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Biogenesis and Function of Nuclear Bodies

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Abstract

Nuclear bodies including nucleoli, Cajal bodies, nuclear speckles, Polycomb bodies, and paraspeckles are membrane-less subnuclear organelles. They are steady-state structures that dynamically respond to basic physiological processes as well as various forms of stress, altered metabolic conditions and alterations in cellular signaling. The formation of specific nuclear bodies has been suggested to follow stochastic and ordered assembly models. In addition, a seeding mechanism has been proposed to assemble, maintain, and regulate particular nuclear bodies. In coordination with noncoding RNAs, chromatin modifiers and other machineries, various nuclear bodies have been shown to sequester and modify proteins, process RNAs and assemble ribonucleoprotein complexes, as well as epigenetically regulate gene expression. Understanding the functional relationships between the three-dimensional organization of the genome and nuclear bodies is essential to fully uncover the regulation of gene expression and its implications in human diseases.

The cell nucleus is highly organized and harbors many nuclear bodies

The nucleus contains the vast majority of the cell's genetic material, organized as multiple chromosomes. Individual chromosomes reside in limited and nonrandom regions of the interphase nucleus, known as chromosome territories (Box 1) [1, 2]. Studies of how the nucleus is physically and functionally organized within the interchromatin space have revealed that the nucleus is very organized and highly dynamic. One prominent feature of the nuclear landscape is the ability to harbor a variety of discrete subnuclear organelles, collectively referred to as nuclear bodies [3, 4] (Figure 1). Structures of nuclear bodies have been identified as distinct nuclear foci at both light and electron microscopic levels. Numerous nuclear bodies have been characterized thus far including nucleoli, Cajal bodies (CBs), nuclear speckles, paraspeckles, Polycomb bodies, etc. (Table 1).

Box 1

Chromosome Territories

During interphase, individual chromosomes occupy distinct regions known as chromosome territories. Neighboring chromosome territories have varying levels of overlap and chromatin can loop out from one territory to another territory. The interior of chromosome territories contains spaces, allowing access of gene regulatory factors [1] (Figure 1). The intranuclear radical distribution of chromosome territories seems to be nonrandom, with an evolutionarily conserved feature that gene-poor chromosomes tend

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to be oriented to the nuclear periphery and gene-rich chromosomes tend to localize in more internal nuclear regions [2]. However, these structures and distributions are not static. The mobility of chromatin allows dynamic interactions between genomic loci and loci with other nuclear structures. It has been shown that in some cases the repositioning of specific loci with respect to nuclear domains is correlated with their transcriptional activity [111]. Whether these specific interactions provide means to regulate gene expression or are the consequences of changes in transcriptional activity remains to be determined. The higher order organization of chromosome territories and its relationship with the regulation of gene expression merit further investigation with a higher spatial and temporal resolution.

Nuclear bodies spatially compartmentalize the nuclear environment and create distinct sites within a confined volume, thereby concentrating reactants and substrates to potentially facilitate more efficient biological reactions. This compartmentalization paradigm is similar to that which occurs in the cytoplasm, where intracellular organelles carry out various metabolic processes in isolated areas demarcated by membranes to achieve specificity and efficiency. However, unlike cytoplasmic organelles, nuclear bodies lack a defining membrane to separate them from their surroundings. Recent studies are beginning to elucidate the molecular mechanisms responsible for the assembly and maintenance of several nuclear bodies [5-7].

In this review, we highlight recent advances in our understanding of the biogenesis and organization of nuclear bodies, focusing primarily on mechanisms of the assembly process. We emphasize the functional implications of several nuclear bodies during cellular differentiation and development. The significance of some nuclear bodies in response to stress and their connections with human diseases are also discussed.

Nuclear bodies are highly organized and dynamically regulated

Although not membrane-bound, nuclear bodies maintain their structural integrity at steady-state, implicating a biogenesis and maintenance mechanism that differs from membrane-bound cytoplasmic organelles [8, 9]. Elucidating the mechanism(s) of how cells assemble, maintain, and regulate nuclear bodies in fine molecular details is critical to understanding their biological functions in different metabolic conditions and during development, and their potential contribution to human diseases.

Nuclear bodies are dynamic steady-state structures

The absence of membranes around nuclear bodies allows their components to exchange more freely with the surrounding nucleoplasm. Most protein components of nuclear bodies are also diffusely distributed in the interchromatin spaces at lower concentrations. Fluorescence recovery after photobleaching (FRAP) studies have demonstrated the rapid exchange of many components between a nuclear body and the nucleoplasm. For example, all three paraspeckle core protein components (PSP1, p54nrb and PSF) show a similar mobile fraction (60-70%) within the paraspeckle. The recovery half life after photobleaching ($t_{1/2}$) of these mobile populations, which is on the order of seconds, is larger than their nucleoplasmic populations [6]. The delayed mobility of proteins within paraspeckles as compared to the nucleoplasmic population is similar to what has been shown for SRSF1 (SF2/ASF) in speckles and fibrillarin in nucleoli [10, 11], reflecting the intricate protein-protein and protein-RNA interactions within paraspeckles. These protein-protein/RNA interactions account for the structural integrity of the paraspeckle. In fact, paraspeckle proteins form homo- or hetero-oligomers by coiled-coil domains and associate with RNAs by RNA recognition motifs, and the deletion of these interacting motifs results in the loss of

paraspeckles [12]. These self-association properties have also been shown for many other components in most other nuclear bodies [8]. Thus, the rapid and free exchange of individual components and their self-association properties determine that nuclear bodies are steady-state structures formed by dynamic interactions of protein-protein and/or protein-RNA components in the nucleoplasm.

