myopathies.[115] The most common cause of familial ALS is the intronic G_4C_2 repeat expansion in the C9orf72 gene whose transcript drives the formation of aberrant nuclear condensates that sequester splicing factors and give rise to, through RAN translation, toxic dipeptide repeats that may perturb function of physiological condensates.[116] Over 50 neurological or neuromuscular diseases have been identified that are associated with repeat expansion mutations.[117] These repeats can be harbored in noncoding regions of the gene (for example in DM1, FXTAS, SCA10, and SCA31) and scaffold pathological condensates when transcribed. They may also be embedded in coding regions (as in HD and SCA2), that can be translated into toxic polypeptide tracts forming insoluble aggregates.[89] Understanding the condensate pathology in each case may point to novel therapeutic approaches. For example, disruption of the repeat RNA condensate in myotonic dystrophy is effective to restore MBNL splicing activity.[118] However, in diseases like Huntington's where mutant polyQ proteins are produced from repeat expansion translation, it may be strategic to maintain or even promote the sequestration of the repeat RNA within the condensates to inhibit nuclear export and translation into toxic protein products.

Transcription can be enhanced through the condensation of transcription factors, co-activators, RNA polymerase, and cofactors at active enhancer sites.[119] Dysregulation of these transcriptional condensates is associated with many cancers. For example, FET proteins (FUS, EWS, and TAF15) are all found in deleterious genomic rearrangements that cause sarcomas and acute leukemia.[120] The chimeric proteins that result from translocation contain the condensate-promoting IDRs of the FET proteins fused to the DNA-binding domain of transcription factors. EWS-FLI1 is a classic example of this, which drives Ewing's sarcoma[120,121] by aberrantly recruiting transcriptional coactivators to oncogenes. Similarly, the intrinsically disordered FG-repeat domain of nucleoporin Nup98 can rearrange to the DNA-binding domains of HOX genes, chromatin-modifying domains, and helicase domains of over 30 fusion partners and underlie many forms of pediatric and adult leukemias.[43,121,122]

Cancers can employ condensate mechanisms that confer resistance to radiation and chemotherapy,[123] such as upregulating stress granule assemble through activating G3BP1 translation.[121] Knock down of G3BP1 in non-small-cell lung cancer (NSCLC) enhances radiation-induced DNA damage and cell death.[123] In addition, sorafenib induces ER stress that activates phosphorylation of eIF2a through PKR-like ER kinase (PERK) and SG formation leads to drug resistance.[123] In addition, several other chemotherapeutic drugs including lapatinib, 5-fluorouracil, bortezomib, vinca alkaloids, cisplatin, and paclitaxel have been documented to facilitate SG assembly.[123]

Viruses use condensate mechanisms for essential life cycle processes including uncoating after cellular entry, replication, and evasion from host immune surveillance. Influenza A virus (IAV) packages its genomic RNA with phase-separating nucleoprotein (NP) and RNA-dependent RNA polymerase and hijacks host protein TNPO1, which attenuates FUS condensation in the cytosol, to release its genome for replication.[124] Replication machinery can also be found assembled in condensates called inclusion bodies or virus factories in viruses such as respiratory syncytial virus (RSV) and rabies (RABV) and are thought to promote enzymatic activity.[124] Additionally, replication condensates may help viruses evade host immune response by shielding their genomic material.[43] Stress granules are induced by host antiviral response through activation of protein kinase R (PKR) via dsRNA sensing to phosphorylate eIF2α. Since many antiviral proteins are recruited to SGs, they may serve as scaffolds for antiviral complex assembly. Consistent with this hypothesis, knock down of SG induction attenuates antiviral response in IAV-infected cells.[125] In addition, the SARS-CoV-2 nucleocapsid (N) protein binds G3BP1 and prevents SG formation during infection. When the interaction between N and G3BP1 is abolished, SGs are restored and sequester viral RNA, which suggests that SARS-CoV-2 employs this tactic to evade condensation and translational repression of its genomic RNA by host SGs.[125]

During normal aging, nucleolar function exhibits gradual decline. rRNA transcription diminishes and consequently, the total nucleolar volume decreases since rRNA represents the majority of the constituent bulk.[126] Aging-related diseases, neurodegenerative diseases, in particular, are often associated with nucleolar dysfunction. For example, in Alzheimer's disease (AD), a decrease in total nucleolar volume and rDNA transcription has been reported for patient postmortem brain tissue compared to age-matched healthy controls.[126] In C9orf72 ALS patient-derived cells, the dipeptide repeats that result from translation of the G_4C_2 repeat expansion accumulate in nucleoli, cause mislocalization of nucleolar proteins, and impair rRNA processing.[127] Furthermore, in HD, mutant HTT has been shown to interfere with RNA polymerase I to disrupt nucleolar function and CAG repeat expansion condensates observed to sequester nucleolin, a major nucleolar protein that is involved in pre-rRNA transcription.[127] Interestingly, rDNA methylation, which silences pre-rRNA transcription and downregulates ribosome biogenesis, has been proposed as a predictor of biological age[128] and correlates with aging and senescence in yeast.[129] Condensate material properties can change from liquid-like droplet behavior to a solid-like or gel-like state with time which is analogous to the physical aging of synthetic polymers long established in the polymer science field.[130] Mutations in condensate proteins associated with aging-related disorders can accelerate condensate hardening,[130] which has been described in detail for the ALS-associated RNA-binding protein FUS,[131] potentially linking biological aging with changes to condensate material properties.

MODALITIES TO MODULATE RNA CONDENSATES

In this section, we will discuss approaches to target RNA condensates: small molecules, nucleic acids, and protein-based therapeutics (Figure 4, top panel).

Although most small molecule therapeutics have been developed for proteins, a number of small molecules have been identified to target RNAs and alter RNA condensate function. Earlier work this century made advances with antibiotics that target

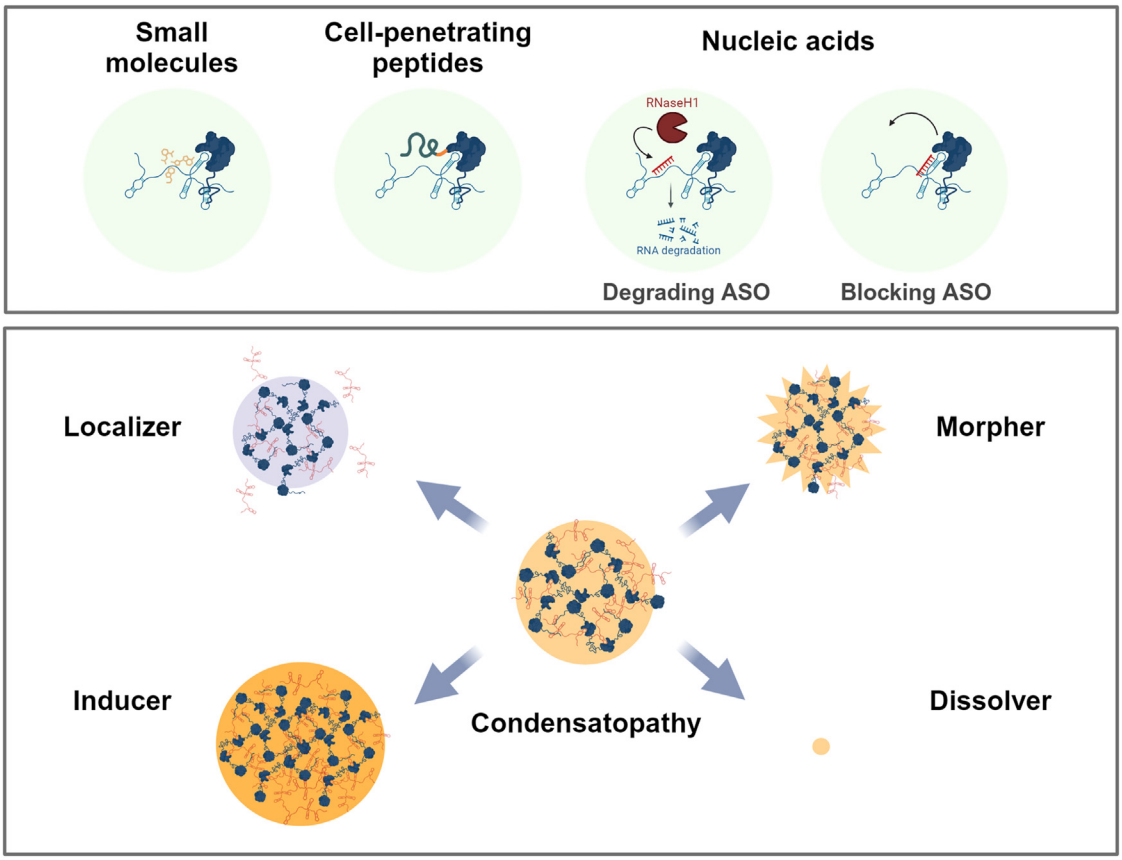

Figure 4. Modalities to modulate RNA condensates

Structure and function of RNA-containing biomolecular condensates can be modulated with small molecules, nucleic acid, and peptide modalities (top panels). These modalities can be classified based on the mechanism by which condensates are modulated (bottom panel). Localizers alter condensate composition either by departitioning proteins aberrantly sequestered or sequestering toxic factors. Morphers alter emergent properties which impacts the regulation of the condensate community. Inducers and dissolvers restore or inhibit condensate activity, respectively. Figure created with BioRender.

bacterial ribosomal RNA,[132] but more recent progress has been seen with diseases caused by repeat expansions. Several small molecules have been shown to either disrupt RNA-protein interactions or dissolve the RNA repeat condensate to release MBNL protein and restore splicing regulation in DM1[133–135] and similarly, small molecules have been developed to disrupt repeat RNA condensates in DM2,[136] FXTAS,[137] C9orf72 ALS/FTD,[138] SCA10,[113] and SCA31.[80] In addition to dissolving aberrant condensates caused by toxic repeat expansions, small molecules have also been identified that attenuate stress granule assembly and modulate TDP-43 recruitment, since persistent TDP-43 accumulation in the cytoplasm is thought to lead to disease-associated aggregation.[139]

In HD, a trinucleotide CAG repeat expansion in HTT mRNA drives the formation of aberrant nuclear condensates and results in an expanded polyglutamine tract in the HTT protein, a phase-separating protein that is aggregation prone.[140] Branaplam and PTC518 are small molecule splice modulators that promote the inclusion of a pseudoexon in HTT pre-mRNA that leads to a reduction in HTT protein,[141,142] which alleviates HD symptoms in animal models. Insufficient SMN1, an RNA-binding protein associated with the assembly of small nuclear ribonuclear particles (snRNPs) in Cajal bodies and accessory gem condensates,[143] underlies motor neurodegeneration and progressive muscle weakness in SMA.[144] Small molecules including risdiplam,[145] TEC-1,[146] and piridazines[147] have been identified that modulate splicing of its nearly identical paralog SMN2 to increase protein levels and restore SMN activity. Interestingly, both branaplam and risdiplam have an advantageous off-target effect in promoting pseudoexon inclusion in PMS1, a key protein in the DNA mismatch repair pathway that impacts HD age of onset. This leads to loss of PMS1 protein function and is suggested to reduce somatic instability of the CAG repeat expansion,[142] thus further limiting toxicity of the repeat expansion and disease progression.

