Trends in Genetics

CellPress
REVIEWS

Review

Phase Separation as a Melting Pot for DNA Repeats

Amanda C. Hall [1,3], Lauren A. Ostrowski [1,3] and Karim Mekhail [1,2,*]

Genome expression and stability are dependent on biological processes that control repetitive DNA sequences and nuclear compartmentalization. The phase separation of macromolecules has recently emerged as a major player in the control of biological pathways. Here, we summarize recent studies that collectively reveal intersections between phase separation, repetitive DNA elements, and nuclear compartments. These intersections modulate fundamental processes, including gene expression, DNA repair, and cellular lifespan, in the context of health and diseases such as cancer and neurodegeneration.

Highlights

The function and stability of rDNA repeats is intimately linked to the phase separation of various nucleolar factors.

By creating Cajal bodies or chromosome end shields, phase separation modulates the length of telomeres and their crosstalk with the DNA damage response.

Intersections between phase-separated nuclear compartments and factors encoded by mobile genetic elements impact genome stability and cellular lifespan.

Aberrant phase transition of factors linked to diseases, including cancer and neurodegeneration, intersects with repetitive DNA loci and their nuclear compartments.

Repetitive DNA Loci, Heterochromatin, and Phase Separation

Genomes contain large amounts of repetitive DNA sequences that constitute, for example, >70% of the human genome [1]. Previously referred to as 'junk' DNA, these sequences, which comprise both coding and noncoding sequences, are now clearly vital for genome function. Key examples of repetitive DNA regions include the life-sustaining **rDNA** (see Glossary) loci, chromosome-protecting **telomeres**, and the genome-reorganizing **transposable elements (TEs)**. Due to their vulnerability to DNA damage and aberrant recombination, cells have evolved mechanisms to safeguard repetitive regions, such as their epigenetic silencing within heterochromatic domains. **Heterochromatin** itself is critical for global genome architecture, expression, and stability [2].

An emerging mechanism linked to the formation of heterochromatin involves a phenomenon known as **phase separation** [3,4]. Phase separation occurs when macromolecules, such as proteins and/or nucleic acids, form a local environment that gives rise to nonmembrane-bound compartments that can be either liquid, gelatinous (dynamic), or solid (nondynamic) [5]. Phase separation of a given macromolecule can be driven by the formation of weak noncovalent bonds with binding partners and the presence of intrinsically disordered domains that induce self-interactions [6]. Although the processes regulating phase separation are still being actively characterized, post-translational modifications and the enzymes that deposit these modifications may emerge as central players [7–11]. Importantly, cells utilize phase separation to compartmentalize complex biochemical processes, resulting in membraneless organelles or compartments [12]. Recent studies revealed that heterochromatin protein 1 of *Drosophila melanogaster* (HP1a), *Homo sapiens* (HP1α), and *Schizosaccharomyces pombe* (Swi6) mediates the formation of heterochromatic domains via phase separation [3,4,13]. The phase separation of heterochromatin is compatible with its long-appreciated dynamic nature [14,15]. Additionally, the phase separation of heterochromatin allows both the enrichment and exclusion of heterochromatin-promoting and -destabilizing factors, respectively [15]. These findings appear to be broadly applicable to not only constitutive heterochromatin, but also facultative heterochromatin. Additional studies have found that factors related to facultative heterochromatin formation (e.g., polycomb proteins) also form phase-separated condensates, suggesting that phase separation is a broad regulator of heterochromatin formation [16]. The role of phase separation in heterochromatin formation has already been carefully reviewed elsewhere [17].

[1]Department of Laboratory Medicine and Pathobiology, Faculty of Medicine, University of Toronto, 661 University Avenue, Toronto, ONT, M5G 1M1, Canada
[2]Canada Research Chairs Program, Faculty of Medicine, University of Toronto, 1 King's College Circle, Toronto, ONT, M5S 1A8, Canada
[3]These authors contributed equally

*Correspondence:
karim.mekhail@utoronto.ca (K. Mekhail).

In particular, phase separation is emerging as a regulator of repetitive DNA loci and their associated nuclear compartments. Here, we review the relationship between phase separation and major repetitive DNA loci in the context of health, aging, and disease. We review how phase separation impacts rDNA, telomeres, and TEs, and explore connections between these loci, phase separation, and human disease.

Roles of Phase Separation at rDNA Repeats and the Nucleolus

rDNA loci are spatially isolated from the rest of the nuclear DNA, forming one or a small number of membraneless nucleolar compartments. In human cells, rDNA is tandemly arranged across chromosomes 13, 14, 15, 21, and 22, with ~70 units per chromosome [18]. The **rRNA** genes within rDNA are transcribed by RNA polymerase I (Pol I) into a precursor ribosomal RNA (pre-rRNA), which undergoes processing and maturation into 18S, 5.8S, and 28S rRNAs as it migrates from the center to the periphery of the **nucleolus**. Mature rRNA subunits are then assembled into the 40S and 60S ribosome subunits. The stages of rRNA biogenesis occur at different sites within the nucleolus, which exhibits a tripartite architecture comprising fibrillar centers (FCs) that are surrounded by dense fibrillar components (DFCs), which are in turn enveloped by a granular component (GC) [19]. The transcription of rRNA is thought to occur at the FC–DFC interface, while early rRNA processing takes place within the DFC. Finally, the GC is the site of late rRNA processing and maturation. This internal organization into the FC–DFC–GC is known as the nucleolar microstructure, while the nucleolar macrostructure refers to the separation of the entire nucleolus from the surrounding nucleus. Recently, phase separation has emerged as an important regulator of nucleolar microstructure and macrostructure.

Phase separation has been shown to regulate three key aspects of the nucleolar macrostructure: the sequestration of rDNA from bulk chromatin, nucleolar formation, and nucleolar fusion (Figure 1). Modeling studies suggest that, in the budding yeast *Saccharomyces cerevisiae*, rDNA is phase separated from bulk chromatin through the formation of dynamic chromosomal crosslinks between different rDNA units (Figure 1Ai) [20]. The rDNA repeats within the nucleolus are also tethered to the nuclear envelope via chromosomal complexes and inner nuclear membrane proteins [21]. Therefore, the combination of rDNA phase separation and perinuclear tethering likely explains the crescent shape of the budding yeast nucleolus, which may result from wetting against the nuclear envelope [20,21]. Disrupting this spatial organization of yeast rDNA or the nucleolus causes rDNA repeats to undergo untimely exposure to nucleoplasmic DNA repair and recombination factors and to engage in aberrant recombination events that shorten cellular lifespan [21–30]. Typically, yeast and mammalian cells move damaged rDNA repeats to the nucleolar periphery or even to the nucleoplasm to access functional DNA repair factors that are repressed in the nucleolus but enriched in the nucleoplasm [23,31]. Studies in *D. melanogaster* embryos suggest that two independent mechanisms are responsible for the formation of the nucleolus: the active recruitment and phase separation of nucleolar factors [32]. For example, Modulo (Mod) and Nucleostemin1 (Ns1) are actively recruited proteins, whereas other nucleolar proteins are recruited to the nucleolus via thermodynamic liquid–liquid phase separation (LLPS). These proteins include RNA Pol I subunit 135 (Rpl135), pitchoune (Pit), nucleolar phosphoprotein 140 (Nopp140) and fibrillarin (Fib), all of which, with the exception of Rpl135, have **intrinsically disordered domains**. Interestingly, rRNA transcription seeds the assembly of phase-separated Fib and Rpl135, is responsible for the spatiotemporal regulation of nucleolar LLPS, and is involved in the active recruitment of nucleolar proteins (Figure 1Aii) [32]. This evidence indicates that rRNA transcription coordinates multiple mechanisms underlying nucleolar formation. Studies in model organisms demonstrated that, in addition to chromatin sequestration and nucleolar formation, phase separation has a role in nucleolar fusion, a behavior first described over a century ago [33,34]. In *Xenopus laevis* oocytes, nucleolar size and shape reflect the

