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Nuclear bodies: new insights into assembly/dynamics and disease relevance

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Eukaryotic cells enclose their genome within a dedicated organelle, the nucleus, which is the site of major cellular events such as messenger RNA synthesis and processing, ribosome subunit biogenesis and DNA replication. Like the cytoplasm, the nucleus is compartmentalized to facilitate efficient coordination of these pathways, although subnuclear compartments form without the use of membranes. Numerous disease states have been linked to dysfunction of these compartments or 'nuclear bodies'. Recent advances have shed light on the formation and maintenance of key structures, including nucleoli, splicing speckles, paraspeckles, Cajal bodies, histone locus bodies and promyelocytic leukemia bodies. Here, we review the impact of these findings, which provide major insights into dynamic processes that affect both structure and function within the nucleus.

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Subnuclear organization and nuclear body formation

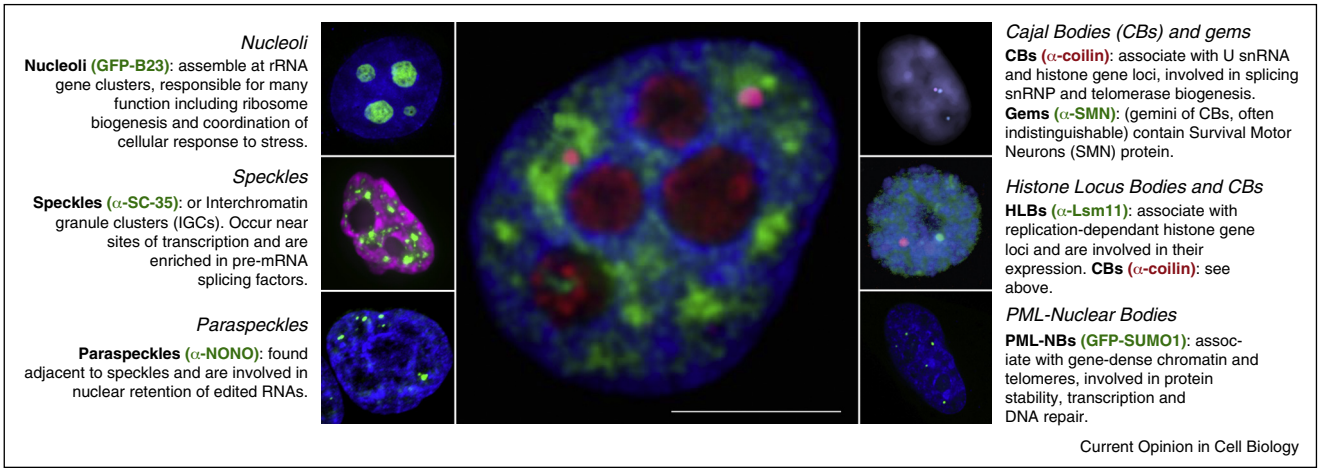
Nuclear functions such as DNA replication and transcription are carried out within a highly organized three-dimensional environment, which starts with the packaging of DNA and associated proteins into chromatin. Varying compaction levels create euchromatic and heterochromatic domains, with further organization into distinct chromosome domains or 'territories' (see [1,2] for review). Nuclear bodies, which include nucleoli, Cajal bodies (CBs), histone locus bodies (HLBs), PML nuclear bodies (PML-NBs), splicing speckles (also known as interchromatin granule clusters) and paraspeckles, primarily occupy the interchromatin space, and several have been shown to be associated with specific gene loci and/or their RNA products (Figure 1).

As a general rule, these bodies serve to concentrate proteins (and in most cases RNAs) involved in similar processes in a constrained space, presumably to enhance reaction efficiency and facilitate regulation. Although many of the mechanisms controlling their formation, organization, and movement remain unclear, cataloguing their respective components has provided valuable clues to their distinct functions. Direct analysis of protein composition has been carried out on purified nucleoli [3,4], enriched fractions of speckles/interchromatin granule clusters [5] and various complexes that associate with specific bodies. Approaches such as imaging-based screening for proteins that show distinct subnuclear localization patterns [6,7•] and proximity labeling of gene loci associated with specific bodies for identification by microarray or deep sequencing [8•] are helping to fill in the gaps.