A natural product cyclopamine has been shown to inhibit respiratory syncytial virus (RSV) replication *in vitro* and in mouse models[103,148] by altering condensate material properties. RNA synthesis in RSV occurs in cytoplasmic condensates called inclusion bodies (IB) which concentrate viral genomic RNA, the viral polymerase and co-factor P, and transcription factor M2-1.[149] Cyclopamine stabilizes the interactions between proteins, which prevents RNA binding, and solidifies the viral condensate thus inhibiting transcription and replication.[103,149] Similarly, nucleozin is a small molecule that inhibits Influenza A viral replication in cells and in mice by aggregating viral RNPs

and hardening inclusion body condensates.[105] In both examples, small molecules alter protein-protein interactions; however, the structure and function of the RNAs are consequently modulated because the effective drug target is the condensate and its emergent properties that affect the entire condensate community.

RNA-based therapeutics may have an advantage compared to small molecules in that they obey simple Watson-Crick base-pairing rules and rational design can be more straightforward. However, since they are highly hydrophilic, it can be difficult to effectively deliver RNAs to certain tissues and furthermore, stability may be an issue.[150–152] Several modifications have been developed to address these concerns, including modifications to the phosphodiester bond, ribose, and nucleobase which can improve both target selectivity and potency as well as resistance to cellular nucleases.[153,154] In addition, nucleic acid-based therapeutics can be conjugated to receptor-specific ligands, antibodies, or lipids to facilitate uptake and promote tissue specificity.[154,155]

siRNAs are double-stranded molecules that engage the RNAi pathway via assembly onto RNA-induced silencing complex (RISC) and targeting mRNAs for enzymatic degradation.[153] They can be designed to mimic or inhibit naturally occurring endogenous miRNAs that are encoded in the human genome but can be optimized to enhance target engagement.[156] For example, AOC 1001 (also known as del-desiran) is an siRNA-based therapeutic conjugated to an antibody that recognizes the transferrin receptor TfR1, facilitating cellular uptake.[155] AOC 1001 downregulates expression level of both normal and repeat expansion containing transcripts of the DMPK gene, thus dissolving the aberrant CUG repeat RNA condensate and restoring splicing regulation and is currently undergoing clinical trials for treatment of DM1.[157] In addition, AMT-130 delivers miRNA that targets HTT by adeno-associated virus as a form of gene therapy for treatment of HD which has shown efficacy in lowering HTT levels in animal models.[158] This intervention not only inhibits the CAG repeat RNA condensates but also limits mutant HTT protein levels.

Antisense oligonucleotides (ASOs) are single-stranded nucleic acid molecules that, like siRNAs, engage their targets via complementary base pairing. ASOs can be divided into two main classes: degrading ASOs that recruit endogenous RNase H to degrade target RNAs and blocking ASOs that can sterically hinder RNA-RNA or RNA-protein interaction. Either may be deployed to perturb condensate formation or composition. Degrading ASOs have been developed to target toxic repeat expansion containing transcripts that scaffold disease-associated condensates and, in some cases, give rise to harmful translation products. Examples include tominersen,[140] which is a non-allele specific ASO that lowers total HTT protein levels, IONIS-DMPKRx for DM1[118] and afinersen,[159] which targets the G_4C_2 repeat expansion in the C9orf72 gene in ALS. In addition, degrading ASOs have been designed to target other factors implicated in ALS such as tofersen,[160] which downregulates toxic SOD1 mutant protein, the second most common cause of familial ALS, and ION363[161] which downregulates the RNA-binding protein FUS which leads to aggressive early onset ALS when mutated. Both SOD1 and FUS are phase-separating proteins and disease-associated mutant forms can be more prone to aggregation.[162,163] In other cases where it might be important to preserve the translation product or where dissolving the RNA condensate is detrimental, blocking ASOs may be the preferred therapeutic strategy. Interestingly for DM1, it has been suggested that blocking ASOs may have enhanced efficacy in splicing rescue and fewer off-target effects compared to degrading ASOs.[164] An example of this approach is VX-670 currently in clinical trials (NCT06185764). VX-670 combines phosphorodiamidate morpholino oligomers (PMOs), which are ASOs with stabilizing modifications in the sugar-phosphate backbone, that target DM1 repeat expansions conjugated to a cyclic peptide designed to promote cellular uptake. Blocking ASOs can also be deployed to modulate splicing as seen with nusinersen[165] which, like risdiplam, increases SMN protein levels through altering SMN2 pre-mRNA splicing. By degrading the RNA that scaffolds aberrant condensates, one might expect to dissolve the condensates altogether and release the molecular community of proteins and nucleic acids whose sequestration may also contribute to pathology. Even if perturbation is limited to selectively inhibiting a specific protein/RNA interaction, as is in the case of blocking ASOs, there may still be potential to affect other members of the condensate community.

Two examples of protein-based approaches to targeting RNA condensates are small peptides that induce therapeutic condensation. Axin proteins scaffold condensates that promote degradation of β-catenin, which is dysregulated in colorectal cancer.[166] While axin readily phase separates to form these destruction depots, axin homolog conductin/axin2 is inhibited by an aggregation site within its regulator of G protein signaling (RGS) domain.[167] Bernkopf et al. identified a small peptide that masks this region in conductin and induces condensate formation, which downregulates the β-catenin signaling pathway.[167] This provides proof of concept that induction of endogenous condensates can promote inactivation of pathogenic proteins. PSD-95 is an important modulator of synaptic transmission that scaffolds key postsynaptic proteins like AMPA receptors (AMPAR) and associated regulatory proteins. PSD-95 undergoes phase separation *in vitro* in complex with its client proteins and peptide-based pharmacological inhibitors have been developed to mimic this interaction.[168] NA-1 and its bivalent derivative AVLX-144 both contain 9 amino acid residues from the C-terminus of the Glu2B NMDA receptor (NMDAR) subunit linked to an arginine-rich cell-penetrating peptide to facilitate cellular uptake.[168] Both peptides are in clinical trials as potential therapeutics for acute ischemic stroke (NCT02930018 and NCT04689035).

We propose that condensate modulators can be classified as localizers, morphers, dissolvers, and inducers based on mechanism (Figure 4, bottom panel).[139] Localizers either restore or remove components from the condensate environment. Departitioning MBNL protein from aberrant repeat expansion and restoring splicing regulation in DM1 exemplify this category of modulators.[164] Morphers alter condensate material properties and thus regulate the entire condensate community, such as the small molecules that harden viral RNA condensates.[105,149] Dissolvers disassemble or prevent the formation of aberrant, pathological condensates and may be employed as a strategy against repeat expansion disorders such as DM1[118,133–135] and HD.[140–142] Inducers, conversely, promote condensate assembly to sequester pathological factors or restore condensate

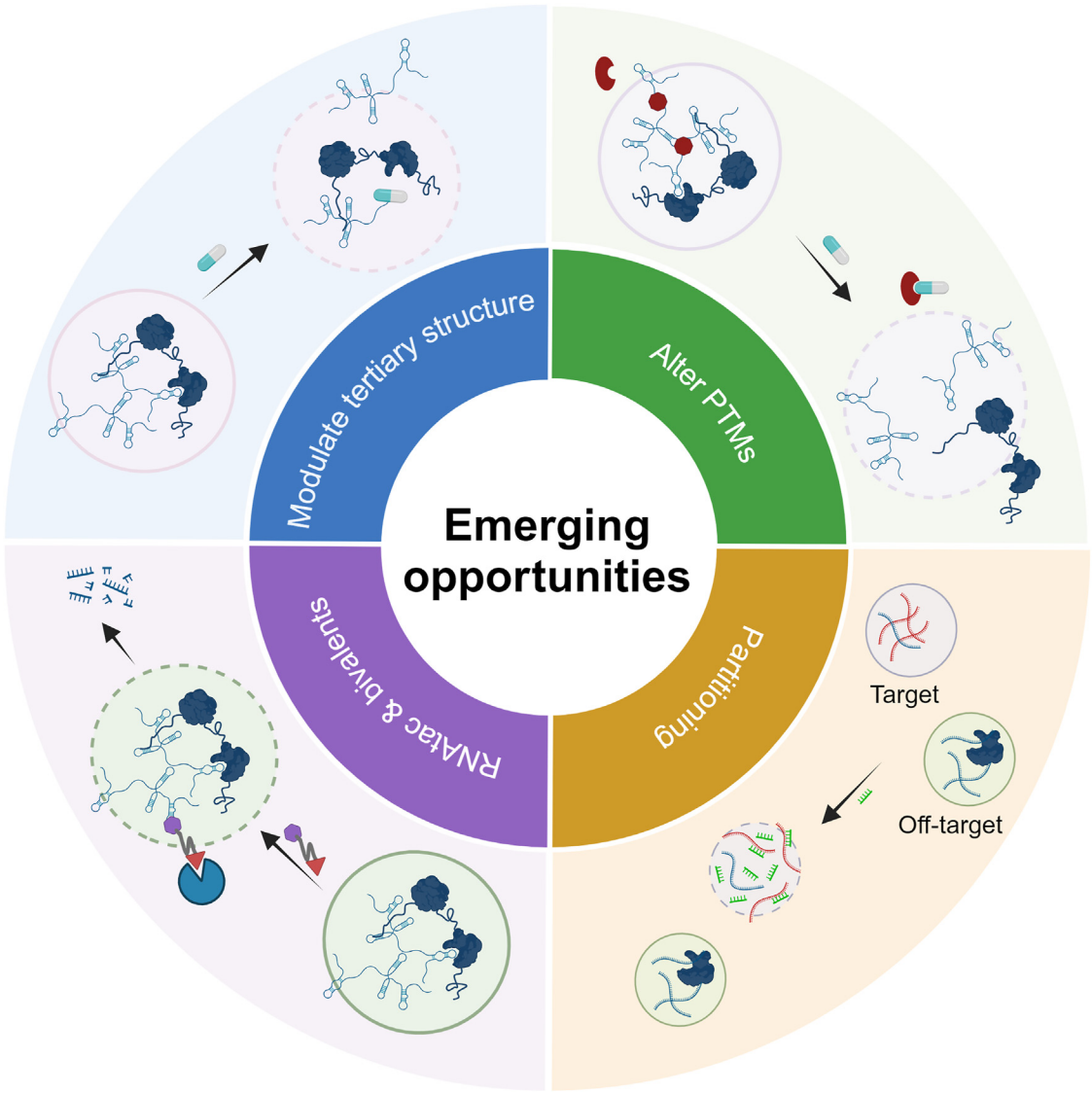

Figure 5. Emerging opportunities in drugging RNA condensates

New and developing approaches involve (i) targeting the condensate indirectly, by modulating the function of upstream post-transcriptional modification (PTM) enzymes (green), (ii) targeting the condensate directly by modulating the tertiary structure of RNA (blue), or with bivalent molecules, such as RNAtacs for selective degradation of scaffolding RNAs (purple), and (iii) improving efficacy and minimizing off-target effects by optimizing partitioning of drugs, e.g., ASOs, into the target condensate (orange). Figure created with BioRender.

function. Examples include enhancing axin function to promote formation of the β-catenin destruction complex[167] and restoring Cajal body function through upregulation of SMN activity.[145,165]

EMERGING OPPORTUNITIES IN DRUGGING RNA CONDENSATES

RNA is a key regulator of condensation, its dysregulation can manifest pathogenesis through its impact on condensates, and various therapeutics may function via their effects on condensates. In this section, we speculate on how this knowledge could lead to innovative therapeutic strategies (Figure 5).