Glossary

Cajal body (CB): subnuclear membraneless compartments that are the site of small nuclear RNA (snRNA) and small nucleolar RNA (snoRNA) modification and the assembly site of their respective RNPs.
Heterochromatin: epigenetic silencing mechanism where DNA is highly condensed, thereby inhibiting transcription at these regions. Heterochromatin can be either constitutive (consistently silent) or facultative (with gene expression potential).
Intrinsically disordered domain: polypeptide domain sequences that cannot undergo cooperative folding into a tertiary structure due to the lack of sufficient hydrophobic amino acids.
Nucleolus: a membraneless structure within the nucleus that forms around rDNA units and that is critical for ribosome biogenesis. The nucleolus has a tripartite architecture comprising fibrillar centers (FCs) that are surrounded by dense fibrillar components (DFCs), which are in turn enveloped by a granular component (GC).
Phase separation: occurs when macromolecules, such as proteins and/or nucleic acids, form a local environment that gives rise to nonmembrane-bound compartments that can be either liquid, gelatinous (dynamic), or solid (nondynamic).
Polyglutamine (polyQ) tract: a segment of a protein that comprises CAG/CAA trinucleotide repeats-encoded glutamines. This tract can vary in length and proteins that have expanded polyQ tracts are often linked to fatal neurodegenerative diseases, such as SCA2 and ALS.
Protein aggregation: occurs when misfolded proteins clump together and give rise to a nonmembrane-bound solid-state compartment; it is implicated in various neurodegenerative diseases.
rDNA: tandemly arranged repetitive housekeeping genes that encode ribosomal RNA.
rRNA: transcribed by RNA Pol I into precursor RNA, which undergoes processing and maturation and then assembles into ribosomes that are required for protein synthesis.
Stress granules: membraneless cytoplasmic RNP compartments that are the sites of mRNA triage under endogenous and exogenous cellular stress conditions.

existence of liquid-like droplets that comprise protein and RNA [33]. Small spherical nucleoli were shown to behave like liquid droplets and fuse to form larger spherical nucleoli (Figure 1Aiii). Additionally, dynamic chromosomal crosslinks can drive multiple rDNA loci on separate chromosomes to coalesce and form a nucleolus [20]. Taken together, these findings indicate that phase separation controls the formation of nucleoli and the positioning of rDNA inside the nucleolus as well as within the broader nucleus. This control is critical to rDNA stability and cellular lifespan.

In addition to regulating the nucleolar macrostructure, phase separation has been shown to underlie the nucleolar microstructure. Although membraneless, the nucleolus exhibits distinct separation between the FC, DFC, and GC (Figure 1). Recent research provides convincing evidence that the separation of these nucleolar subcompartments is driven largely by phase separation. A seminal study found that purified nucleolar RNA-binding proteins from *X. laevis* oocytes form liquid droplets *in vitro* [35]. The DFC component FIB1 and the GC component nucleophosmin (NPM1) were found to phase separate into droplets that recapitulated the *in vivo* layering effect exhibited by DFC and/or GC in nucleoli. Specifically, the FIB1 droplets were enveloped by the NPM1 droplets when mixed, a layering phenomenon that is caused by differences in surface tension between these droplets [i.e., the DFC (FIB1) phase exhibits higher surface tension than the GC (NPM1) phase]. The authors suggested that differences in droplet surface tensions arise from sequence-encoded features of the molecules involved. Interestingly, it was demonstrated that disordered

Telomeres: repetitive DNA sequences positioned at the ends of linear chromosomes to prevent their attrition and fusion.

Transposable elements (TEs): jumping genes that often drive genome reorganization. They can move through copy-and-paste (retrotransposons) and cut-and-paste (DNA transposons) mechanisms. They arguably comprise the greatest source of dispersed repetitive DNA sequences.

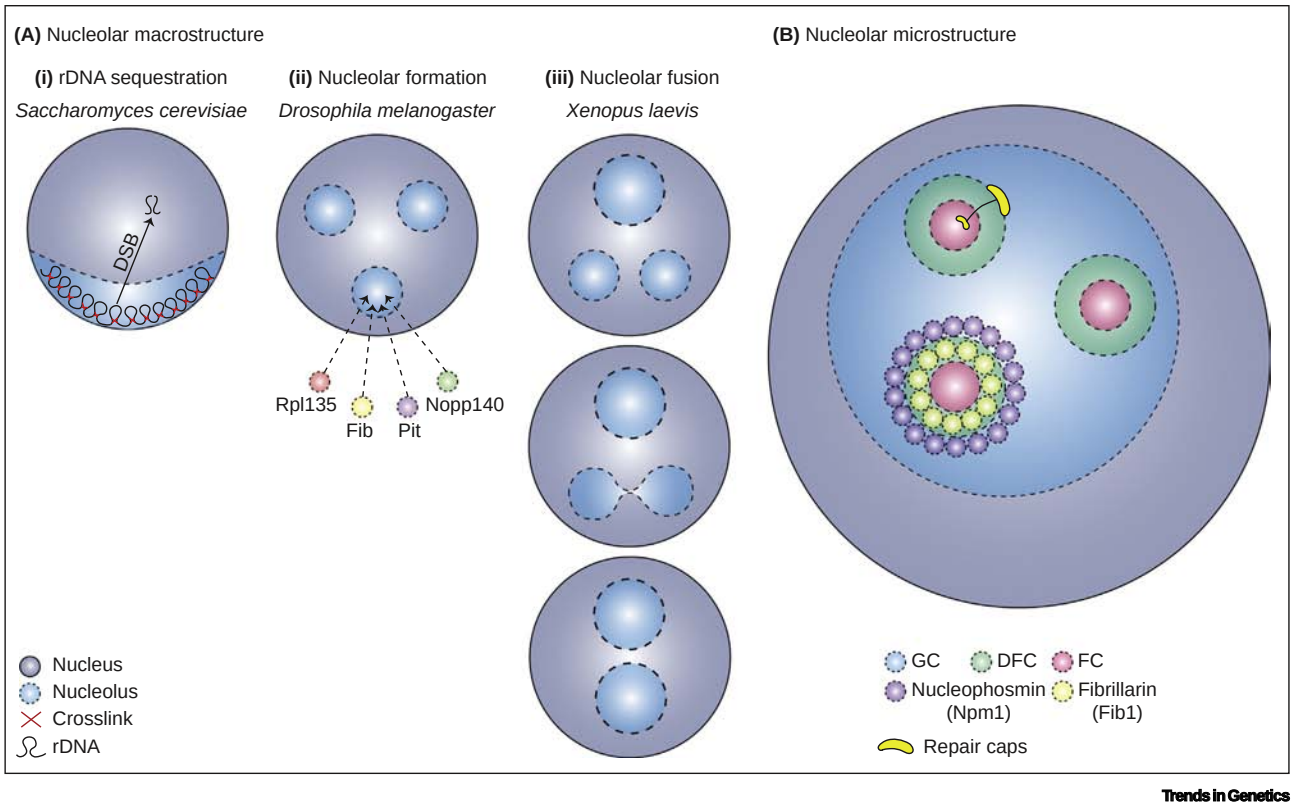

Figure 1. Liquid–Liquid Phase Separation Regulates rDNA Repeats by Establishing Nucleolar Macrostructure and Microstructure. (A) In the budding yeast *Saccharomyces cerevisiae*, the rDNA repeats are phase separated from bulk chromatin through dynamic rDNA–rDNA crosslinks and rDNA repeat tethering to the nuclear envelope (i). In higher organisms, such as *Drosophila melanogaster* and *Xenopus laevis*, nucleolar formation is mediated, in part, by the phase separation-driven recruitment of various proteins to nucleoli (ii), and phase separation can drive nucleolar fusion (iii). (B) The phase separation of key nucleolar proteins underlies the layering of nucleolar fibrillar centers (FCs), dense fibrillar components (DFCs), and granular components (GCs). This layering is critical to the modulation of rRNA synthesis and processing and to global ribosome biogenesis and protein synthesis.

protein domains are required for the formation of phase separation, while RNA recognition motifs are required for the maintenance of phase separation. Taken together, these findings suggest that the different nucleolar compartments comprise proteins that exhibit differences in miscibility, which separates the compartments and produces the trilayered droplet architecture characteristic of nucleoli (Figure 1B).