On the basis of observations that most nuclear body constituents are highly dynamic and in constant flux, the idea of 'self-organization' was proposed, with distinct nuclear subcompartments thought to represent the collective sum of protein and RNA interactions occurring within them [9,10]. Dynamic turnover allows for rapid changes in the composition of nuclear bodies in response to cellular perturbations and their breakdown and reformation during cell division. In a simple stochastic model, body formation results from random associations and can follow multiple pathways, whereas in a hierarchical model there are defined steps that follow a precise order. Recent evidence suggests that a mix of both can occur, for example with initial nucleation provided by an RNA or protein 'seeding' event that is then followed by either stochastic association of other components or formation of subcomplexes that then associate in a hierarchical manner.

Nucleoli are the quintessential 'RNA-seeded' nuclear bodies, with their assembly triggered by activation of rDNA transcription [11]. An elegant demonstration of the ability of other nascent RNA transcripts, both coding and noncoding (nc), to nucleate nuclear bodies was provided by Shevtsov and Dundr [12••], who showed *in vivo* formation of *de novo* HLBs and associated CBs (*via* tethered histone pre-mRNA), speckles (*via* a tethered β-globin minigene that is efficiently spliced), paraspeckles (*via* tethered NEAT1/Men ε/β long ncRNA; Figure 2a) and nuclear stress bodies (*via* tethered satellite III transcripts). While the recruitment of known components does not necessarily indicate a functional body, Spector and

Figure 1



Major subnuclear bodies. The central panel demonstrates the spatial relationships between different structures in a HeLa cell nucleus. Chromatin is stained with DAPI (blue) in a cell expressing the core snRNP protein, SmB, tagged with YFP (green). The dense fibrillar components of nucleoli are stained with antibodies to fibrillarin (red) while anti-fibrillarin and anti-coilin both stain Cajal bodies (pink). Bar = 10 μm. The smaller side panels show the nucleolus (reviewed in [19]) in HeLa cells (with a marker for the granular component), speckles (reviewed in [16]) in human lens epithelial cells, paraspeckles (reviewed in [66]) in HeLa cells, Cajal bodies and gems (reviewed in [67]) in SH-SY5Y neuroblastoma cells, histone locus bodies and Cajal bodies (reviewed in [68]) in *Drosophila* tissues and PML nuclear bodies (reviewed in [69]) in HeLa cells.

colleagues carried out a comprehensive molecular dissection of the co-transcriptional assembly of paraspeckles on Men ε/β long ncRNA [13••] and showed that *de novo* paraspeckles are dependent on ongoing Men ε/β transcription and share many features with endogenous paraspeckles, including similar kinetics and retention of specific hyperedited mRNAs.