RNA tertiary structures might be modulated by small molecules to alter condensate structure and function. The ability to form tertiary structures is a key feature of RNAs, and this property may contribute to condensate formation. These structures may regulate biological pathways by their impact on condensate formation, and when dysfunctional, may cause disease.[5] The discovery that small molecules can bind to tertiary RNA structures suggests that these molecules might also modulate RNA folding.[169] This modulation might change the condensing properties of the bound RNA, providing a novel mechanism of action for small molecules against the condensate-forming tertiary structure of RNA.

Post-transcriptional modifications of RNA drive pathogenesis, modulate condensate structure and function, and present potential druggable properties of condensate-forming RNA. Post-transcriptional modification is a well-established pathogenic

event, modulating RNA interaction partners and condensation. As discussed earlier, dysregulation of m^6A addition to RNA, which is essential and the most abundant RNA modification, plays a key role in cancer through several mechanisms.[170] m^6A has been shown to cause phase separation with YTHCD1 to facilitate transcriptional condensate formation and drive oncogene expression.[65] Modifications such as m^6A are catalyzed by effector proteins that could be targeted by small molecules, potentially leading to novel enzymatic inhibitors of RNA condensation,[171] although m^6A methylation inhibitors like STM2457 may also impact global mRNA decay rates.[172] The interaction between modified RNA and cognate proteins may also serve as an important druggable interface to prevent condensate formation, when the modified RNA-protein interaction contributes to pathogenic condensate formation.

The development of artificial intelligence models to understand the structure and interaction space of RNAs may benefit from understanding that RNAs exist in condensates. Machine learning models have revolutionized our understanding of protein structure and function by allowing for the determination of protein folding based solely on protein sequence.[173] These models now also enable the modeling of protein-protein interfaces and protein-ligand interactions. Similar models are being developed for RNA, facilitating the prediction of RNA structure and interactions, and potentially guiding the AI-aided design of small molecule drugs targeting RNAs.[174] Recognizing that RNAs reside within condensates may improve these models. Condensates have distinct physicochemical properties that can influence RNA folding, as in the nucleolus, or unfolding, as in condensates formed by the disordered domains of RNA helicases.[36,175] Understanding the environment in which these models predict folding could enhance their predictive accuracy. Some models seek to predict interactors and interaction interfaces.[176] Condensates concentrate and exclude specific biomolecules, forming a local milieu of potential RNA interaction partners. This understanding could restrict the interaction space for a particular RNA, improving predictions of interactors, interaction interfaces, and potentially the design of small molecules to disrupt these interactions.

Conventional drug discovery approaches do not typically consider the intracellular distribution of drugs. However, recent findings show that the partitioning of drugs into specific cellular compartments can significantly influence drug efficacy and impact resistance. For example, several clinically approved cancer therapies concentrate in cellular condensates containing their drug targets.[177,178] The common cancer drug, oxaliplatin, works by targeting the nucleolar scaffold protein, FBL, disrupting the layered architecture of the nucleolus, and indirectly blocking rRNA synthesis.[177] *In vitro*, overexpression of FBL overcomes the toxic effects of oxaliplatin.[177] The breast cancer drug, tamoxifen, partitions into transcriptional condensates containing both its target, estrogen receptor (ER) and the mediator subunit, Med1.[178] Tamoxifen binding to ER blocks associating with Med1, and cells compensate and develop tamoxifen resistance by over-expressing Med1.[178] This property of small molecules can be defined both *in vitro* and *in vivo* by leveraging small molecule probes and can be predicted using machine learning approaches.[179] It has been postulated that this partitioning property can be utilized to develop small molecule therapeutics with optimal subcellular distribution. In the case that small molecules interact with RNA present in specific condensates, an understanding of the properties of small molecules that can be optimized to encourage or discourage partitioning can improve effective local RNA-targeted drug concentrations, target engagement, and efficacy. Furthermore, a recent study discovered selective partitioning of certain metabolites within condensates, in particular phospholipids and amphipathic molecules.[180] Metabolomics in condensate biology may uncover mechanisms for enzymatic reactions in phase-separated compartments.

Similar to the small molecule partitioning case discussed previously, we envision a future for RNA therapeutics where we understand the modifications on ASOs that can alter their subcellular distribution in condensates. Supporting this possibility, it has been shown that modifications on ASO can alter subcellular distribution, and even concentrate ASOs in cellular bodies that are now understood to be condensates. It has been shown that the ASO backbone and hydrophobic 2′ sugar modifications govern interactions with FUS, causing ASOs to be retained in specific condensates.[181] Condensate-directing RNA modifications, once understood and employed, might be utilized to direct an oligonucleotide therapeutic to concentrate within the condensate containing its target, potentially increasing its efficacy. Furthermore, preventing concentration in condensate compartments by modifying partitioning might prevent toxic consequences associated with the mislocalization of an RNA therapeutic.

RNAs that scaffold condensates are unlikely to be catalytic, and so binding RNA folds with small molecules may not elicit a desired therapeutic response. PROTACs, bifunctional molecules that recruit E3 ubiquitin ligases to target and degrade proteins via the ubiquitin-proteosome pathway, may provide a solution to this problem.[182] One approach to translate binding to function is ‘‘RIBOTACs’’ or ‘‘RNATACs’’ the RNA analog of PROTACs whereby inert RNA-targeting small molecules are functionalized with a molecule that recruits and activates RNA-degrading enzymes.[183,184] This modality may be applicable in the context of aberrant RNA condensates where degradation of scaffolding RNA below a threshold concentration could elicit highly cooperative effects on condensate formation. RIBOTACs may be specifically attractive for RNA repeat-expansion diseases where repeat length may be inversely correlated to the amount of drug required to degrade the pathologic RNA due to the increased number of binding sites and processive degradation of RNA by recruited enzymes. Such an approach could functionalize a growing number of small molecules identified to engage disease-associated RNAs, such as those associated with ALS and DM1[185,186]

Understanding that drug targets such as RNA reside within condensates opens the possibility of generating more effective bivalent drugs. ZXH-3-2, a PROTAC molecule developed to selectively degrade phase-separating transcriptional regulator BRD4, reversibly perturbed transcriptional condensates within hours of treatment, demonstrating proof of concept that PROTACs can modulate condensates.[182] Phase separation enhanced PROTAC (PSETAC) advances this concept by introducing a phase-separating intrinsically disordered region (IDR) as the linker between the ligand for target recognition and a

proteosome degradation tag. This is then packaged in lipid nanoparticles as mRNA for delivery.[187] Condensing the target protein and proteosome in this manner enhanced degradation, and choice of IDR influenced subcellular compartmentalization of degradation activity.[187] This raises the possibility that condensate formation may be catalyzed or engineered to enhance intracellular processes, including those that govern the activity of drugs targeting proteins and RNA in condensates.

DECLARATION OF INTERESTS

T.W.H. and B.P. are employees and shareholders of Dewpoint Therapeutics. A.B. and I.K. are employees and shareholders of Dewpoint Therapeutics and hold multiple patents related to the work discussed in this review. R.A.Y. is a founder and shareholder of Syros Pharmaceuticals, Camp4 Therapeutics, Omega Therapeutics, Dewpoint Therapeutics, and Paratus Sciences, has consulting or advisory roles at Precede Biosciences and Novo Nordisk, and holds multiple patents related to the work discussed in this review.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this work, the authors occasionally used ChatGPT 4o in order to improve the readability and language of the manuscript. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

REFERENCES

1. Brangwynne, C.P., Eckmann, C.R., Courson, D.S., Rybarska, A., Hoege, C., Gharakhani, J., Jülicher, F., and Hyman, A.A. (2009). Germline P Granules Are Liquid Droplets That Localize by Controlled Dissolution/Condensation. Science *324*, 1729–1732. https://doi.org/10.1126/science.1172046.

2. Wang, J., Choi, J.-M., Holehouse, A.S., Lee, H.O., Zhang, X., Jahnel, M., Maharana, S., Lemaitre, R., Pozniakovsky, A., Drechsel, D., et al. (2018). A Molecular Grammar Governing the Driving Forces for Phase Separation of Prion-like RNA Binding Proteins. Cell *174*, 688–699.e16. https://doi.org/10.1016/j.cell.2018.06.006.

3. Ginell, G.M., Emenecker, R.J., Lotthammer, J.M., Usher, E.T., and Holehouse, A.S. (2024). Direct prediction of intermolecular interactions driven by disordered regions. Preprint at bioRxiv. https://doi.org/10.1101/2024.06.03.597104.

4. Dai, X., Zhang, S., and Zaleta-Rivera, K. (2020). RNA: interactions drive functionalities. Mol. Biol. Rep. *47*, 1413–1434. https://doi.org/10.1007/s11033-019-05230-7.

5. Roden, C., and Gladfelter, A.S. (2021). RNA contributions to the form and function of biomolecular condensates. Nat. Rev. Mol. Cell Biol. *22*, 183–195. https://doi.org/10.1038/s41580-020-0264-6.

6. Tauber, D., Tauber, G., and Parker, R. (2020). Mechanisms and Regulation of RNA Condensation in RNP Granule Formation. Trends Biochem. Sci. *45*, 764–778. https://doi.org/10.1016/j.tibs.2020.05.002.

7. Jain, A., and Vale, R.D. (2017). RNA phase transitions in repeat expansion disorders. Nature *546*, 243–247. https://doi.org/10.1038/nature22386.

8. Wadsworth, G.M., Zahurancik, W.J., Zeng, X., Pullara, P., Lai, L.B., Sidharthan, V., Pappu, R.V., Gopalan, V., and Banerjee, P.R. (2023). RNAs undergo phase transitions with lower critical solution temperatures. Nat. Chem. *15*, 1693–1704. https://doi.org/10.1038/s41557-023-01353-4.

9. Poudyal, R.R., Sieg, J.P., Portz, B., Keating, C.D., and Bevilacqua, P.C. (2021). RNA sequence and structure control assembly and function of RNA condensates. RNA *27*, 1589–1601. https://doi.org/10.1261/rna.078875.121.

10. Zhang, Y., Yang, M., Duncan, S., Yang, X., Abdelhamid, M.A.S., Huang, L., Zhang, H., Benfey, P.N., Waller, Z.A.E., and Ding, Y. (2019). G-quadruplex structures trigger RNA phase separation. Nucleic Acids Res. *47*, 11746–11754. https://doi.org/10.1093/nar/gkz978.

11. Aumiller, W.M., and Keating, C.D. (2016). Phosphorylation-mediated RNA/peptide complex coacervation as a model for intracellular liquid organelles. Nat. Chem. *8*, 129–137. https://doi.org/10.1038/nchem.2414.

12. Ries, R.J., Zaccara, S., Klein, P., Olarerin-George, A., Namkoong, S., Pickering, B.F., Patil, D.P., Kwak, H., Lee, J.H., and Jaffrey, S.R. (2019). m6A enhances the phase separation potential of mRNA. Nature *571*, 424–428. https://doi.org/10.1038/s41586-019-1374-1.

13. Tauber, D., Tauber, G., Khong, A., Van Treeck, B., Pelletier, J., and Parker, R. (2020). Modulation of RNA Condensation by the DEAD-Box Protein eIF4A. Cell *180*, 411–426.e16. https://doi.org/10.1016/j.cell.2019.12.031.

14. Linsenmeier, M., Hondele, M., Grigolato, F., Secchi, E., Weis, K., and Arosio, P. (2022). Dynamic arrest and aging of biomolecular condensates are modulated by low-complexity domains, RNA and biochemical activity. Nat. Commun. *13*, 3030. https://doi.org/10.1038/s41467-022-30521-2.