In addition, these findings suggest a role for phase separation in the regulation of nucleolar processes. Phase separation underlies rDNA sequestration as well as nucleolar formation and fusion. The tripartite structure of the nucleolus, separating the FC, DFC, and GC, also depends on the phase separation of different protein constituents. Therefore, LLPS helps establish the microstructure and macrostructure of the nucleolus and, thus, is key to the stability and function of rDNA repeats and their nucleolar home.

Phase Separation Impacts Telomeres via Heterochromatin Modulation, Nuclear Compartmentalization, and DNA Repair Control

Telomeres are repetitive DNA sequences that are positioned at the ends of linear chromosomes to prevent their attrition and fusion. Recent evidence suggests that telomeres are regulated by phase separation-dependent mechanisms. For example, HP1, which phase separates and promotes the formation of liquid-like heterochromatin domains, is required for the establishment of telomeric silent chromatin [3,4,36]. This silencing is integral to the dynamic nature of telomere elongation. Specifically, HP1-dependent chromatin silencing at long telomeres limits access to the telomere-elongating telomerase enzyme, while the loss of silencing at short telomeres makes them more accessible to telomerase [37].

In addition to heterochromatin domains, other phase-separated membraneless nuclear compartments are also important for telomere maintenance. Human telomerase comprises the protein telomerase reverse transcriptase (hTERT) and telomeric RNA component (hTR; aka hTERC). Interestingly, hTR has a **Cajal body (CB)** localization signal (CAB box) and localizes to CBs in a CAB box-dependent manner [38–40]. Further research demonstrated that over a quarter of telomeres that accumulate hTR also colocalize with CBs [41]. These findings suggest that CBs sequester hTR to regulate telomerase biogenesis and/or telomere elongation (Figure 2A). Indeed, the accumulation of hTR in CBs is important for the regulation of telomere elongation [42]. Specifically, preventing the accumulation of hTR in CBs by mutating the CAB box did not prevent the assembly of catalytically active telomerase but compromised telomerase-dependent telomere extension. The CB scaffold protein coilin also appears to be essential for telomerase association with telomeres [43]. Taken together, these findings suggest that CBs function to promote telomere elongation. Importantly, several studies have provided evidence that CBs are phase-separated nuclear organelles. First, CBs are membraneless and undergo fusion and fission events [44]. Second, CB formation is repressed by the LLPS disruptor 1,6-hexanediol [45,46]. Third, CBs have been described as ‘sponge-like structures’ that rapidly exchange their constituent macromolecules with the surrounding nucleoplasm [47]. For example, this description is supported by photobleaching microscopy revealing the rapid recovery of CB-associated fluorescence in cells expressing the GFP-tagged CB component coilin, which also harbors a low-complexity domain, self-associates, and binds to RNA molecules [48–52]. These studies reveal that phase separation underlies the formation of CBs, which have an important role in telomere elongation.

In addition to regulating telomere elongation, phase separation may also modulate the DNA damage response (DDR) at chromosome ends. In human cells, the telomeric complex shelterin helps arrange telomeric chromatin into compact globular domains that block access to DDR factors [53]. It has also been proposed that, upon removal of the shelterin complex, DDR components

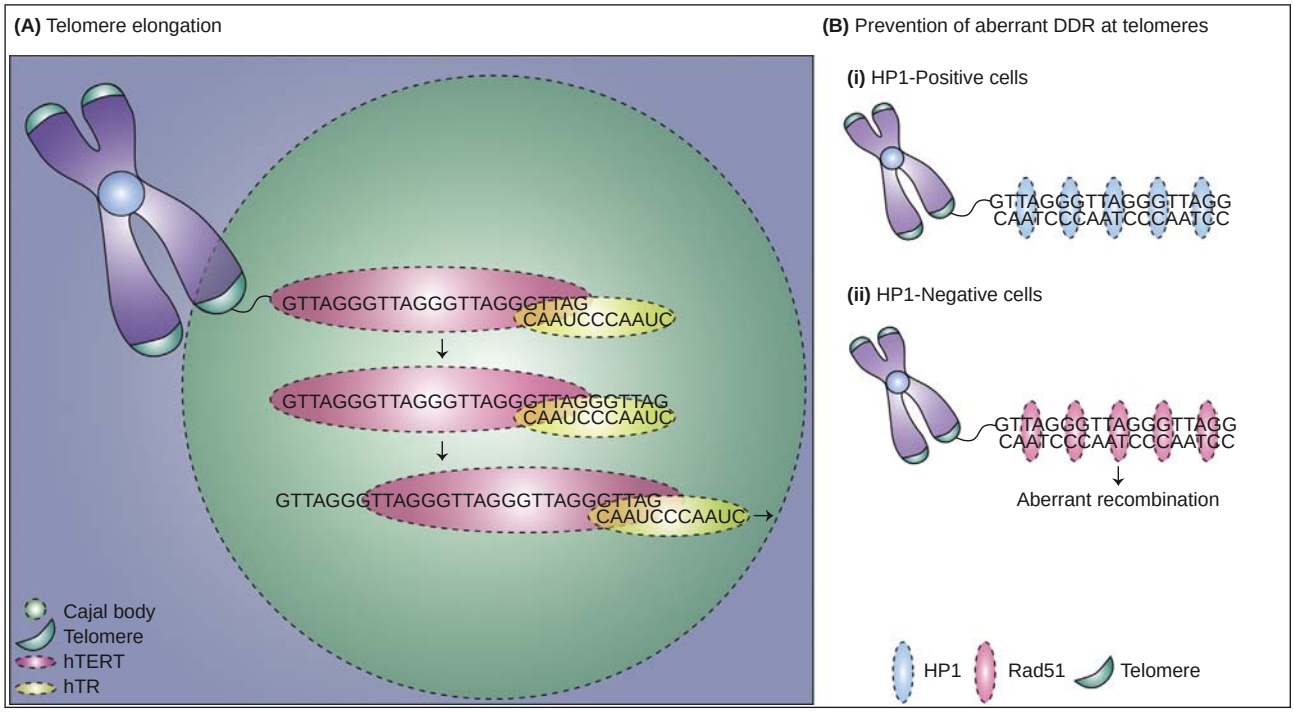

Figure 2. Roles for Phase Separation at Telomeres. (A) Telomere elongation is regulated by phase-separated Cajal bodies (CBs). Telomerase comprises reverse transcriptase (hTERT) and structural telomere RNA (hTR). Telomerase-bound telomeres are sequestered to CBs, which is important for telomere elongation. (B) Heterochromatin protein 1 (HP1)-mediated phase separation of heterochromatic telomeres (i) prevents the aberrant recruitment of the Rad51 DNA repair protein to telomeric DNA double-strand breaks (DSBs) (ii). Abbreviation: DDR, DNA damage response.

may access telomeres as a result of a change in the phase separation properties of the shelterin-dependent globular domains at chromosome ends [53]. In *S. cerevisiae*, telomeres often form foci that are spherical, dynamic, and fuse into telomeric clusters [22]. Telomeric proteins within these clusters dynamically exchange with the surrounding nucleoplasmic environment [54,55]. Thus, these clusters exhibit some liquid-like behavior. Importantly, this clustering promotes the efficient tethering of telomeres to the nuclear envelope and the local enrichment of Sir2, the sirtuin histone deacetylase that mediates telomeric chromatin silencing, which in turn limits deleterious telomere-telomere recombination events [22]. Telomeres experiencing DNA damage appear to leave the repair-repressive clusters and undergo motor protein-dependent mobilization on nuclear microtubule filaments towards the repair-conducive nuclear pore complexes [22,56,57]. Taken together, these findings indicate that phase separation may constitute an evolutionarily conserved mechanism that regulates DDR at the chromosome ends of different organisms (Figure 2B). In addition, they suggest that phase separation maintains telomeres by establishing local heterochromatin, forming CBs, and controlling the exposure of telomeres to DDR factors.