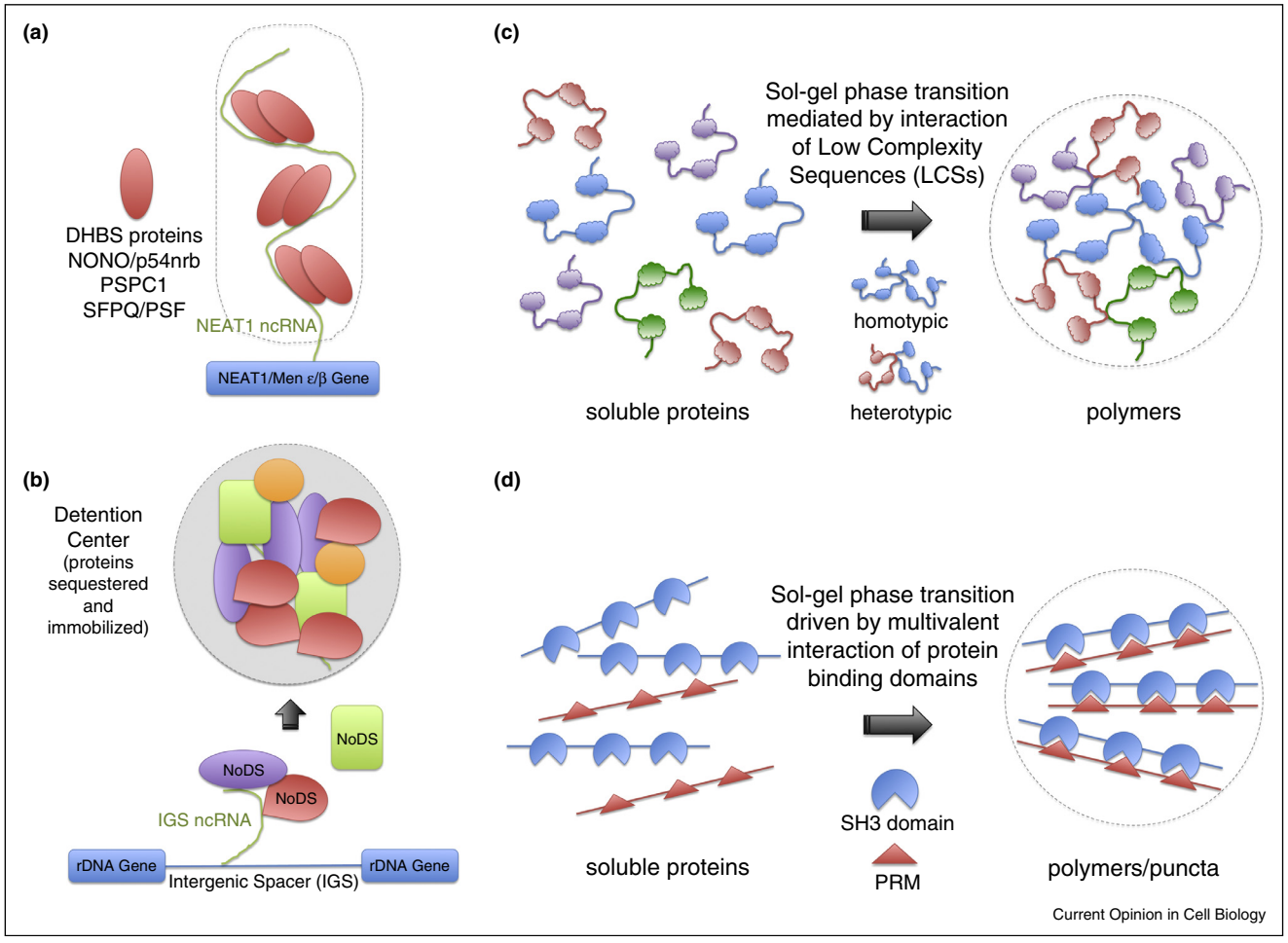
The formation of *Drosophila* HLBs, in contrast, is proposed to involve a protein-based seeding event governed by Mxc and FLASH, which can occur independently of histone gene expression [14]. The sequential recruitment of proteins to HLBs observed during development in the early embryo supports the idea of hierarchical assembly following this initial nucleation step. Chung *et al.* [15] recently proposed that the trigger event in the formation of ALT-associated PML bodies at telomeric DNA is a post-transcriptional modification, namely SUMOylation of telomeric proteins followed by recruitment of PML and Sp100. Other post-translational modifications, in particular reversible protein phosphorylation, have been shown to play roles in body assembly/maintenance [16,17]. Given the complexity and dynamic nature of these structures and their essential roles in key nuclear processes, the molecular events that determine recruitment and retention of individual components warrant further investigation.

A large body of work has also demonstrated that new nuclear bodies form and the properties of existing bodies change in response to cellular stress [18,19]. Recently, a family of long ncRNAs was implicated in a surprising new role for the nucleolus, namely the reversible, stress-induced global sequestration and immobilization of a diverse range of cytoplasmic and nuclear proteins. Lee and colleagues showed that sequestration is driven by induction of transcription of long ncRNA from discrete regions of the rDNA intergenic spacer [20••]. The transcripts are stress-specific and interact directly with proteins that contain nucleolar detention sequences. The functional consequence is the formation, by further stabilization of the initial long ncRNA nucleation event *via* high affinity hydrophobic interactions, of a distinct subnucleolar structure termed the 'Detention Center' (Figure 2b) that serves to immobilize proteins and thus deprive them of their intrinsic dynamic nature [21]. This temporary imprisonment allows for rapid inhibition of diverse cellular processes in response to stress, and provides further evidence of the dynamic nature of nuclear bodies and the generality of RNA-based seeding events.