15. Aumiller, W.M., Pir Cakmak, F., Davis, B.W., and Keating, C.D. (2016). RNA-Based Coacervates as a Model for Membraneless Organelles: Formation, Properties, and Interfacial Liposome Assembly. Langmuir *32*, 10042–10053. https://doi.org/10.1021/acs.langmuir.6b02499.

16. Treeck, B.V., Protter, D.S.W., Matheny, T., Khong, A., Link, C.D., and Parker, R. (2018). RNA self-assembly contributes to stress granule formation and defining the stress granule transcriptome. Proc. Natl. Acad. Sci. USA *115*, 2734–2739. https://doi.org/10.1073/pnas.1800038115.

17. Boeynaems, S., Bogaert, E., Kovacs, D., Konijnenberg, A., Timmerman, E., Volkov, A., Guharoy, M., De Decker, M., Jaspers, T., Ryan, V.H., et al. (2017). Phase Separation of C9orf72 Dipeptide Repeats Perturbs Stress Granule Dynamics. Mol. Cell *65*, 1044–1055.e5. https://doi.org/10.1016/j.molcel.2017.02.013.

18. Boeynaems, S., Holehouse, A.S., Weinhardt, V., Kovacs, D., Van Lindt, J., Larabell, C., Van Den Bosch, L., Das, R., Tompa, P.S., Pappu, R.V., and Gitler, A.D. (2019). Spontaneous driving forces give rise to protein–RNA condensates with coexisting phases and complex material properties. Proc. Natl. Acad. Sci. USA *116*, 7889–7898. https://doi.org/10.1073/pnas.1821038116.

19. Zu, T., Liu, Y., Bañez-Coronel, M., Reid, T., Pletnikova, O., Lewis, J., Miller, T.M., Harms, M.B., Falchook, A.E., Subramony, S.H., et al. (2013). RAN proteins and RNA foci from antisense transcripts in C9ORF72 ALS and frontotemporal dementia. Proc. Natl. Acad. Sci. USA *110*, E4968–E4977. https://doi.org/10.1073/pnas.1315438110.

20. Alshareedah, I., Moosa, M.M., Raju, M., Potoyan, D.A., and Banerjee, P.R. (2020). Phase transition of RNA–protein complexes into ordered hollow condensates. Proc. Natl. Acad. Sci. USA *117*, 15650–15658. https://doi.org/10.1073/pnas.1922365117.

21. Alshareedah, I., Moosa, M.M., Pham, M., Potoyan, D.A., and Banerjee, P.R. (2021). Programmable viscoelasticity in protein-RNA condensates with disordered sticker-spacer polypeptides. Nat. Commun. *12*, 6620. https://doi.org/10.1038/s41467-021-26733-7.

22. Poudyal, R.R., Pir Cakmak, F., Keating, C.D., and Bevilacqua, P.C. (2018). Physical Principles and Extant Biology Reveal Roles for RNA-Containing Membraneless Compartments in Origins of Life Chemistry. Biochemistry *57*, 2509–2519. https://doi.org/10.1021/acs.biochem.8b00081.

23. Shevtsov, S.P., and Dundr, M. (2011). Nucleation of nuclear bodies by RNA. Nat. Cell Biol. *13*, 167–173. https://doi.org/10.1038/ncb2157.

24. Burke, K.A., Janke, A.M., Rhine, C.L., and Fawzi, N.L. (2015). Residue-by-Residue View of In Vitro FUS Granules that Bind the C-Terminal Domain of RNA Polymerase II. Mol. Cell *60*, 231–241. https://doi.org/10.1016/j.molcel.2015.09.006.

25. Zhang, H., Elbaum-Garfinkle, S., Langdon, E.M., Taylor, N., Occhipinti, P., Bridges, A.A., Brangwynne, C.P., and Gladfelter, A.S. (2015). RNA Controls PolyQ Protein Phase Transitions. Mol. Cell *60*, 220–230. https://doi.org/10.1016/j.molcel.2015.09.017.

26. Portz, B., and Shorter, J. (2021). Biochemical Timekeeping Via Reentrant Phase Transitions. J. Mol. Biol. *433*, 166794. https://doi.org/10.1016/j.jmb.2020.166794.

27. Maharana, S., Wang, J., Papadopoulos, D.K., Richter, D., Pozniakovsky, A., Poser, I., Bickle, M., Rizk, S., Guillén-Boixet, J., Franzmann, T.M., et al. (2018). RNA buffers the phase separation behavior of prion-like RNA binding proteins. Science *360*, 918–921. https://doi.org/10.1126/science.aar7366.

28. Fare, C.M., Villani, A., Drake, L.E., and Shorter, J. (2021). Higher-order organization of biomolecular condensates. Open Biol. *11*, 210137. https://doi.org/10.1098/rsob.210137.

29. Wheeler, J.R., Matheny, T., Jain, S., Abrisch, R., and Parker, R. (2016). Distinct stages in stress granule assembly and disassembly. Elife *5*, e18413. https://doi.org/10.7554/elife.18413.

30. Yu, H., Lu, S., Gasior, K., Singh, D., Vazquez-Sanchez, S., Tapia, O., Toprani, D., Beccari, M.S., Yates, J.R., 3rd, Da Cruz, S., et al. (2021). HSP70 chaperones RNA-free TDP-43 into anisotropic intranuclear liquid spherical shells. Science *371*, eabb4309. https://doi.org/10.1126/science.abb4309.

31. Folkmann, A.W., Putnam, A., Lee, C.F., and Seydoux, G. (2021). Regulation of biomolecular condensates by interfacial protein clusters. Science *373*, 1218–1224. https://doi.org/10.1126/science.abg7071.

32. Henninger, J.E., Oksuz, O., Shrinivas, K., Sagi, I., LeRoy, G., Zheng, M.M., Andrews, J.O., Zamudio, A.V., Lazaris, C., Hannett, N.M., et al. (2021). RNA-Mediated Feedback Control of Transcriptional Condensates. Cell *184*, 207–225.e24. https://doi.org/10.1016/j.cell.2020.11.030.

33. Garcia-Jove Navarro, M., Kashida, S., Chouaib, R., Souquere, S., Pierron, G., Weil, D., and Gueroui, Z. (2019). RNA is a critical element for the sizing and the composition of phase-separated RNA–protein condensates. Nat. Commun. *10*, 3230. https://doi.org/10.1038/s41467-019-11241-6.

34. Erkamp, N.A., Farag, M., Qian, D., Sneideris, T., Welsh, T.J., Ausserwöger, H., Weitz, D.A., Pappu, R.V., and Knowles, T.P.J. (2023). Adsorption of RNA to interfaces of biomolecular condensates enables wetting transitions. Preprint at bioRxiv. https://doi.org/10.1101/2023.01.12.523837.

35. Farag, M., Cohen, S.R., Borcherds, W.M., Bremer, A., Mittag, T., and Pappu, R.V. (2022). Condensates formed by prion-like low-complexity domains have small-world network structures and interfaces defined by expanded conformations. Nat. Commun. *13*, 7722. https://doi.org/10.1038/s41467-022-35370-7.

36. Riback, J.A., Zhu, L., Ferrolino, M.C., Tolbert, M., Mitrea, D.M., Sanders, D.W., Wei, M.-T., Kriwacki, R.W., and Brangwynne, C.P. (2020). Composition-dependent thermodynamics of intracellular phase separation. Nature *581*, 209–214. https://doi.org/10.1038/s41586-020-2256-2.

37. Riback, J.A., Eeftens, J.M., Lee, D.S.W., Quinodoz, S.A., Donlic, A., Orlovsky, N., Wiesner, L., Beckers, L., Becker, L.A., Strom, A.R., et al. (2023). Viscoelasticity and advective flow of RNA underlies nucleolar form and function. Mol. Cell *83*, 3095–3107.e9. https://doi.org/10.1016/j.molcel.2023.08.006.

38. Ries, R.J., Pickering, B.F., Poh, H.X., Namkoong, S., and Jaffrey, S.R. (2023). m6A governs length-dependent enrichment of mRNAs in stress granules. Nat. Struct. Mol. Biol. *30*, 1525–1535. https://doi.org/10.1038/s41594-023-01089-2.

39. Fu, Y., and Zhuang, X. (2020). m6A-binding YTHDF proteins promote stress granule formation. Nat. Chem. Biol. *16*, 955–963. https://doi.org/10.1038/s41589-020-0524-y.

40. Gao, Y., Pei, G., Li, D., Li, R., Shao, Y., Zhang, Q.C., and Li, P. (2019). Multivalent m6A motifs promote phase separation of YTHDF proteins. Cell Res. *29*, 767–769. https://doi.org/10.1038/s41422-019-0210-3.

41. Cheng, Y., Xie, W., Pickering, B.F., Chu, K.L., Savino, A.M., Yang, X., Luo, H., Nguyen, D.T., Mo, S., Barin, E., et al. (2021). N6-Methyladenosine on mRNA facilitates a phase-separated nuclear body that suppresses myeloid leukemic differentiation. Cancer Cell *39*, 958–972.e8. https://doi.org/10.1016/j.ccell.2021.04.017.

42. Mann, J.R., Gleixner, A.M., Mauna, J.C., Gomes, E., DeChellis-Marks, M.R., Needham, P.G., Copley, K.E., Hurtle, B., Portz, B., Pyles, N.J., et al. (2019). RNA Binding Antagonizes Neurotoxic Phase Transitions of TDP-43. Neuron *102*, 321–338.e8. https://doi.org/10.1016/j.neuron.2019.01.048.

43. Mitrea, D.M., Mittasch, M., Gomes, B.F., Klein, I.A., and Murcko, M.A. (2022). Modulating biomolecular condensates: a novel approach to drug discovery. Nat. Rev. Drug Discov. *21*, 841–862. https://doi.org/10.1038/s41573-022-00505-4.

44. Tian, S., Curnutte, H.A., and Trcek, T. (2020). RNA Granules: A View from the RNA Perspective. Molecules *25*, 3130. https://doi.org/10.3390/molecules25143130.

45. Mattick, J.S., Amaral, P.P., Carninci, P., Carpenter, S., Chang, H.Y., Chen, L.-L., Chen, R., Dean, C., Dinger, M.E., Fitzgerald, K.A., et al. (2023). Long non-coding RNAs: definitions, functions, challenges and recommendations. Nat. Rev. Mol. Cell Biol. *24*, 430–447. https://doi.org/10.1038/s41580-022-00566-8.

46. Quinodoz, S.A., Jachowicz, J.W., Bhat, P., Ollikainen, N., Banerjee, A.K., Goronzy, I.N., Blanco, M.R., Chovanec, P., Chow, A., Markaki, Y., et al. (2021). RNA promotes the formation of spatial compartments in the nucleus. Cell *184*, 5775–5790.e30. https://doi.org/10.1016/j.cell.2021.10.014.

47. Johnston, R., Aldrich, A., and Lyons, S.M. (2024). Roles of ribosomal RNA in health and disease. Front. RNA Res. *1*, 1331185. https://doi.org/10.3389/frnar.2023.1331185.

48. Lafontaine, D.L.J., Riback, J.A., Bascetin, R., and Brangwynne, C.P. (2021). The nucleolus as a multiphase liquid condensate. Nat. Rev. Mol. Cell Biol. *22*, 165–182. https://doi.org/10.1038/s41580-020-0272-6.