Transposable Elements, Nuclear Compartments, and Phase Separation

TEs are jumping genes that often drive genome reorganization. TEs can move through copy-and-paste (retrotransposons) and cut-and-paste (DNA transposons) mechanisms, with the former requiring an RNA intermediate [58]. Retrotransposons comprise up to 45% of the human genome, and their dysregulation is implicated in various diseases, including neurodegeneration and cancer, as well as aging [1,59,60]. Human autonomous long interspersed element 1 (LINE1) encodes two proteins, ORF1p and ORF2p, that bind LINE1 RNA, forming L1 ribonucleoproteins (RNPs),

which are required for retrotransposition [58]. Due to their ability to reorganize the genome, retrotransposons are commonly packaged into HP1-dependent heterochromatin [61]. Considering the role of HP1 phase separation in heterochromatin formation [3,4], it is possible that crosstalk exists between TEs and phase separation.

Indeed, a link has been shown between phase separation and LINE1 retrotransposition. **Stress granules (SGs)** are well established as liquid phase-separated membraneless compartments [12]. Under drug-induced stress, arsenite, and thapsigargin treatment, ORFp1 colocalizes to SGs and interacts with various SG proteins, including YB-1 and hnRNPA1, suggesting that LINE1 retrotransposition is inhibited by the sequestration of L1 RNPs to the phase-separated SGs (Figure 3A) [62–64]. Supporting evidence for this concept revealed that the retrotransposition-inhibiting SAM and HD domain-containing protein 1 (SAMHD1) inhibits LINE1 by inducing SG assembly and the subsequent sequestration of L1 RNPs [65]. Interestingly, when LINE1 is overexpressed, the formation of ORF1p foci and colocalization with SG components occurs even in the absence of environmental stress [63], suggesting that overexpression of LINE1 is sufficient to trigger a stress response and SG formation.

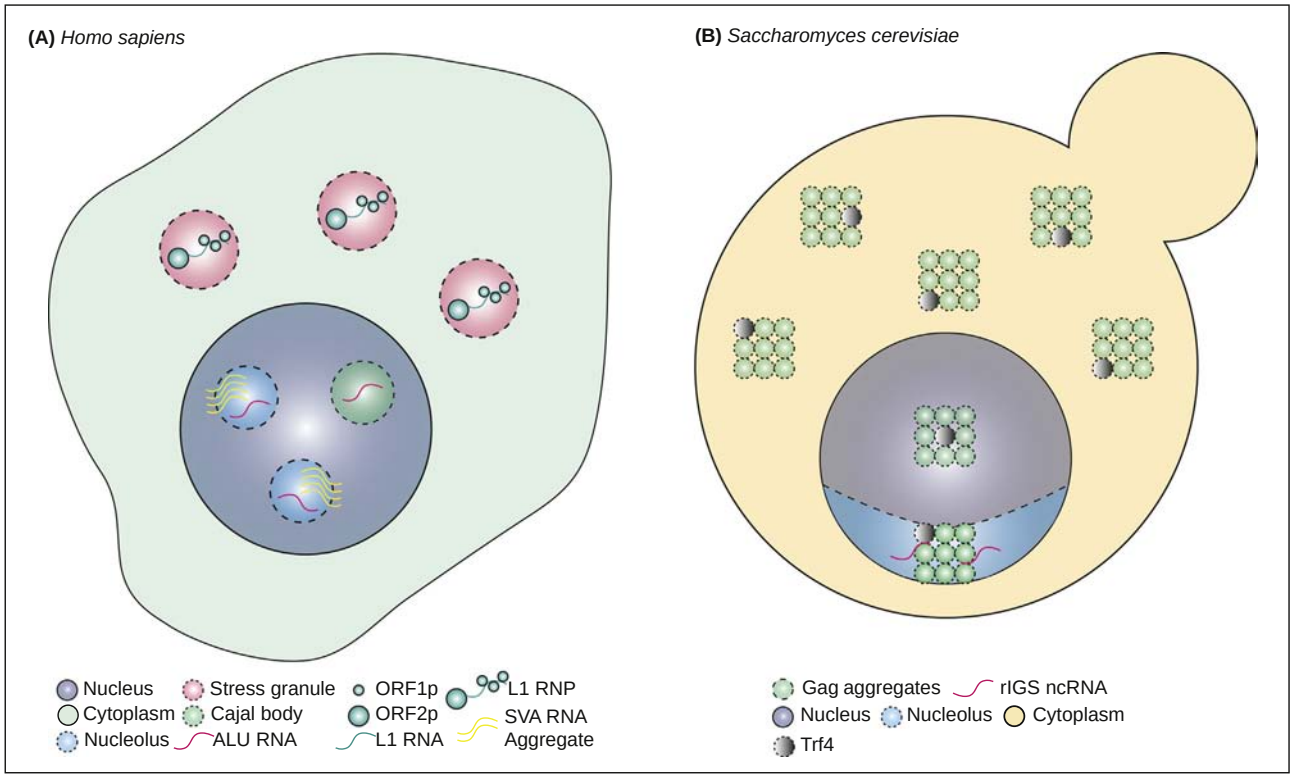

Figure 3. Phase Separation-Mediated Regulation of Retroelements. (A) Autonomous long interspersed element 1 (LINE1) and nonautonomous *Arthrobacter luteus* elements (ALU) and SINE-VNTR-Alu (SVA) retroelements in mammalian cells crosstalk with liquid phase-separated organelles. Stress granules (SGs) can inhibit LINE1 retromobility by sequestering the L1 ribonucleoproteins (RNPs), which are composed of LINE1 proteins, ORF1p/ORF2p, and L1 RNA. The localization of ALU RNAs to nucleoli maintains nucleolar structure and function. ALU transcripts also localize to Cajal bodies (CBs), while SVA transcripts aggregate and localize to the nucleolar periphery. (B) Studies using novel spinocerebellar ataxia type 2 (SCA2)-modeling *Saccharomyces cerevisiae* and human cells revealed that retrotransposon proteins, such as yeast Gag, form phase-separated Trf4-containing aggregates that localize to the nucleolus, where they associate with intergenic noncoding (nc)RNA at rDNA repeats. This allows Trf4 to mediate the hyperdegradation of ncRNAs, thereby decreasing cohesin loading onto rDNA repeats, increasing repeat instability, and shortening cellular lifespan. Abbreviation: rIGS, rDNA intergenic spacer.

In addition to being controlled by phase-separated cytoplasmic SGs, retrotransposons crosstalk with various compartments within the nucleus [63,66]. While the ORF1p and ORF2p proteins are predominantly cytoplasmic, a small fraction has been shown to localize to the nucleoli [63,66]. In addition to autonomous LINE1, mammalian retrotransposons include the nonautonomous short interspersed elements (SINE), Alu (*Arthrobacter luteus* elements), and SINE-VNTR-Alus (SVA), which require LINE1 proteins for retrotransposition [67]. Although these retrotransposons require and exploit the LINE1 proteins to retrotranspose, the transcripts emerging from these elements localize to various phase-separated subcellular compartments (Figure 3A). Alu RNA localizes to membraneless and phase-separated nuclear CBs and nucleoli, while SVAs concentrate in large perinucleolar rings [67]. In fact, the localization of Alu RNA to the nucleoli is required for the maintenance of the phase separation-driven nucleolar macrostructure and microstructure that are critical to overall rRNA biogenesis [68]. Similarly, LINE1 RNA mediates the proper localization of nucleolar proteins to promote rRNA synthesis and self-renewal in mouse embryonic stem cells [69]. Additionally, the retromobility of Ty1 elements, a retrotransposon of *S. cerevisiae*, is repressed by the aggregation of Ty1 proteins under certain conditions and, similar to SVAs, these aggregates can colocalize with the nucleolus (Figure 3B) [70]. These Ty1 protein aggregates compromise rDNA repeat stability and shorten cellular lifespan [70]. Taken together, these studies reveal a role for phase separation in the regulation of retrotransposition.