Physical properties of RNA–protein assemblies

With the field long dominated by protein biochemistry and molecular biology approaches, it is only recently that attention has turned to the physical properties of RNA–protein assemblies. This has provided surprising insight into the consequences of particular protein–protein and protein–RNA interactions, in particular highlighting their ability to drive sol–gel phase transitions as a possible explanation for the rapid and reversible formation of

Figure 2



(a) Proposed model for the assembly of paraspeckles on nascent NEAT1 long ncRNA transcripts. On the basis of high resolution *in situ* hybridization studies, the molecular size of the transcript is believed to define the width of the body [70•], with increased protein accumulation resulting in elongation [71]. Dimerization of DHBS (*Drosophila* behavior, human splicing) proteins into higher-order structures and subsequent association with NEAT1 has been proposed to drive paraspeckle self-assembly [71]. (b) Proposed model for the stress-induced assembly of nucleolar Detention Centers, driven by induction of specific transcripts from the rDNA intergenic spacer and sequestration/immobilization of proteins containing nucleolar detention sequences. (c) Phase transition induced by interaction of LCSs (regions of low amino acid diversity that are normally disordered) in RNA binding proteins. While distinct from pathological amyloid fibrils, this state may represent a physiological balance that is disrupted in neurodegenerative diseases. (d) Phase transition driven by multivalent interactions between SH3 (Src homology 3) and PRM (proline-rich motif) protein binding domains.

non-membranous bodies from soluble constituents (Figure 2c–d).

A series of recent papers explored the contribution of intrinsically disordered low complexity sequences (LCSs), which are enriched in many RNA and DNA binding proteins, to the formation of higher-order RNA-containing structures. By assessing the protein and RNA composition of *in vitro* cytoplasmic precipitates that bear striking similarities to endogenous RNA granules, McKnight and colleagues demonstrated an enrichment of RNA binding proteins and distinct mRNAs [22•,23•]. They showed that interaction of LCSs in a range of these RNA binding proteins can drive phase transitions to hydrogel droplets composed, at the EM level, of uniformly polymerized amyloid-like fibers (Figure 2c). In contrast to pathological amyloid fibrils, these LCS-driven hydrogels are reversible and dynamic, can be heterotypic, and are SDS soluble. Having noted that both LCSs and RNA binding domains are required for retention of mRNAs in hydrogels, the authors propose a biological model whereby the LC domains of RNA binding proteins mediate reversible association into granules, while their RNA binding domains recruit their target mRNAs

These observations were further extended to nuclear RNA binding proteins by demonstration of LCS-mediated polymerization of the RNA binding protein

TAF15 [24]. Recruitment of the C-terminal domain (CTD) of RNA polymerase II by TAF15 hydrogels suggests a scaffolding/recruitment role that has the potential to directly affect transcription. Although a caveat to these experiments is the use of high concentrations of proteins at low temperatures, Schwartz *et al.* [25••] showed that formation of higher-order assemblies of the RNA binding protein FUS could be triggered at more physiological protein levels through cooperative binding to RNA, with the RNA binding domain mediating the initial nucleation step and LCSs mediating the binding-induced phase transition. These assemblies were also shown to bind the CTD of RNA polymerase II. Taken together, these studies provide a universal/unifying model for the formation of cytoplasmic granules and nuclear bodies that contain both RNA and protein. Furthermore, Li and colleagues [26••] have extended this model to protein-based structures, showed that multivalent protein–protein interactions can drive phase transitions to polymers *in vitro* and organize proteins into puncta *in vivo* (Figure 2d).