49. Brockdorff, N. (2018). Local Tandem Repeat Expansion in Xist RNA as a Model for the Functionalisation of ncRNA. Noncoding. RNA *4*, 28. https://doi.org/10.3390/ncrna4040028.

50. Clemson, C.M., Hutchinson, J.N., Sara, S.A., Ensminger, A.W., Fox, A.H., Chess, A., and Lawrence, J.B. (2009). An Architectural Role for a Nuclear Noncoding RNA: NEAT1 RNA Is Essential for the Structure of Paraspeckles. Mol. Cell *33*, 717–726. https://doi.org/10.1016/j.molcel.2009.01.026.

51. Lee, S., Kopp, F., Chang, T.-C., Sataluri, A., Chen, B., Sivakumar, S., Yu, H., Xie, Y., and Mendell, J.T. (2016). Noncoding RNA NORAD Regulates Genomic Stability by Sequestering PUMILIO Proteins. Cell *164*, 69–80. https://doi.org/10.1016/j.cell.2015.12.017.

52. Fox, A.H., Nakagawa, S., Hirose, T., and Bond, C.S. (2018). Paraspeckles: Where Long Noncoding RNA Meets Phase Separation. Trends Biochem. Sci. *43*, 124–135. https://doi.org/10.1016/j.tibs.2017.12.001.

53. Hirose, T., Virnicchi, G., Tanigawa, A., Naganuma, T., Li, R., Kimura, H., Yokoi, T., Nakagawa, S., Bénard, M., Fox, A.H., and Pierron, G. (2014). NEAT1 long noncoding RNA regulates transcription via protein sequestration within subnuclear bodies. Mol. Biol. Cell *25*, 169–183. https://doi.org/10.1091/mbc.e13-09-0558.

54. Elguindy, M.M., and Mendell, J.T. (2021). NORAD-induced Pumilio phase separation is required for genome stability. Nature *595*, 303–308. https://doi.org/10.1038/s41586-021-03633-w.

55. Zhang, J., Li, X.-Y., Hu, P., and Ding, Y.-S. (2018). lncRNA NORAD Contributes to Colorectal Cancer Progression by Inhibition of miR-202-5p. Oncol. Res. *26*, 1411–1418. https://doi.org/10.3727/096504018x15190844870055.

56. Alves-Vale, C., Capela, A.M., Tavares-Marcos, C., Domingues-Silva, B., Pereira, B., Santos, F., Gomes, C.P., Espadas, G., Vitorino, R., Sabidó, E., et al. (2023). Expression of NORAD correlates with breast cancer aggressiveness and protects breast cancer cells from chemotherapy. Mol. Ther. Nucleic Acids *33*, 910–924. https://doi.org/10.1016/j.omtn.2023.08.019.

57. Wang, W., Min, L., Qiu, X., Wu, X., Liu, C., Ma, J., Zhang, D., and Zhu, L. (2021). Biological Function of Long Non-coding RNA (LncRNA) Xist. Front. Cell Dev. Biol. *9*, 645647. https://doi.org/10.3389/fcell.2021.645647.

58. Pandya-Jones, A., Markaki, Y., Serizay, J., Chitiashvili, T., Mancia Leon, W.R., Damianov, A., Chronis, C., Papp, B., Chen, C.-K., McKee, R., et al.

(2020). A protein assembly mediates Xist localization and gene silencing. Nature *587*, 145–151. https://doi.org/10.1038/s41586-020-2703-0.

59. Yang, J., Qi, M., Fei, X., Wang, X., and Wang, K. (2021). Long non-coding RNA XIST: a novel oncogene in multiple cancers. Mol. Med. *27*, 159. https://doi.org/10.1186/s10020-021-00421-0.

60. Dou, D.R., Zhao, Y., Belk, J.A., Zhao, Y., Casey, K.M., Chen, D.C., Li, R., Yu, B., Srinivasan, S., Abe, B.T., et al. (2024). Xist ribonucleoproteins promote female sex-biased autoimmunity. Cell *187*, 733–749.e16. https://doi.org/10.1016/j.cell.2023.12.037.

61. Sharp, P.A., Chakraborty, A.K., Henninger, J.E., and Young, R.A. (2022). RNA in formation and regulation of transcriptional condensates. RNA *28*, 52–57. https://doi.org/10.1261/rna.078997.121.

62. Harrison, L.J., and Bose, D. (2022). Enhancer RNAs step forward: new insights into enhancer function. Development *149*, dev200398. https://doi.org/10.1242/dev.200398.

63. Cho, W.-K., Spille, J.-H., Hecht, M., Lee, C., Li, C., Grube, V., and Cisse, I.I. (2018). Mediator and RNA polymerase II clusters associate in transcription-dependent condensates. Science *361*, 412–415. https://doi.org/10.1126/science.aar4199.

64. Sabari, B.R., Dall'Agnese, A., Boija, A., Klein, I.A., Coffey, E.L., Shrinivas, K., Abraham, B.J., Hannett, N.M., Zamudio, A.V., Manteiga, J.C., et al. (2018). Coactivator condensation at super-enhancers links phase separation and gene control. Science *361*, eaar3958. https://doi.org/10.1126/science.aar3958.

65. Lee, J.-H., Wang, R., Xiong, F., Krakowiak, J., Liao, Z., Nguyen, P.T., Moroz-Omori, E.V., Shao, J., Zhu, X., Bolt, M.J., et al. (2021). Enhancer RNA m6A methylation facilitates transcriptional condensate formation and gene activation. Mol. Cell *81*, 3368–3385.e9. https://doi.org/10.1016/j.molcel.2021.07.024.

66. Decker, C.J., Burke, J.M., Mulvaney, P.K., and Parker, R. (2022). RNA is required for the integrity of multiple nuclear and cytoplasmic membraneless RNP granules. EMBO J. *41*, e110137. https://doi.org/10.15252/embj.2021110137.

67. Hur, W., Kemp, J.P., Tarzia, M., Deneke, V.E., Marzluff, W.F., Duronio, R.J., and Di Talia, S. (2020). CDK-Regulated Phase Separation Seeded by Histone Genes Ensures Precise Growth and Function of Histone Locus Bodies. Dev. Cell *54*, 379–394.e6. https://doi.org/10.1016/j.devcel.2020.06.003.

68. Geisler, M.S., Kemp, J.P., and Duronio, R.J. (2023). Histone locus bodies: a paradigm for how nuclear biomolecular condensates control cell cycle regulated gene expression. Nucleus *14*, 2293604. https://doi.org/10.1080/19491034.2023.2293604.

69. Ohn, T., Kedersha, N., Hickman, T., Tisdale, S., and Anderson, P. (2008). A functional RNAi screen links O-GlcNAc modification of ribosomal proteins to stress granule and processing body assembly. Nat. Cell Biol. *10*, 1224–1231. https://doi.org/10.1038/ncb1783.

70. Bounedjah, O., Desforges, B., Wu, T.-D., Pioche-Durieu, C., Marco, S., Hamon, L., Curmi, P.A., Guerquin-Kern, J.-L., Piétrement, O., and Pastré, D. (2014). Free mRNA in excess upon polysome dissociation is a scaffold for protein multimerization to form stress granules. Nucleic Acids Res. *42*, 8678–8691. https://doi.org/10.1093/nar/gku582.

71. Sun, L., Zuo, Z., Qiu, X., Wang, G., Li, Q., Qiu, J., and Peng, Q. (2023). Recent advances in the interplay between stress granules and m6A RNA modification. Curr. Opin. Solid State Mater. Sci. *27*, 101119. https://doi.org/10.1016/j.cossms.2023.101119.

72. Altemose, N. (2022). A classical revival: Human satellite DNAs enter the genomics era. Semin. Cell Dev. Biol. *128*, 2–14. https://doi.org/10.1016/j.semcdb.2022.04.012.

73. Ninomiya, K., Yamazaki, T., and Hirose, T. (2023). Satellite RNAs: emerging players in subnuclear architecture and gene regulation. EMBO J. *42*, e114331. https://doi.org/10.15252/embj.2023114331.

74. Altemose, N., Logsdon, G.A., Bzikadze, A.V., Sidhwani, P., Langley, S.A., Caldas, G.V., Hoyt, S.J., Uralsky, L., Ryabov, F.D., Shew, C.J., et al. (2022). Complete genomic and epigenetic maps of human centromeres. Science *376*, eabl4178. https://doi.org/10.1126/science.abl4178.

75. Corless, S., Höcker, S., and Erhardt, S. (2020). Centromeric RNA and Its Function at and Beyond Centromeric Chromatin. J. Mol. Biol. *432*, 4257–4269. https://doi.org/10.1016/j.jmb.2020.03.027.

76. Trivedi, P., Palomba, F., Niedzialkowska, E., Digman, M.A., Gratton, E., and Stukenberg, P.T. (2019). The inner centromere is a biomolecular condensate scaffolded by the chromosomal passenger complex. Nat. Cell Biol. *21*, 1127–1137. https://doi.org/10.1038/s41556-019-0376-4.

77. Landers, C.C., Rabeler, C.A., Ferrari, E.K., D'Alessandro, L.R., Kang, D.D., Malisa, J., Bashir, S.M., and Carone, D.M. (2021). Ectopic expression of pericentric HSATII RNA results in nuclear RNA accumulation, MeCP2 recruitment, and cell division defects. Chromosoma *130*, 75–90. https://doi.org/10.1007/s00412-021-00753-0.

78. Hall, L.L., Byron, M., Carone, D.M., Whitfield, T.W., Pouliot, G.P., Fischer, A., Jones, P., and Lawrence, J.B. (2017). Demethylated HSATII DNA and HSATII RNA Foci Sequester PRC1 and MeCP2 into Cancer-Specific Nuclear Bodies. Cell Rep. *18*, 2943–2956. https://doi.org/10.1016/j.celrep.2017.02.072.

79. Ninomiya, K., Adachi, S., Natsume, T., Iwakiri, J., Terai, G., Asai, K., and Hirose, T. (2020). LncRNA-dependent nuclear stress bodies promote intron retention through SR protein phosphorylation. EMBO J. *39*, e102729. https://doi.org/10.15252/embj.2019102729.

80. Shibata, T., Nagano, K., Ueyama, M., Ninomiya, K., Hirose, T., Nagai, Y., Ishikawa, K., Kawai, G., and Nakatani, K. (2021). Small molecule targeting r(UGGAA)n disrupts RNA foci and alleviates disease phenotype in Drosophila model. Nat. Commun. *12*, 236. https://doi.org/10.1038/s41467-020-20487-4.

81. Sil, S., Keegan, S., Ettefa, F., Denes, L.T., Boeke, J.D., and Holt, L.J. (2023). Condensation of LINE-1 is critical for retrotransposition. Elife *12*, e82991. https://doi.org/10.7554/elife.82991.

82. Mendez-Dorantes, C., and Burns, K.H. (2023). LINE-1 retrotransposition and its deregulation in cancers: implications for therapeutic opportunities. Genes Dev. *37*, 948–967. https://doi.org/10.1101/gad.351051.123.

83. Nogalski, M.T., Solovyov, A., Kulkarni, A.S., Desai, N., Oberstein, A., Levine, A.J., Ting, D.T., Shenk, T., and Greenbaum, B.D. (2019). A tumor-specific endogenous repetitive element is induced by herpesviruses. Nat. Commun. *10*, 90. https://doi.org/10.1038/s41467-018-07944-x.