Our review thus far illustrates a role for phase separation in the regulation of repetitive nuclear domains, such as rDNA repeats, telomeres, and TEs. Table 1 provides a summary of the phase separation-mediated regulation of repetitive loci. We now discuss a potential link between phase separation and repetitive DNA in health and disease.

Phase Separation and Repetitive DNA in Human Disease

Thus far, we have discussed connections between repetitive DNA loci and compartments that are phase separated and liquid like. However, phase separation can yield compartments with a

Table 1. Phase Separation at Repetitive Loci

Repetitive locus	Mechanism	Effect	Species	Refs
Heterochromatin	HP1/Swi6	Heterochromatin formation	*Drosophila melanogaster*, human, *Schizosaccharomyces pombe*	[3,4,13]
rDNA	Chromosomal crosslink-mediated phase separation of rDNA	Sequestration of rDNA from bulk chromatin	*Saccharomyces cerevisiae*	[20]
	LLPS-mediated nucleolar recruitment of Rpl135, Pit, Nopp140, Fib	Nucleolar formation	*Drosophila melanogaster*	[32]
	Small spherical liquid-like nucleoli fuse to form larger nucleoli	Nucleolar fusion	*Xenopus laevis*	[33]
	LLPS of FIB1 and NPM1	Separation of FC, DFC, and GC	*Xenopus laevis*	[35]
Telomeres	Colocalization of hTR to phase-separated CBs	Telomere elongation	Human	[42]
	HP1-mediated prevention of Rad51 accumulation at heterochromatin	Prevents DDR at telomeres	*Drosophila melanogaster*	[3,4,14]
Transposable elements	Sequestration of LINE1 RNPs to phase-separated SGs	Inhibition of LINE1 retromobility	Human	[62–64]
	Localization of ORF1p and ORF2p to the nucleolus	Unknown	Human	[63,66]
	Localization of Alu and SVA RNAs to phase-separated CBs and nucleoli	Alu RNAs maintains nucleoli	Human	[63,67,68]
	Aggregation of Ty1 Gag protein	rDNA instability, reduced lifespan, inhibition of Ty1 retromobility	*Saccharomyces cerevisiae*	[70]

range of physical properties. For example, the contents can range from low- to high-viscosity liquids and even solid-like structures. In some cases, phase-separated molecules can transition back and forth through these phases. In human disease, these phase separations and transitions can be defining pathological features that provide unique insights or suggest novel therapeutic approaches. For example, solid-like phase compartments often arise from **protein aggregation**, a well-established hallmark of neurodegenerative diseases.

Proteins with regions of low sequence complexity (LC) are termed 'prion-like' proteins due to their propensity to aggregate, which gives rise to age-associated neurodegenerative diseases [71]. Many RNA and DNA-binding proteins are prion-like LC proteins. Mutations in these proteins lead to their misfolding and subsequent aggregation, giving rise to a nonmembrane-bound solid-state compartment. Studies have revealed that aggregates form through a liquid-to-solid phase transition process [72–75]. Many prion-like proteins exist as liquid droplets and, under normal conditions, these droplets age and eventually transition to a solid-state. However, mutations, including **polyglutamine (polyQ) tract** expansions, can speed up the phase transition process, inducing premature cellular aging (Figure 4A). The conversion of liquid droplets to solid fibers is accelerated by mutations in the fused in sarcoma (FUS) protein or polyQ expansion of the Huntingtin (HTT) protein, which are implicated in amyotrophic lateral sclerosis (ALS) and Huntington's disease, respectively [72,74]. These findings suggest that aberrant or untimely phase transitions are a determining factor in neurodegenerative disorders.

Phase transitioning from a liquid to a solid state has been detected at the rDNA-harboring nucleoli. In human cells, LC noncoding (nc)RNA transcribed from the rDNA intergenic spacer (rIGSRNA) has a role in this phase transition [75]. The bulk of rIGSRNAs comprise dinucleotide (CT or AG) repeats. Under heat shock or acidosis, these rIGSRNAs are transcribed and interact with short cationic peptides, giving rise to new stress-induced and liquid-like foci inside the phase-separated nucleolus [75–79]. Proteins with a propensity to form fibrils, including the von Hippel–Lindau (VHL) tumor suppressor and the Alzheimer disease (AD)-linked β-amyloid protein, localize within these stress-inducible droplets and promote their transition into solid-like nondynamic aggregates known as amyloid bodies (A-bodies). These A-bodies comprise immobile, amyloid-like proteins and can induce cellular dormancy, a process that can drive cancer cell resistance to therapeutic strategies [75,78]. These stress responses are likely evolutionarily conserved, because the intergenic ncRNAs driving them were initially described and have been extensively studied in *S. cerevisiae,* where these RNAs modulate rDNA repeat stability and cellular lifespan [80–87].

In fact, a recent report revealed unexpected connections between the intergenic ncRNAs of *S. cerevisiae* rDNA repeats and retrotransposon protein aggregation in the context of a new genetic model of spinocerebellar ataxia type 2 (SCA2) [70]. Previously, it was shown that loss of the *S. cerevisiae* protein Pbp1 and its human ortholog ATXN2 leads to the accumulation of intergenic ncRNA–DNA hybrid-containing triplex nucleic acid structures called R-loops [80,81]. This R-loop accumulation interferes with replication fork progression, leading to aberrant recombination within the rDNA repeats and a consequent shortening of replicative lifespan [81]. However, it is the expansion of the polyQ tract of ATXN2, not simply ATXN2 loss, that is associated with neurodegenerative disorders. Specifically, moderate polyQ expansions (from ~22/23 to 27–33 Qs) are a genetic risk factor for ALS, while longer expansions (>34 Qs) are the genetic cause of SCA2 [88]. Therefore, *S. cerevisiae* was humanized to introduce different Pbp1 forms with expanded polyQ tracts to model ALS and SCA2 [70]. Surprisingly, all of the polyQ-expanded Pbp1 forms retained the ability to suppress R-loops at rDNA, yet the rDNA repeats were highly destabilized and replicative lifespan was severely shortened in SCA2-modeling Pbp1 [70]. Mechanistic analyses revealed that, in cells with SCA2-modeling Pbp1, these polyQ-expanded proteins did not