If non-membraneous organelles form by sol–gel phase transitions, it is reasonable to assume that they might behave to some extent like liquid droplets. Following on from an earlier demonstration that cytoplasmic RNA-rich and protein-rich P granules in *Caenorhabditis elegans* localize by controlled dissolution/condensation and exhibit liquid-like behaviors [27], the Hyman group later showed the same for nucleoli in *Xenopus laevis* oocytes [28•]. The dynamic and liquid-like nature of these nuclear bodies was demonstrated by their viscous relaxation into a spherical shape following mechanical deformation, and by the observation that they fuse when they touch, resulting in a larger, single body. This behavior is consistent with their *in vivo* morphology and distribution. They further showed that viscosity is ATP-dependent, suggesting a role for active processes in maintaining the fluidity of the contents of this key nuclear structure. This suggests a model in which the formation of nucleoli results from RNA–protein complexes (seeded by initial rRNA transcription events) condensing into small droplets which then fuse with neighboring droplets to form larger structures. Whether this model can be extended to other nuclear structures remains to be determined, although novel bodies showing similar behavior have recently been experimentally induced in *Drosophila* egg chambers [29].

Nuclear bodies and disease

A key impetus for gaining a better understanding of nuclear body formation and maintenance is our increased appreciation of their essential roles in maintenance of cellular homeostasis. Given that most play roles in the regulation of gene expression, it is not surprising that their dysfunction has been linked to numerous disease states, including cancer and degenerative disorders.

Telomere regulation

The importance of regulation of chromosomal telomeres is clear both from the range of degenerative disorders, including dyskeratosis congenital (DC), that have been linked to defects in telomeres [30], and from the pathological stabilization and lengthening of telomeres seen in many cancers [31]. Chromosomal ends are protected from degradation and recognition as double-stranded DNA breaks by telomeres [32]. These highly specialized structures are formed and maintained by the shelterin complex [33], which contains a number of proteins with various functions in DNA repair and regulation of the action of telomerase, the RNP (ribonucleoprotein) responsible for maintenance of telomeric DNA. Both PML-NBs and CBs are linked to telomere maintenance, with many recent reports aiming to dissect the molecular interactions involved in telomere maintenance under physiological and pathological conditions.

Cajal bodies and telomerase

In the majority of tumors, telomere stabilization is achieved by the reactivation of telomerase, which in mammals is usually active only during development and in adult stem cells. Co-localization has been observed between a subset of CBs and telomeres during S-phase [34] and the core human telomerase RNP (human telomerase RNA/hTR plus human telomerase reverse transcriptase/hTERT) accumulates in CBs in human cancer cells [35]. Telomerase is delivered to CBs by the telomerase Cajal body protein 1 (TCAB1; [36]), with missense mutations in this protein in DC resulting in mislocalization of telomerase from CBs to nucleoli [37•]. Disruption of CBs by reduction of the signature protein coilin was recently shown to abolish telomerase accumulation at telomeres [38•]. While this accumulation defect is not associated with a significant reduction in telomerase levels, overexpression of hTERT and hTR together can restore telomerase recruitment to telomeres in coilin-depleted cells. This suggests a role for coilin in correct recruitment of telomerase to telomeres, although the role of the CB as a structure remains unclear. It has been proposed to play a role in the maturation of telomerase RNP [39], analogous to its role in the maturation of splicing snRNPs (small nuclear ribonucleoproteins). The current body of literature suggests that CBs are not essential for snRNP maturation, although they may contribute to its efficiency by enabling local concentration of maturation factors [40]. This may also prove to be the case for the clearly dynamic relationship between telomerase and CBs.

PML bodies, the ALTernate pathway and epigenetics

In some tumors, rather than reactivation of telomerase, an alternate (ALT) pathway operates to maintain telomere length [41]. The ALT pathway operates through DNA repair and recombination processes, resulting in heterogeneous telomere lengths, and has been linked to a subset

of PML-NBs termed ALT-associated PML-nuclear bodies (APBs). These are found at some telomeres in cells positive for the ALT pathway [42]. The role of APBs in telomere elongation is not yet fully understood, although they have been suggested to actively promote alternative telomere lengthening [41]. The recent identification of small PML-NBs associated with telomeres in non-neoplastic cells [43•] suggests that PML-NBs may play a more general role in telomere maintenance. In mouse embryonic stem (ES) cells, telomere-associated PML bodies have recently been implicated in maintaining the lower levels of heterochromatin that are characteristic of telomeres in pluripotent cells [44••], leading to the intriguing proposal that telomere-associated PML bodies in ES cells have a role in maintaining epigenetic chromatin states, rather than in telomeric recombination.