84. Wang, R., Cao, L., Thorne, R.F., Zhang, X.D., Li, J., Shao, F., Zhang, L., and Wu, M. (2021). LncRNA GIRGL drives CAPRIN1-mediated phase separation to suppress glutaminase-1 translation under glutamine deprivation. Sci. Adv. *7*, eabe5708. https://doi.org/10.1126/sciadv.abe5708.

85. Michelini, F., Pitchiaya, S., Vitelli, V., Sharma, S., Gioia, U., Pessina, F., Cabrini, M., Wang, Y., Capozzo, I., Iannelli, F., et al. (2017). Damage-induced lncRNAs control the DNA damage response through interaction with DDRNAs at individual double-strand breaks. Nat. Cell Biol. *19*, 1400–1411. https://doi.org/10.1038/ncb3643.

86. Marenda, M., Michieletto, D., Czapiewski, R., Stocks, J., Winterbourne, S.M., Miles, J., Fleming, O.C., Lazarova, E., Chiang, M., Grimes, G.R., et al. (2024). Nuclear RNA forms an interconnected network of transcription-dependent and tunable microgels. Preprint at bioRxiv. https://doi.org/10.1101/2024.06.16.599208.

87. Oksuz, O., Henninger, J.E., Warneford-Thomson, R., Zheng, M.M., Erb, H., Vancura, A., Overholt, K.J., Hawken, S.W., Banani, S.F., Lauman, R., et al. (2023). Transcription factors interact with RNA to regulate genes. Mol. Cell *83*, 2449–2463.e13. https://doi.org/10.1016/j.molcel.2023.06.012.

88. Wang, Z., Yang, C., Guan, D., Li, J., and Zhang, H. (2023). Cellular proteins act as surfactants to control the interfacial behavior and function of biological condensates. Dev. Cell *58*, 919–932.e5. https://doi.org/10.1016/j.devcel.2023.04.004.

89. Fujino, Y., and Nagai, Y. (2022). The molecular pathogenesis of repeat expansion diseases. Biochem. Soc. Trans. *50*, 119–134. https://doi.org/10.1042/bst20200143.

90. Lee, J.E., and Cooper, T.A. (2009). Pathogenic mechanisms of myotonic dystrophy. Biochem. Soc. Trans. *37*, 1281–1286. https://doi.org/10.1042/bst0371281.

91. Yum, K., Wang, E.T., and Kalsotra, A. (2017). Myotonic dystrophy: disease repeat range, penetrance, age of onset, and relationship between repeat size and phenotypes. Curr. Opin. Genet. Dev. *44*, 30–37. https://doi.org/10.1016/j.gde.2017.01.007.

92. Bates, G. (2003). Huntingtin aggregation and toxicity in Huntington's disease. Lancet *361*, 1642–1644. https://doi.org/10.1016/s0140-6736(03)13304-1.

93. Mori, K., Weng, S.-M., Arzberger, T., May, S., Rentzsch, K., Kremmer, E., Schmid, B., Kretzschmar, H.A., Cruts, M., Van Broeckhoven, C., et al. (2013). The C9orf72 GGGGCC Repeat Is Translated into Aggregating Dipeptide-Repeat Proteins in FTLD/ALS. Science *339*, 1335–1338. https://doi.org/10.1126/science.1232927.

94. Todd, P.K., Oh, S.Y., Krans, A., He, F., Sellier, C., Frazer, M., Renoux, A.J., Chen, K.c., Scaglione, K.M., Basrur, V., et al. (2013). CGG Repeat-Associated Translation Mediates Neurodegeneration in Fragile X Tremor Ataxia Syndrome. Neuron *78*, 440–455. https://doi.org/10.1016/j.neuron.2013.03.026.

95. Ayhan, F., Perez, B.A., Shorrock, H.K., Zu, T., Banez-Coronel, M., Reid, T., Furuya, H., Clark, H.B., Troncoso, J.C., Ross, C.A., et al. (2018). SCA8 RAN polySer protein preferentially accumulates in white matter regions and is regulated by eIF3F. EMBO J. *37*, e99023. https://doi.org/10.15252/embj.201899023.

96. Zu, T., Gibbens, B., Doty, N.S., Gomes-Pereira, M., Huguet, A., Stone, M.D., Margolis, J., Peterson, M., Markowski, T.W., Ingram, M.A.C., et al. (2011). Non-ATG–initiated translation directed by microsatellite expansions. Proc. Natl. Acad. Sci. USA *108*, 260–265. https://doi.org/10.1073/pnas.1013343108.

97. Hnisz, D., Shrinivas, K., Young, R.A., Chakraborty, A.K., and Sharp, P.A. (2017). A Phase Separation Model for Transcriptional Control. Cell *169*, 13–23. https://doi.org/10.1016/j.cell.2017.02.007.

98. Linder, P., and Jankowsky, E. (2011). From unwinding to clamping — the DEAD box RNA helicase family. Nat. Rev. Mol. Cell Biol. *12*, 505–516. https://doi.org/10.1038/nrm3154.

99. Malik, I., Kelley, C.P., Wang, E.T., and Todd, P.K. (2021). Molecular mechanisms underlying nucleotide repeat expansion disorders. Nat. Rev. Mol. Cell Biol. *22*, 589–607. https://doi.org/10.1038/s41580-021-00382-6.

100. Ishiguro, T., Sato, N., Ueyama, M., Fujikake, N., Sellier, C., Kanegami, A., Tokuda, E., Zamiri, B., Gall-Duncan, T., Mirceta, M., et al. (2017). Regulatory Role of RNA Chaperone TDP-43 for RNA Misfolding and Repeat-Associated Translation in SCA31. Neuron *94*, 108–124.e7. https://doi.org/10.1016/j.neuron.2017.02.046.

101. Hou, J., Zhang, G., Wang, X., Wang, Y., and Wang, K. (2023). Functions and mechanisms of lncRNA MALAT1 in cancer chemotherapy resistance. Biomark. Res. *11*, 23. https://doi.org/10.1186/s40364-023-00467-8.

102. Liao, M., Liao, W., Xu, N., Li, B., Liu, F., Zhang, S., Wang, Y., Wang, S., Zhu, Y., Chen, D., et al. (2019). LncRNA EPB41L4A-AS1 regulates glycolysis and glutaminolysis by mediating nucleolar translocation of HDAC2. EBioMedicine *41*, 200–213. https://doi.org/10.1016/j.ebiom.2019.01.035.

103. Risso-Ballester, J., Galloux, M., Cao, J., Le Goffic, R., Hontonnou, F., Jobart-Malfait, A., Desquesnes, A., Sake, S.M., Haid, S., Du, M., et al. (2021). A condensate-hardening drug blocks RSV replication in vivo. Nature *595*, 596–599. https://doi.org/10.1038/s41586-021-03703-z.

104. Monette, A., Niu, M., Nijhoff Asser, M., Gorelick, R.J., and Mouland, A.J. (2022). Scaffolding viral protein NC nucleates phase separation of the HIV-1 biomolecular condensate. Cell Rep. *40*, 111251. https://doi.org/10.1016/j.celrep.2022.111251.

105. Etibor, T.A., Vale-Costa, S., Sridharan, S., Brás, D., Becher, I., Mello, V.H., Ferreira, F., Alenquer, M., Savitski, M.M., and Amorim, M.-J. (2023). Defining basic rules for hardening influenza A virus liquid condensates. Elife *12*, e85182. https://doi.org/10.7554/elife.85182.

106. Nevers, Q., Albertini, A.A., Lagaudrière-Gesbert, C., and Gaudin, Y. (2020). Negri bodies and other virus membrane-less replication compartments. Biochim. Biophys. Acta. Mol. Cell Res. *1867*, 118831. https://doi.org/10.1016/j.bbamcr.2020.118831.

107. Iserman, C., Roden, C.A., Boerneke, M.A., Sealfon, R.S.G., McLaughlin, G.A., Jungreis, I., Fritch, E.J., Hou, Y.J., Ekena, J., Weidmann, C.A., et al. (2020). Genomic RNA Elements Drive Phase Separation of the SARS-CoV-2 Nucleocapsid. Mol. Cell *80*, 1078–1091.e6. https://doi.org/10.1016/j.molcel.2020.11.041.

108. Bajc Česnik, A., Darovic, S., Prpar Mihevc, S., Štalekar, M., Malnar, M., Motaln, H., Lee, Y.-B., Mazej, J., Pohleven, J., Grosch, M., et al. (2019). Nuclear RNA foci from C9ORF72 expansion mutation form paraspeckle-like bodies. J. Cell Sci. *132*, jcs224303. https://doi.org/10.1242/jcs.224303.

109. Taneja, K.L., McCurrach, M., Schalling, M., Housman, D., and Singer, R.H. (1995). Foci of trinucleotide repeat transcripts in nuclei of myotonic dystrophy cells and tissues. J. Cell Biol. *128*, 995–1002. https://doi.org/10.1083/jcb.128.6.995.

110. Margolis, J.M., Schoser, B.G., Moseley, M.L., Day, J.W., and Ranum, L.P.W. (2006). DM2 intronic expansions: evidence for CCUG accumulation without flanking sequence or effects on ZNF9 mRNA processing or protein expression. Hum. Mol. Genet. *15*, 1808–1815. https://doi.org/10.1093/hmg/ddl103.

111. Mootha, V.V., Hussain, I., Cunnusamy, K., Graham, E., Gong, X., Neelam, S., Xing, C., Kittler, R., and Petroll, W.M. (2015). TCF4 Triplet Repeat Expansion and Nuclear RNA Foci in Fuchs' Endothelial Corneal Dystrophy. Invest. Ophthalmol. Vis. Sci. *56*, 2003–2011. https://doi.org/10.1167/iovs.14-16222.

112. Sellier, C., Rau, F., Liu, Y., Tassone, F., Hukema, R.K., Gattoni, R., Schneider, A., Richard, S., Willemsen, R., Elliott, D.J., et al. (2010). Sam68 sequestration and partial loss of function are associated with splicing alterations in FXTAS patients. Embo J. *29*, 1248–1261. https://doi.org/10.1038/emboj.2010.21.

113. Yang, W.-Y., Gao, R., Southern, M., Sarkar, P.S., and Disney, M.D. (2016). Design of a bioactive small molecule that targets r(AUUCU) repeats in spinocerebellar ataxia 10. Nat. Commun. *7*, 11647. https://doi.org/10.1038/ncomms11647.

114. Arends, T., Tsuchida, H., Adeyemi, R.O., and Tapscott, S.J. (2024). DUX4-induced HSATII transcription causes KDM2A/B–PRC1 nuclear foci and impairs DNA damage response. J. Cell Biol. *223*, e202303141. https://doi.org/10.1083/jcb.202303141.

115. Hayes, L.R., and Kalab, P. (2022). Emerging Therapies and Novel Targets for TDP-43 Proteinopathy in ALS/FTD. Neurotherapeutics *19*, 1061–1084. https://doi.org/10.1007/s13311-022-01260-5.

116. Solomon, D.A., Smikle, R., Reid, M.J., and Mizielinska, S. (2021). Altered Phase Separation and Cellular Impact in C9orf72-Linked ALS/FTD. Front. Cell. Neurosci. *15*, 664151. https://doi.org/10.3389/fncel.2021.664151.

117. Figueiredo, A.S., Loureiro, J.R., Macedo-Ribeiro, S., and Silveira, I. (2023). Advances in Nucleotide Repeat Expansion Diseases: Transcription Gets in Phase. Cells *12*, 826. https://doi.org/10.3390/cells12060826.