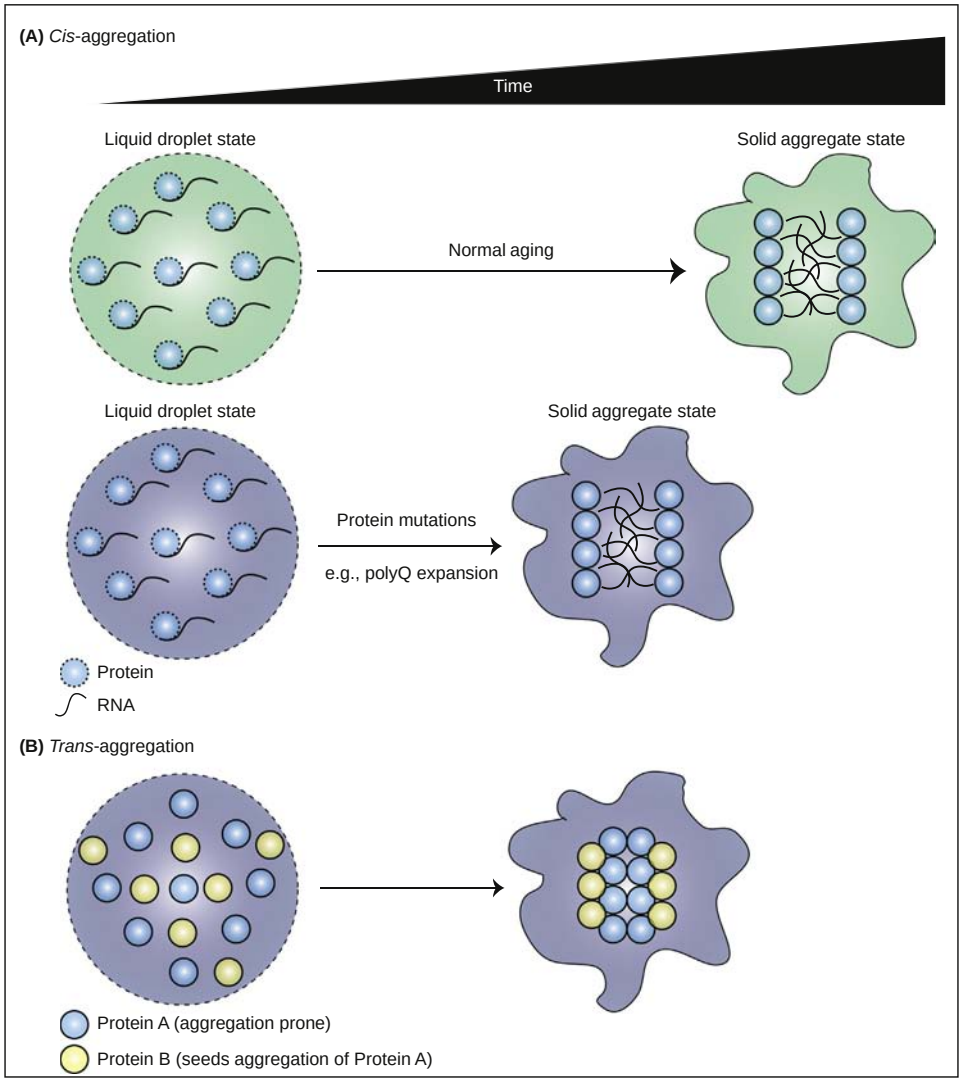

Figure 4. Phase Transitions in Human Disease. (A) Several RNA-binding proteins form liquid phase-separated compartments that, under normal aging conditions, transition into solid-like or fibrous aggregates. Mutations and polyglutamine (polyQ) expansions associated with neurodegenerative diseases can accelerate and exacerbate this phase transition. (B) Protein B, in yellow, acts in *trans* to induce the aggregation of Protein A, in blue.

aggregate but instead acted in *trans* and induced the aggregation of Gag, a Ty1 retrotransposon protein that is required for retromobility (Figure 4B). This aggregation halted retromobility. Interestingly, Gag aggregates formed dynamic foci that often localized to the nucleolus. These Gag aggregates were enriched in the RNA polyadenylation and degradation factor Trf4. The nucleolar-enriched Trf4 then drove the hyperelimination of longer intergenic ncRNAs that are in fact important for the loading of the cohesin complex to rDNA repeats. The loss of cohesin–rDNA binding resulted in rDNA repeat destabilization and lifespan shortening [70,89]. Importantly, overexpression of the protein disaggregase Hsp104 or suppression of Gag protein production in cells with SCA2-modeling Pbp1 countered Gag aggregation, restored rDNA stability, and

rescued the replicative lifespan. In addition, SCA2-modeling polyQ expansions of ATXN2 similarly connected retrotransposons and rDNA repeats in human cells [70]. Other polyQ proteins, including human HTT and yeast Whi3, have been shown to aggregate and phase separate [74,90–92], and expansion of the HTT polyQ tract phase transitions the protein from a liquid to a solid state [74]. These findings suggest that neurodegeneration-linked proteins with varying polyQ tract lengths exist in different states that can directly and sometimes simultaneously impact the function and stability of different types of repetitive DNA.

In addition to Pbp1/ATXN2, the prion-like protein Tau has also been independently implicated in the regulation of rDNA and retrotransposons [93–96]. Tau aggregates or tangles are implicated in AD and frontotemporal dementias (FTDs). Interestingly, similar to *bona fide* nucleolar components, Tau undergoes LLPS and helps recruit upstream binding factor 1 (UBF1), a transcription initiation factor of Pol I, to rDNA to mediate rRNA synthesis [93,94,96]. Disease-associated mutations in Tau cause the protein to mature into a viscous gel-like phase; therefore, it will be important to test how this phase transition impacts the nucleolar role of Tau [96]. In addition, in human brain tissue and *D. melanogaster*, Tau pathology de-represses retrotransposition [95]. On another front, the TAR DNA-binding protein 43 (TDP-43) is a phase-separating protein that drives cellular aging and neurodegeneration in a *D. melanogaster* model of ALS by de-repressing retrotransposon activity [59,97]. These studies suggest that neurodegenerative disease-associated alterations in phase transition-prone factors, such as ATXN2/Pbp1, Tau, and TDP-43, promote cellular toxicity, aging, and disease by, at least in part, impacting ribosomal DNA and/or retrotransposons. Future studies should carefully dissect how the phase separation and transition behaviors of these factors progress over the course of disease and how such progression may impact DNA repeat stability and overall cellular fitness.

Concluding Remarks

Here, we have highlighted crosstalks between phase separation, nuclear compartmentalization, and major repetitive DNA loci that are critical to genome organization, chromosome protection, and cellular lifespan. We speculate that DNA repeats are particularly susceptible to regulation by phase separation for two main reasons. First, DNA repeats form the bulk of eukaryotic genomes and represent a risk to genome stability. Phase separation may provide the cell with a broad and energy-efficient mechanism to sequester, control, and coregulate the repeats. Second, colocalization of DNA repeats into relatively small nuclear domains may in turn increase the local concentration of repeat-associated factors that can reinforce phase separation. However, we do not believe that the forces underlying phase separation-based regulation of DNA repeats need to be completely different from those acting across the rest of the genome. In addition, we have reviewed how phase separation, specifically phase transition from liquid-like to solid-like states at rDNA and retrotransposons, is implicated in various neurodegenerative diseases. Disease-associated mutations or environmental conditions, such as heat shock, may contribute to such phase transitions. Future studies should provide a detailed understanding of these pathological mechanisms, which may point towards novel effective treatments for these debilitating and lethal diseases (see Outstanding Questions). Taken together, our review of the critical connections between DNA repeats, phase separation, and nuclear compartmentalization highlights the rapidly growing role of phase separation in the regulation of genome function and human health.

Outstanding Questions

What are the signals controlling the phase separation or transition of factors that establish nuclear compartments critical to the regulation of repetitive DNA loci?

Following cell division, how does the cell coordinate chromosome folding or chromatin tethering to nuclear landmarks with the phase separation-driven genesis of nuclear compartments?

Can aging and diseases related to the dysfunction of repetitive DNA loci be countered or halted by using molecules that alter the phase separation properties of specific disease-related macromolecules? Can we develop new research and therapeutic tools to reach this goal?

Acknowledgments

A.C.H is funded by a Doctoral Award (152283) from the Canadian Institutes of Health Research (CIHR). L.A.O. is funded by an Ontario Graduate Scholarship (OGS). This work was supported by grants to K.M. from the CIHR (388041, 399687) and Canada Research Chairs Program (CRC; 950-230661). We thank members of the Mekhail laboratory for fruitful discussions.