CBs/gems in neurodegeneration

The complexity of the links between nuclear bodies and human disease is exemplified by the pathology of the inherited neurodegenerative condition, Spinal Muscular Atrophy (SMA). SMA affects, at least predominantly, cells of the nervous system [45], but is caused by reduction in the expression of the Survival of Motor Neurons (SMN) protein [46], which has a housekeeping role in splicing snRNP maturation [47,48]. SMN localizes to nuclear gems, which are often, but not always, coincident with CBs, and SMA patient cells show disruption of CBs [49]. Despite this, a great deal of work in recent years has concentrated on two theories concerning cytoplasmic roles for SMN. The first is that failure of SMN to assemble the Sm protein core onto splicing snRNPs in the cytoplasm leads to splicing abnormalities to which neural cells, particularly motor neurons, are particularly sensitive. Aberrant splicing of mRNA for the motor circuit protein Stasimon has, for example, been identified in models of SMA [50••,51]. The second is that SMN has a role in mRNA trafficking, unrelated to its role in snRNP assembly, which is of particular importance in neural cells. In support of this idea, both β-actin [52] and candidate plasticity-related gene 15 (Cpg15) [53] mRNA levels are reduced in neurites of SMN-depleted neurons. A more extensive genome-wide analysis identified RNAs from many different classes as putative SMN-interaction partners although more than half of those identified were mRNAs [54••]. Several were shown to co-localize with SMN in mouse motor neuron-like NSC-34 cells, with the localization of some being disrupted by SMN depletion.

Recent research on proteins that are involved in some cases of amyotrophic lateral sclerosis (ALS) has, however, has thrown the focus partially back onto nuclear bodies or, more precisely, onto the intricate dynamic balance between nuclear and cytoplasmic roles of proteins involved in RNA metabolism. Expression of mutants of the FUS (fused in sarcoma) protein associated with ALS prevents SMN from forming nuclear gems [55••], linking molecular pathways involved in two separate neurodegenerative conditions. This result may, however, be an indirect effect of changes in the rate of snRNP maturation, as another study [56•] demonstrates that FUS mutants or overexpressed wild type FUS can trap snRNPs in the cytoplasm. Modulating the expression of snRNP proteins can negatively or positively regulate gem formation [57,58]. Both FUS and TDP-43 (TAR DNA-binding protein 43), an RNA-binding protein mutated in some cases of ALS, co-localize with CBs/gems, while in ALS patients, snRNA expression is upregulated [59•]. This is in marked contrast to SMA, in which snRNA levels are decreased [60,61], but suggests that compromised integrity of the spliceosome and, consequently, splicing may be at the root of both conditions. Interestingly, TDP-43 has roles in mRNA splicing regulation [62] and the control of mRNA stability [63], while FUS has functions that include regulation of transcription and splicing [64,65]. Both can form abnormal cytoplasmic aggregates in ALS patients. As for SMN, the balance between the localizations of these two proteins in nuclear and cytoplasmic bodies reflecting their complex roles in different stages of RNA metabolism may prove critical for correct cellular function.

Conclusion

As shown here, recent technological advances in the molecular dissection of nuclear body composition, assembly and maintenance, coupled with a growing appreciation of the pathological implications of their dysfunction, are providing new insights into their roles in the regulation of key cellular events and a better understanding of their contribution to cellular homeostasis and disease progression.

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In this study the authors assess the protein composition of precipitates that bear striking similarities to RNA granules, demonstrating that interaction of intrinsically disordered low complexity sequences in a range of RNA binding proteins can drive phase transitions to hydrogel droplets. This suggests a physical model for the formation of RNA-containing bodies in both the cytoplasm and the nucleus.

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