118. De Serres-Bérard, T., Ait Benichou, S., Jauvin, D., Boutjdir, M., Puymirat, J., and Chahine, M. (2022). Recent Progress and Challenges in the Development of Antisense Therapies for Myotonic Dystrophy Type 1. Int. J. Mol. Sci. *23*, 13359. https://doi.org/10.3390/ijms232113359.

119. Boija, A., Klein, I.A., and Young, R.A. (2021). Biomolecular condensates and cancer. Cancer Cell *39*, 174–192. https://doi.org/10.1016/j.ccell.2020.12.003.

120. Kovar, H. (2011). Dr. Jekyll and Mr. Hyde: The Two Faces of the FUS/EWS/TAF15 Protein Family. Sarcoma *2011*, 837474. https://doi.org/10.1155/2011/837474.

121. Cai, D., Liu, Z., and Lippincott-Schwartz, J. (2021). Biomolecular Condensates and Their Links to Cancer Progression. Trends Biochem. Sci. *46*, 535–549. https://doi.org/10.1016/j.tibs.2021.01.002.

122. Michmerhuizen, N.L., Klco, J.M., and Mullighan, C.G. (2020). Mechanistic insights and potential therapeutic approaches for NUP98-rearranged hematologic malignancies. Blood *136*, 2275–2289. https://doi.org/10.1182/blood.2020007093.

123. Zhou, H., Luo, J., Mou, K., Peng, L., Li, X., Lei, Y., Wang, J., Lin, S., Luo, Y., and Xiang, L. (2023). Stress granules: functions and mechanisms in

cancer. Cell Biosci. *13*, 86. https://doi.org/10.1186/s13578-023-01030-6.

124. Etibor, T.A., Yamauchi, Y., and Amorim, M.J. (2021). Liquid Biomolecular Condensates and Viral Lifecycles: Review and Perspectives. Viruses *13*, 366. https://doi.org/10.3390/v13030366.

125. Watkins, J.M., and Burke, J.M. (2024). A closer look at mammalian antiviral condensates. Biochem. Soc. Trans. *52*, 1393–1404. https://doi.org/10.1042/bst20231296.

126. Nyhus, C., Pihl, M., Hyttel, P., and Hall, V.J. (2019). Evidence for nucleolar dysfunction in Alzheimer's disease. Rev. Neurosci. *30*, 685–700. https://doi.org/10.1515/revneuro-2018-0104.

127. Parlato, R., and Bierhoff, H. (2015). Role of nucleolar dysfunction in neurodegenerative disorders: a game of genes? AIMS Mol. Sci. *2*, 211–224. https://doi.org/10.3934/molsci.2015.3.211.

128. Wang, M., and Lemos, B. (2019). Ribosomal DNA harbors an evolutionarily conserved clock of biological aging. Genome Res. *29*, 325–333. https://doi.org/10.1101/gr.241745.118.

129. Ganley, A.R.D., and Kobayashi, T. (2014). Ribosomal DNA and cellular senescence: new evidence supporting the connection between rDNA and aging. FEMS Yeast Res. *14*, 49–59. https://doi.org/10.1111/1567-1364.12133.

130. Alberti, S., and Hyman, A.A. (2021). Biomolecular condensates at the nexus of cellular stress, protein aggregation disease and ageing. Nat. Rev. Mol. Cell Bio. *22*, 196–213. https://doi.org/10.1038/s41580-020-00326-6.

131. Patel, A., Lee, H.O., Jawerth, L., Maharana, S., Jahnel, M., Hein, M.Y., Stoynov, S., Mahamid, J., Saha, S., Franzmann, T.M., et al. (2015). A Liquid-to-Solid Phase Transition of the ALS Protein FUS Accelerated by Disease Mutation. Cell *162*, 1066–1077. https://doi.org/10.1016/j.cell.2015.07.047.

132. Moellering, R.C. (2003). Linezolid: The First Oxazolidinone Antimicrobial. Ann. Intern. Med. *138*, 135–142. https://doi.org/10.7326/0003-4819-138-2-200301210-00015.

133. Warf, M.B., Nakamori, M., Matthys, C.M., Thornton, C.A., and Berglund, J.A. (2009). Pentamidine reverses the splicing defects associated with myotonic dystrophy. Proc. Natl. Acad. Sci. USA *106*, 18551–18556. https://doi.org/10.1073/pnas.0903234106.

134. Nguyen, L., Luu, L.M., Peng, S., Serrano, J.F., Chan, H.Y.E., and Zimmerman, S.C. (2015). Rationally Designed Small Molecules That Target Both the DNA and RNA Causing Myotonic Dystrophy Type 1. J. Am. Chem. Soc. *137*, 14180–14189. https://doi.org/10.1021/jacs.5b09266.

135. Rzuczek, S.G., Colgan, L.A., Nakai, Y., Cameron, M.D., Furling, D., Yasuda, R., and Disney, M.D. (2017). Precise small-molecule recognition of a toxic CUG RNA repeat expansion. Nat. Chem. Biol. *13*, 188–193. https://doi.org/10.1038/nchembio.2251.

136. Nguyen, L., Lee, J., Wong, C.H., and Zimmerman, S.C. (2014). Small Molecules that Target the Toxic RNA in Myotonic Dystrophy Type 2. ChemMedChem *9*, 2455–2462. https://doi.org/10.1002/cmdc.201402095.

137. Yang, W.-Y., He, F., Strack, R.L., Oh, S.Y., Frazer, M., Jaffrey, S.R., Todd, P.K., and Disney, M.D. (2016). Small Molecule Recognition and Tools to Study Modulation of r(CGG)exp in Fragile X-Associated Tremor Ataxia Syndrome. ACS Chem. Biol. *11*, 2456–2465. https://doi.org/10.1021/acschembio.6b00147.

138. Simone, R., Balendra, R., Moens, T.G., Preza, E., Wilson, K.M., Heslegrave, A., Woodling, N.S., Niccoli, T., Gilbert-Jaramillo, J., Abdelkarim, S., et al. (2018). G-quadruplex-binding small molecules ameliorate C9orf72 FTD/ALS pathology in vitro and in vivo. EMBO Mol. Med. *10*, 22–31. https://doi.org/10.15252/emmm.201707850.

139. Patel, A., Mitrea, D., Namasivayam, V., Murcko, M.A., Wagner, M., and Klein, I.A. (2022). Principles and functions of condensate modifying drugs. Front. Mol. Biosci. *9*, 1007744. https://doi.org/10.3389/fmolb.2022.1007744.

140. Saade, J., and Mestre, T.A. (2024). Huntington's Disease: Latest Frontiers in Therapeutics. Curr. Neurol. Neurosci. Rep. *24*, 255–264. https://doi.org/10.1007/s11910-024-01345-y.

141. Keller, C.G., Shin, Y., Monteys, A.M., Renaud, N., Beibel, M., Teider, N., Peters, T., Faller, T., St-Cyr, S., Knehr, J., et al. (2022). An orally available, brain penetrant, small molecule lowers huntingtin levels by enhancing pseudoexon inclusion. Nat. Commun. *13*, 1150. https://doi.org/10.1038/s41467-022-28653-6.

142. McLean, Z.L., Gao, D., Correia, K., Roy, J.C.L., Shibata, S., Farnum, I.N., Valdepenas-Mellor, Z., Kovalenko, M., Rapuru, M., Morini, E., et al. (2024). Splice modulators target PMS1 to reduce somatic expansion of the Huntington's disease-associated CAG repeat. Nat. Commun. *15*, 3182. https://doi.org/10.1038/s41467-024-47485-0.

143. Courchaine, E.M., Barentine, A.E.S., Straube, K., Lee, D.-R., Bewersdorf, J., and Neugebauer, K.M. (2021). DMA-tudor interaction modules control the specificity of in vivo condensates. Cell *184*, 3612–3625.e17. https://doi.org/10.1016/j.cell.2021.05.008.

144. Antonaci, L., Pera, M.C., and Mercuri, E. (2023). New therapies for spinal muscular atrophy: where we stand and what is next. Eur. J. Pediatr. *182*, 2935–2942. https://doi.org/10.1007/s00431-023-04883-8.

145. Sturm, S., Günther, A., Jaber, B., Jordan, P., Al Kotbi, N., Parkar, N., Cleary, Y., Frances, N., Bergauer, T., Heinig, K., et al. (2019). A phase 1 healthy male volunteer single escalating dose study of the pharmacokinetics and pharmacodynamics of risdiplam (RG7916, RO7034067), a SMN2 splicing modifier. Br. J. Clin. Pharmacol. *85*, 181–193. https://doi.org/10.1111/bcp.13786.

146. Ando, S., Suzuki, S., Okubo, S., Ohuchi, K., Takahashi, K., Nakamura, S., Shimazawa, M., Fuji, K., and Hara, H. (2020). Discovery of a CNS penetrant small molecule SMN2 splicing modulator with improved tolerability for spinal muscular atrophy. Sci. Rep. *10*, 17472. https://doi.org/10.1038/s41598-020-74346-9.

147. Palacino, J., Swalley, S.E., Song, C., Cheung, A.K., Shu, L., Zhang, X., Van Hoosear, M., Shin, Y., Chin, D.N., Keller, C.G., et al. (2015). SMN2 splice modulators enhance U1–pre-mRNA association and rescue SMA mice. Nat. Chem. Biol. *11*, 511–517. https://doi.org/10.1038/nchembio.1837.

148. Bailly, B., Richard, C.-A., Sharma, G., Wang, L., Johansen, L., Cao, J., Pendharkar, V., Sharma, D.-C., Galloux, M., Wang, Y., et al. (2016). Targeting human respiratory syncytial virus transcription anti-termination factor M2-1 to inhibit in vivo viral replication. Sci. Rep. *6*, 25806. https://doi.org/10.1038/srep25806.

149. Diot, C., Richard, C.-A., Risso-Ballester, J., Martin, D., Fix, J., Eléouët, J.-F., Sizun, C., Rameix-Welti, M.-A., and Galloux, M. (2023). Hardening of Respiratory Syncytial Virus Inclusion Bodies by Cyclopamine Proceeds through Perturbation of the Interactions of the M2-1 Protein with RNA and the P Protein. Int. J. Mol. Sci. *24*, 13862. https://doi.org/10.3390/ijms241813862.

150. Damase, T.R., Sukhovershin, R., Boada, C., Taraballi, F., Pettigrew, R.I., and Cooke, J.P. (2021). The Limitless Future of RNA Therapeutics. Front. Bioeng. Biotechnol. *9*, 628137. https://doi.org/10.3389/fbioe.2021.628137.

151. Kim, Y.-K. (2022). RNA therapy: rich history, various applications and unlimited future prospects. Exp. Mol. Med. *54*, 455–465. https://doi.org/10.1038/s12276-022-00757-5.

152. Zhu, Y., Zhu, L., Wang, X., and Jin, H. (2022). RNA-based therapeutics: an overview and prospectus. Cell Death Dis. *13*, 644. https://doi.org/10.1038/s41419-022-05075-2.

153. Androsavich, J.R. (2024). Frameworks for transformational breakthroughs in RNA-based medicines. Nat. Rev. Drug Discov. *23*, 421–444. https://doi.org/10.1038/s41573-024-00943-2.

154. Fàbrega, C., Aviñó, A., Navarro, N., Jorge, A.F., Grijalvo, S., and Eritja, R. (2023). Lipid and Peptide-Oligonucleotide Conjugates for Therapeutic Purposes: From Simple Hybrids to Complex Multifunctional Assemblies. Pharmaceutics *15*, 320. https://doi.org/10.3390/pharmaceutics15020320.