References

1. Lander, E.S. *et al.* (2001) Initial sequencing and analysis of the human genome. *Nature* 409, 860–921
2. Nair, N. *et al.* (2017) Chromatin dynamics in genome stability: roles in suppressing endogenous DNA damage and facilitating DNA repair. *Int. J. Mol. Sci.* 18, E1486
3. Strom, A.R. *et al.* (2017) Phase separation drives heterochromatin domain formation. *Nature* 547, 241–245
4. Larson, A.G. *et al.* (2017) Liquid droplet formation by HP1alpha suggests a role for phase separation in heterochromatin. *Nature* 547, 236–240
5. Boeynaems, S. *et al.* (2018) Protein phase separation: a new phase in cell biology. *Trends Cell Biol.* 28, 420–435
6. Falahati, H. and Haji-Akbari, A. (2019) Thermodynamically driven assemblies and liquid-liquid phase separations in biology. *Soft Matter* 15, 1135–1154
7. Bah, A. and Forman-Kay, J.D. (2016) Modulation of intrinsically disordered protein function by post-translational modifications. *J. Biol. Chem.* 291, 6696–6705
8. Ferreon, J.C. *et al.* (2018) Acetylation disfavors Tau phase separation. *Int. J. Mol. Sci.* 19, E1360
9. Hofweber, M. and Dormann, D. (2018) Friend or foe – post-translational modifications as regulators of phase separation and RNP granule dynamics. *J. Biol. Chem.* 294, 7137–7150
10. Rane, J.S. *et al.* (2019) An acetylation mimicking mutation, K274Q, in tau imparts neurotoxicity by enhancing tau aggregation and inhibiting tubulin polymerization. *Biochem. J.* 476, 1401–1417
11. Saito, M. *et al.* (2019) Acetylation of intrinsically disordered regions regulates phase separation. *Nat. Chem. Biol.* 15, 51–61
12. Hyman, A.A. *et al.* (2014) Liquid-liquid phase separation in biology. *Annu. Rev. Cell Dev. Biol.* 30, 39–58
13. Canzio, D. *et al.* (2013) A conformational switch in HP1 releases auto-inhibition to drive heterochromatin assembly. *Nature* 496, 377–381
14. Chiolo, I. *et al.* (2011) Double-strand breaks in heterochromatin move outside of a dynamic HP1a domain to complete recombinational repair. *Cell* 144, 732–744
15. Janssen, A. *et al.* (2018) Heterochromatin: guardian of the genome. *Annu. Rev. Cell Dev. Biol.* 34, 265–288
16. Tatavosian, R. *et al.* (2019) Nuclear condensates of the Polycomb protein chromobox 2 (CBX2) assemble through phase separation. *J. Biol. Chem.* 294, 1451–1463
17. Larson, A.G. and Narlikar, G.J. (2018) The role of phase separation in heterochromatin formation, function, and regulation. *Biochemistry* 57, 2540–2548
18. Mekhail, K. and Moazed, D. (2010) The nuclear envelope in genome organization, expression and stability. *Nat. Rev. Mol. Cell Biol.* 11, 317–328
19. Raska, I. *et al.* (2006) New insights into nucleolar architecture and activity. *Int. Rev. Cytol.* 255, 177–235
20. Hult, C. *et al.* (2017) Enrichment of dynamic chromosomal crosslinks drive phase separation of the nucleolus. *Nucleic Acids Res.* 45, 11159–11173
21. Mekhail, K. *et al.* (2008) Role for perinuclear chromosome tethering in maintenance of genome stability. *Nature* 456, 667–670
22. Chan, J.N. *et al.* (2011) Perinuclear cohibin complexes maintain replicative life span via roles at distinct silent chromatin domains. *Dev. Cell* 20, 867–879
23. Torres-Rosell, J. *et al.* (2007) The Smc5-Smc6 complex and SUMO modification of Rad52 regulates recombinational repair at the ribosomal gene locus. *Nat. Cell Biol.* 9, 923–931
24. Defossez, P.A. *et al.* (1999) Elimination of replication block protein Fob1 extends the life span of yeast mother cells. *Mol. Cell* 3, 447–455
25. Kaeberlein, M. *et al.* (1999) The SIR2/3/4 complex and SIR2 alone promote longevity in *Saccharomyces cerevisiae* by two different mechanisms. *Genes Dev.* 13, 2570–2580
26. Sinclair, D.A. *et al.* (1997) Accelerated aging and nucleolar fragmentation in yeast sgs1 mutants. *Science* 277, 1313–1316
27. Salvi, J.S. *et al.* (2013) Enforcement of a lifespan-sustaining distribution of Sir2 between telomeres, mating-type loci, and rDNA repeats by Rif1. *Aging Cell* 12, 67–75
28. Kennedy, B.K. *et al.* (1997) Redistribution of silencing proteins from telomeres to the nucleolus is associated with extension of life span in *S. cerevisiae*. *Cell* 89, 381–391
29. Sinclair, D.A. and Guarente, L. (1997) Extrachromosomal rDNA circles–a cause of aging in yeast. *Cell* 91, 1033–1042
30. Ganley, A.R. *et al.* (2009) The effect of replication initiation on gene amplification in the rDNA and its relationship to aging. *Mol. Cell* 35, 683–693
31. van Sluis, M. and McStay, B. (2015) A localized nucleolar DNA damage response facilitates recruitment of the homology-directed repair machinery independent of cell cycle stage. *Genes Dev.* 29, 1151–1163
32. Falahati, H. and Wieschaus, E. (2017) Independent active and thermodynamic processes govern the nucleolus assembly in vivo. *Proc. Natl. Acad. Sci. U. S. A.* 114, 1335–1340
33. Brangwynne, C.P. *et al.* (2011) Active liquid-like behavior of nucleoli determines their size and shape in *Xenopus laevis* oocytes. *Proc. Natl. Acad. Sci. U. S. A.* 108, 4334–4339
34. Amenta, P.S. (1961) Fusion of nucleoli in cells cultured from the heart of *Triturus viridescens*. *Anat. Rec.* 139, 155–165
35. Feric, M. *et al.* (2016) Coexisting liquid phases underlie nucleolar subcompartments. *Cell* 165, 1686–1697
36. Canzio, D. *et al.* (2014) Mechanisms of functional promiscuity by HP1 proteins. *Trends Cell Biol.* 24, 377–386
37. Blasco, M.A. (2007) The epigenetic regulation of mammalian telomeres. *Nat. Rev. Genet.* 8, 299–309
38. Richard, P. *et al.* (2003) A common sequence motif determines the Cajal body-specific localization of box H/ACA scaRNAs. *EMBO J.* 22, 4283–4293
39. Jady, B.E. *et al.* (2004) Human telomerase RNA and box H/ACA scaRNAs share a common Cajal body-specific localization signal. *J. Cell Biol.* 164, 647–652
40. Zhu, Y. *et al.* (2004) Telomerase RNA accumulates in Cajal bodies in human cancer cells. *Mol. Biol. Cell* 15, 81–90
41. Jady, B.E. *et al.* (2006) Cell cycle-dependent recruitment of telomerase RNA and Cajal bodies to human telomeres. *Mol. Biol. Cell* 17, 944–954
42. Cristofari, G. *et al.* (2007) Human telomerase RNA accumulation in Cajal bodies facilitates telomerase recruitment to telomeres and telomere elongation. *Mol. Cell* 27, 882–889
43. Stern, J.L. *et al.* (2012) Telomerase recruitment requires both TCAB1 and Cajal bodies independently. *Mol. Cell. Biol.* 32, 2384–2395
44. Platani, M. *et al.* (2000) In vivo analysis of Cajal body movement, separation, and joining in live human cells. *J. Cell Biol.* 151, 1561–1574
45. Lin, Y. *et al.* (2016) Toxic PR poly-dipeptides encoded by the C9orf72 repeat expansion target LC domain polymers. *Cell* 167, 789–802
46. Yamazaki, T. *et al.* (2018) Functional domains of NEAT1 architectural lncRNA induce paraspeckle assembly through phase separation. *Mol. Cell* 70, 1038–1053
47. Handwerger, K.E. *et al.* (2005) Cajal bodies, nucleoli, and speckles in the *Xenopus* oocyte nucleus have a low-density, sponge-like structure. *Mol. Biol. Cell* 16, 202–211
48. Dundr, M. *et al.* (2004) In vivo kinetics of Cajal body components. *J. Cell Biol.* 164, 831–842
49. Machyna, M. *et al.* (2015) Coilin: the first 25 years. *RNA Biol.* 12, 590–596
50. Hebert, M.D. and Matera, A.G. (2000) Self-association of coilin reveals a common theme in nuclear body localization. *Mol. Biol. Cell* 11, 4159–4171
51. Broome, H.J. and Hebert, M.D. (2013) Coilin displays differential affinity for specific RNAs in vivo and is linked to telomerase RNA biogenesis. *J. Mol. Biol.* 425, 713–724
52. Machyna, M. *et al.* (2014) The coilin interactome identifies hundreds of small noncoding RNAs that traffic through Cajal bodies. *Mol. Cell* 56, 389–399
53. Bandaria, J.N. *et al.* (2016) Shelterin protects chromosome ends by compacting telomeric chromatin. *Cell* 164, 735–746
54. Gallardo, F. *et al.* (2011) Live cell imaging of telomerase RNA dynamics reveals cell cycle-dependent clustering of telomerase at elongating telomeres. *Mol. Cell* 44, 819–827

55. Mattern, K.A. *et al.* (2004) Dynamics of protein binding to telomeres in living cells: implications for telomere structure and function. *Mol. Cell. Biol.* 24, 5587–5594
56. Chung, D.K. *et al.* (2015) Perinuclear tethers license telomeric DSBs for a broad kinesin- and NPC-dependent DNA repair process. *Nat. Commun.* 6, 7742
57. Oshidari, R. *et al.* (2018) Nuclear microtubule filaments mediate non-linear directional motion of chromatin and promote DNA repair. *Nat. Commun.* 9, 2567
58. Hall, A.C. *et al.* (2017) Repetitive DNA loci and their modulation by the non-canonical nucleic acid structures R-loops and G-quadruplexes. *Nucleus* 8, 162–181
59. Krug, L. *et al.* (2017) Retrotransposon activation contributes to neurodegeneration in a Drosophila TDP-43 model of ALS. *PLoS Genet.* 13, e1006635
60. Helman, E. *et al.* (2014) Somatic retrotransposition in human cancer revealed by whole-genome and exome sequencing. *Genome Res.* 24, 1053–1063
61. Goodier, J.L. (2016) Restricting retrotransposons: a review. *Mob. DNA* 7, 16
62. Goodier, J.L. *et al.* (2007) LINE-1 ORF1 protein localizes in stress granules with other RNA-binding proteins, including components of RNA interference RNA-induced silencing complex. *Mol. Cell. Biol.* 27, 6469–6483
63. Goodier, J.L. *et al.* (2010) Discrete subcellular partitioning of human retrotransposon RNAs despite a common mechanism of genome insertion. *Hum. Mol. Genet.* 19, 1712–1725
64. Goodier, J.L. *et al.* (2013) Mapping the LINE1 ORF1 protein interactome reveals associated inhibitors of human retrotransposition. *Nucleic Acids Res.* 41, 7401–7419
65. Hu, S. *et al.* (2015) SAMHD1 Inhibits LINE-1 retrotransposition by promoting stress granule formation. *PLoS Genet.* 11, e1005367
66. Goodier, J.L. *et al.* (2004) A potential role for the nucleolus in L1 retrotransposition. *Hum. Mol. Genet.* 13, 1041–1048
67. Hancks, D.C. and Kazazian Jr., H.H. (2016) Roles for retrotransposon insertions in human disease. *Mob. DNA* 7, 9
68. Caudron-Herger, M. *et al.* (2015) Alu element-containing RNAs maintain nucleolar structure and function. *EMBO J.* 34, 2758–2774
69. Percharde, M. *et al.* (2018) A LINE1-nucleolin partnership regulates early development and ESC identity. *Cell* 174, 391–405
70. Ostrowski, L.A. *et al.* (2018) Conserved Pbp1/Ataxin-2 regulates retrotransposon activity and connects polyglutamine expansion-driven protein aggregation to lifespan-controlling rDNA repeats. *Commun. Biol.* 1, 187
71. King, O.D. *et al.* (2012) The tip of the iceberg: RNA-binding proteins with prion-like domains in neurodegenerative disease. *Brain Res.* 1462, 61–80
72. Patel, A. *et al.* (2015) A Liquid-to-solid phase transition of the ALS protein FUS accelerated by disease mutation. *Cell* 162, 1066–1077
73. Gopal, P.P. *et al.* (2017) Amyotrophic lateral sclerosis-linked mutations increase the viscosity of liquid-like TDP-43 RNP granules in neurons. *Proc. Natl. Acad. Sci. U. S. A.* 114, E2466–E2475
74. Peskett, T.R. *et al.* (2018) A liquid to solid phase transition underlying pathological Huntingtin Exon1 aggregation. *Mol. Cell* 70, 588–601
75. Wang, M. *et al.* (2018) Stress-induced low complexity RNA activates physiological amyloidogenesis. *Cell Rep.* 24, 1713–1721
76. Mekhail, K. *et al.* (2004) HIF activation by pH-dependent nucleolar sequestration of VHL. *Nat. Cell Biol.* 6, 642–647
77. Mekhail, K. *et al.* (2005) Regulation of ubiquitin ligase dynamics by the nucleolus. *J. Cell Biol.* 170, 733–744
78. Audas, T.E. *et al.* (2016) Adaptation to stressors by systemic protein amyloidogenesis. *Dev. Cell* 39, 155–168
79. Audas, T.E. *et al.* (2012) Immobilization of proteins in the nucleolus by ribosomal intergenic spacer noncoding RNA. *Mol. Cell* 45, 147–157
80. Abraham, K.J. *et al.* (2016) Intersection of calorie restriction and magnesium in the suppression of genome-destabilizing RNA-DNA hybrids. *Nucleic Acids Res.* 44, 8870–8884
81. Salvi, J.S. *et al.* (2014) Roles for Pbp1 and caloric restriction in genome and lifespan maintenance via suppression of RNA-DNA hybrids. *Dev. Cell* 30, 177–191
82. Bryk, M. *et al.* (1997) Transcriptional silencing of Ty1 elements in the RDN1 locus of yeast. *Genes Dev.* 11, 255–269
83. Houseley, J. *et al.* (2007) Trf4 targets ncRNAs from telomeric and rDNA spacer regions and functions in rDNA copy number control. *EMBO J.* 26, 4996–5006
84. Kobayashi, T. and Ganley, A.R. (2005) Recombination regulation by transcription-induced cohesin dissociation in rDNA repeats. *Science* 309, 1581–1584
85. Vasiljeva, L. *et al.* (2008) Transcription termination and RNA degradation contribute to silencing of RNA polymerase II transcription within heterochromatin. *Mol. Cell* 29, 313–323
86. Parikh, V.S. *et al.* (1989) Interaction between the yeast mitochondrial and nuclear genomes influences the abundance of novel transcripts derived from the spacer region of the nuclear ribosomal DNA repeat. *Mol. Cell. Biol.* 9, 1897–1907
87. Abraham, K.J. *et al.* (2017) Non-coding RNA molecules connect calorie restriction and lifespan. *J. Mol. Biol.* 429, 3196–3214
88. Ostrowski, L.A. *et al.* (2017) Ataxin-2: from RNA control to human health and disease. *Genes* 8, E157
89. Poon, B.P. and Mekhail, K. (2011) Cohesin and related coiled-coil domain-containing complexes physically and functionally connect the dots across the genome. *Cell Cycle* 10, 2669–2682
90. Posey, A.E. *et al.* (2018) Profilin reduces aggregation and phase separation of huntingtin N-terminal fragments by preferentially binding to soluble monomers and oligomers. *J. Biol. Chem.* 293, 3734–3746
91. Zhang, H. *et al.* (2015) RNA Controls PolyQ protein phase transitions. *Mol. Cell* 60, 220–230
92. Schlissel, G. *et al.* (2017) Aggregation of the Whi3 protein, not loss of heterochromatin, causes sterility in old yeast cells. *Science* 355, 1184–1187
93. Bou Samra, E. *et al.* (2017) A role for Tau protein in maintaining ribosomal DNA stability and cytidine deaminase-deficient cell survival. *Nat. Commun.* 8, 693
94. Maina, M.B. *et al.* (2018) The involvement of tau in nucleolar transcription and the stress response. *Acta Neuropathol. Commun.* 6, 70
95. Guo, C. *et al.* (2018) Tau activates transposable elements in Alzheimer's disease. *Cell Rep.* 23, 2874–2880
96. Wegmann, S. *et al.* (2018) Tau protein liquid–liquid phase separation can initiate tau aggregation. *EMBO J.* 37, e98049
97. Gawande, B.N. *et al.* (2017) Selection of DNA aptamers with two modified bases. *Proc. Natl. Acad. Sci. U. S. A.* 114, 2898–2903