155. Malecova, B., Burke, R.S., Cochran, M., Hood, M.D., Johns, R., Kovach, P.R., Doppalapudi, V.R., Erdogan, G., Arias, J.D., Darimont, B., et al. (2023). Targeted tissue delivery of RNA therapeutics using antibody–oligonucleotide conjugates (AOCs). Nucleic Acids Res. *51*, 5901–5910. https://doi.org/10.1093/nar/gkad415.

156. Matsui, M., and Corey, D.R. (2017). Non-coding RNAs as drug targets. Nat. Rev. Drug Discov. *16*, 167–179. https://doi.org/10.1038/nrd.2016.117.

157. Zhu, Y., Kwan, T., Meng, Q., Tai, L., Cho, H., Lee, M., Younis, H., Levin, A., and Flanagan, M. (2023). VP48 AOC 1001 demonstrates DMPK reduction and spliceopathy improvement in a phase 1/2 study in myotonic dystrophy type 1 (DM1) (MARINA). Neuromuscul. Disord. *33*, S70. https://doi.org/10.1016/j.nmd.2023.07.029.

158. Ma, Y.-M., and Zhao, L. (2023). Mechanism and Therapeutic Prospect of miRNAs in Neurodegenerative Diseases. Behav. Neurol. *2023*, 8537296. https://doi.org/10.1155/2023/8537296.

159. Tran, H., Moazami, M.P., Yang, H., McKenna-Yasek, D., Douthwright, C.L., Pinto, C., Metterville, J., Shin, M., Sanil, N., Dooley, C., et al. (2022). Suppression of mutant C9orf72 expression by a potent mixed backbone antisense oligonucleotide. Nat. Med. *28*, 117–124. https://doi.org/10.1038/s41591-021-01557-6.

160. Miller, T.M., Cudkowicz, M.E., Genge, A., Shaw, P.J., Sobue, G., Bucelli, R.C., Chiò, A., Van Damme, P., Ludolph, A.C., Glass, J.D., et al. (2022). Trial of Antisense Oligonucleotide Tofersen for SOD1 ALS. N. Engl. J. Med. *387*, 1099–1110. https://doi.org/10.1056/nejmoa2204705.

161. Korobeynikov, V.A., Lyashchenko, A.K., Blanco-Redondo, B., Jafar-Nejad, P., and Shneider, N.A. (2022). Antisense oligonucleotide silencing of FUS expression as a therapeutic approach in amyotrophic lateral sclerosis. Nat. Med. *28*, 104–116. https://doi.org/10.1038/s41591-021-01615-z.

162. Prudencio, M., Hart, P.J., Borchelt, D.R., and Andersen, P.M. (2009). Variation in aggregation propensities among ALS-associated variants of SOD1: Correlation to human disease. Hum. Mol. Genet. *18*, 3217–3226. https://doi.org/10.1093/hmg/ddp260.

163. Farrawell, N.E., Lambert-Smith, I.A., Warraich, S.T., Blair, I.P., Saunders, D.N., Hatters, D.M., and Yerbury, J.J. (2015). Distinct partitioning of ALS associated TDP-43, FUS and SOD1 mutants into cellular inclusions. Sci. Rep. *5*, 13416. https://doi.org/10.1038/srep13416.

164. Boujnouni, N.E., van der Bent, M.L., Willemse, M., Hoen, P.A.C. 't, Brock, R., and Wansink, D.G. (2023). Block or degrade? Balancing on- and off-target effects of antisense strategies against transcripts with expanded triplet repeats in DM1. Mol. Ther. Nucleic Acids *32*, 622–636. https://doi.org/10.1016/j.omtn.2023.04.010.

165. Qiao, Y., Chi, Y., Gu, J., and Ma, Y. (2023). Safety and Efficacy of Nusinersen and Risdiplam for Spinal Muscular Atrophy: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. Brain Sci. *13*, 1419. https://doi.org/10.3390/brainsci13101419.

166. Nong, J., Kang, K., Shi, Q., Zhu, X., Tao, Q., and Chen, Y.-G. (2021). Phase separation of Axin organizes the β-catenin destruction complex. J. Cell Biol. *220*, e202012112. https://doi.org/10.1083/jcb.202012112.

167. Bernkopf, D.B., Brückner, M., Hadjihannas, M.V., and Behrens, J. (2019). An aggregon in conductin/axin2 regulates Wnt/β-catenin signaling and holds potential for cancer therapy. Nat. Commun. *10*, 4251. https://doi.org/10.1038/s41467-019-12203-8.

168. Christensen, N.R., Pedersen, C.P., Sereikaite, V., Pedersen, J.N., Vistrup-Parry, M., Sørensen, A.T., Otzen, D., Teilum, K., Madsen, K.L., and Strømgaard, K. (2022). Bidirectional protein–protein interactions control liquid–liquid phase separation of PSD-95 and its interaction partners. iScience *25*, 103808. https://doi.org/10.1016/j.isci.2022.103808.

169. Childs-Disney, J.L., Yang, X., Gibaut, Q.M.R., Tong, Y., Batey, R.T., and Disney, M.D. (2022). Targeting RNA structures with small molecules. Nat. Rev. Drug Discov. *21*, 736–762. https://doi.org/10.1038/s41573-022-00521-4.

170. Deng, X., Qing, Y., Horne, D., Huang, H., and Chen, J. (2023). The roles and implications of RNA m6A modification in cancer. Nat. Rev. Clin. Oncol. *20*, 507–526. https://doi.org/10.1038/s41571-023-00774-x.

171. Yankova, E., Blackaby, W., Albertella, M., Rak, J., De Braekeleer, E., Tsagkogeorga, G., Pilka, E.S., Aspris, D., Leggate, D., Hendrick, A.G., et al. (2021). Small-molecule inhibition of METTL3 as a strategy against myeloid leukaemia. Nature *593*, 597–601. https://doi.org/10.1038/s41586-021-03536-w.

172. Sun, Y., Shen, W., Hu, S., Lyu, Q., Wang, Q., Wei, T., Zhu, W., and Zhang, J. (2023). METTL3 promotes chemoresistance in small cell lung cancer by inducing mitophagy. J. Exp. Clin. Cancer Res. *42*, 65. https://doi.org/10.1186/s13046-023-02638-9.

173. Jumper, J., Evans, R., Pritzel, A., Green, T., Figurnov, M., Ronneberger, O., Tunyasuvunakool, K., Bates, R., Žídek, A., Potapenko, A., et al. (2021). Highly accurate protein structure prediction with AlphaFold. Nature *596*, 583–589. https://doi.org/10.1038/s41586-021-03819-2.

174. Manigrasso, J., Marcia, M., and De Vivo, M. (2021). Computer-aided design of RNA-targeted small molecules: A growing need in drug discovery. Chem *7*, 2965–2988. https://doi.org/10.1016/j.chempr.2021.05.021.

175. 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., and Baldwin, A.J. (2015). Phase Transition of a Disordered Nuage Protein Generates Environmentally Responsive Membraneless Organelles. Mol. Cell *57*, 936–947. https://doi.org/10.1016/j.molcel.2015.01.013.

176. Zhong, L., Zhen, M., Sun, J., and Zhao, Q. (2021). Recent advances on the machine learning methods in predicting ncRNA-protein interactions. Mol. Genet. Genom. *296*, 243–258. https://doi.org/10.1007/s00438-020-01727-0.

177. Schmidt, H.B., Jaafar, Z.A., Wulff, B.E., Rodencal, J.J., Hong, K., Aziz-Zanjani, M.O., Jackson, P.K., Leonetti, M.D., Dixon, S.J., Rohatgi, R., and Brandman, O. (2022). Oxaliplatin disrupts nucleolar function through biophysical disintegration. Cell Rep. *41*, 111629. https://doi.org/10.1016/j.celrep.2022.111629.

178. Klein, I.A., Boija, A., Afeyan, L.K., Hawken, S.W., Fan, M., Dall'Agnese, A., Oksuz, O., Henninger, J.E., Shrinivas, K., Sabari, B.R., et al. (2020). Partitioning of cancer therapeutics in nuclear condensates. Science *368*, 1386–1392. https://doi.org/10.1126/science.aaz4427.

179. Kilgore, H.R., Mikhael, P.G., Overholt, K.J., Boija, A., Hannett, N.M., Van Dongen, C., Lee, T.I., Chang, Y.-T., Barzilay, R., and Young, R.A. (2024). Distinct chemical environments in biomolecular condensates. Nat. Chem. Biol. *20*, 291–301. https://doi.org/10.1038/s41589-023-01432-0.

180. Dumelie, J.G., Chen, Q., Miller, D., Attarwala, N., Gross, S.S., and Jaffrey, S.R. (2024). Biomolecular condensates create phospholipid-enriched microenvironments. Nat. Chem. Biol. *20*, 302–313. https://doi.org/10.1038/s41589-023-01474-4.

181. Bailey, J.K., Shen, W., Liang, X.H., and Crooke, S.T. (2017). Nucleic acid binding proteins affect the subcellular distribution of phosphorothioate antisense oligonucleotides. Nucleic Acids Res. *45*, 10649–10671. https://doi.org/10.1093/nar/gkx709.

182. Shi, Y., Liao, Y., Liu, Q., Ni, Z., Zhang, Z., Shi, M., Li, P., Li, H., and Rao, Y. (2023). BRD4-targeting PROTAC as a unique tool to study biomolecular condensates. Cell Discov. *9*, 47. https://doi.org/10.1038/s41421-023-00544-0.

183. Tong, Y., Lee, Y., Liu, X., Childs-Disney, J.L., Suresh, B.M., Benhamou, R.I., Yang, C., Li, W., Costales, M.G., Haniff, H.S., et al. (2023). Programming inactive RNA-binding small molecules into bioactive degraders. Nature *618*, 169–179. https://doi.org/10.1038/s41586-023-06091-8.

184. Song, Y., Cui, J., Zhu, J., Kim, B., Kuo, M.-L., and Potts, P.R. (2024). RNATACs: Multispecific small molecules targeting RNA by induced proximity. Cell Chem. Biol. *31*, 1101–1117. https://doi.org/10.1016/j.chembiol.2024.05.006.

185. Bush, J.A., Meyer, S.M., Fuerst, R., Tong, Y., Li, Y., Benhamou, R.I., Aikawa, H., Zanon, P.R.A., Gibaut, Q.M.R., Angelbello, A.J., et al. (2022). A blood–brain penetrant RNA-targeted small molecule triggers elimination of r(G 4 C 2) exp in c9ALS/FTD via the nuclear RNA exosome. Proc. Natl. Acad. Sci. USA *119*, e2210532119. https://doi.org/10.1073/pnas.2210532119.

186. Chen, J.L., Taghavi, A., Frank, A.J., Fountain, M.A., Choudhary, S., Roy, S., Childs-Disney, J.L., and Disney, M.D. (2024). NMR structures of small molecules bound to a model of an RNA CUG repeat expansion. Preprint at bioRxiv. https://doi.org/10.1101/2024.06.21.600119.

187. Yu, X., Hu, W., Dong, H., Zhao, T., Wang, X., Chen, L., Xue, S., Li, J.-P., and Luo, S.-Z. (2024). Phase Separation Enhanced PROTAC for Highly Efficient Protein Degradation. Biomacromolecules *25*, 4374–4383. https://doi.org/10.1021/acs.biomac.4c00424.