Three models have been proposed to explain how dynamic interactions of individual components in the nucleoplasm results in the assembly of nuclear bodies [6, 8, 9] (Figure 2). In the stochastic assembly model, the structure of nuclear bodies builds up by essentially stochastic interactions of individual components. The assembly process is largely random and with no strict hierarchical order. Each component is equal in terms of assembly order; so multiple pathways can be followed to assemble a nuclear body (Figure 2a). Alternatively, the ordered assembly model posits that the assembly steps follow a tightly controlled sequential order. Individual components are hierarchically different; therefore only one or limited numbers of pathways can lead to the assembly of a nuclear body (Figure 2a). Both models represent extreme theoretical scenarios and it is not yet clear if they are strictly followed in actual biological settings. Alternatively, nuclear body assembly may follow a compromised assembly process. For example, a seeding assembly model has been proposed in which a single or subset of components act hierarchically as a seed to initiate nuclear body formation (Figure 2b).

Stochastic assembly model—An earlier study using a bacterial Lac operator/repressor (LacO/LacI) tethering system, in which LacI fusion protein can be artificially tethered to the integration site of the LacO array within the mammalian cell genome, has provided evidence that the CB might be *de novo* assembled via stochastic interactions [5]. In this system, individual CB components were tethered to a genomic locus in HeLa cells. The immobilization of any given component was sufficient to initiate the recruitment of most, if not all, of the other CB components. The newly formed structures had a similar size, shape, and composition to endogenous CBs and their components also had comparable dissociation kinetics. Thus, these *de novo* formed structures were regarded as “*bona fide*” CBs. However, it is possible that the CBs observed at the tethering sites are endogenous CBs that have recruited the LacO array, similar to what has been shown using a LacI-Lamin B fusion protein to tether a locus to the nuclear lamina [13]. Live cell imaging experiments are required to distinguish between these scenarios. Since many components can initiate the formation of new CBs, there should be no strict requirement for a sequential order of assembly, supporting the conclusion that the assembly of CBs follows the stochastic model. These findings represent a major step forward for understanding the mechanism of nuclear body assembly. However, it remains to be determined if these structures are actually functional CBs, whether this assembly reflects the native *in vivo* situation, and if so, what nuclear elements serve as the endogenous “tethers”. Nevertheless, this experimental system allows the testing of models for nuclear body organization and provides potential ways to probe nuclear body function.

Seeding assembly model—A recent study has adopted a similar strategy to tether individual paraspeckle proteins in C2C12 myoblasts [6]. In this case, protein tethering did not efficiently recruit other paraspeckle protein components or Men ϵ/β (also known as Neat1) noncoding RNAs (ncRNAs), important structural components of paraspeckle. However, some paraspeckle protein components were shown to interact in the absence of paraspeckles demonstrating that recruitment of some protein components to the tethered site is an indication of protein-protein interactions rather than paraspeckle assembly. Finally, the protein complex formed at the tethering site showed no capacity to retain adenosine to inosine (A-to-I) hyperedited mRNAs (Box 2), which is a known feature of paraspeckles. Hence, it was concluded that paraspeckles do not form via a stochastic assembly model.

Box 2**Adenosine to Inosine (A-to-I) RNA Editing**

The deamination of adenosine to inosine (A-to-I) in RNA is catalyzed by members of adenosine deaminase that acts on RNA (ADAR) enzyme family [112]. These enzymes are ubiquitously expressed in the nuclei of higher eukaryotes and target double-stranded structures encompassing exons, intron, and 5' and 3' untranslated regions (UTRs). A-to-I editing can be site-selective or promiscuous. Since inosines are recognized as guanosines by the translation machinery, site-selective A-to-I editing within coding sequences alters codon meaning therefore diversifying the proteome [112]. Mammalian ADAR2 acts on an intron of its own pre-mRNA creating a new splice site which results in the change of splicing pattern [113]. It has been shown that the presence of inverted repeated Alu elements or short interspersed nuclear elements (SINEs) in the 3' UTR can induce A-to-I hyperediting in many mRNAs. This event has been proposed to result in nuclear retention of some hyperedited mRNAs by promoting their binding with p54nrb and PSF, two major paraspeckle components [114]. For example, mouse Cat2 transcribed nuclear RNA (CTN-RNA) is transcribed from the mouse cationic amino acid transporter 2 (mCat2) gene loci and the 3' UTR of CTN-RNA contains SINE elements required for A-to-I hyperediting [115]. Under normal condition, A-to-I hyperedited CTN-RNA is retained in the nucleus, partially within paraspeckles. Under stress, the 3' UTR of CTN-RNA is post-transcriptionally cleaved to produce mCat2 mRNA which is then released to the cytoplasm [115]. However, many endogenous mRNAs with inosines in their 3' UTRs have been found on polysomes within the cytoplasm of mammalian cells and *Caenorhabditis elegans*, suggesting that this nuclear retention phenomenon is not absolute [116]. A-to-I editing alters RNA structure, coding potential, splicing pattern, or cellular distribution, and offers means to regulate gene expression at a variety of post-transcriptional levels.

Studies to understand paraspeckle assembly turned to analyses of Men ϵ/β ncRNAs, as the depletion of these ncRNAs results in paraspeckle disassembly [14-18]. To address the role of Men ϵ/β ncRNAs in paraspeckle assembly, a live cell imaging system was developed that allows for the inducible transcription of Men ϵ/β ncRNAs and the direct visualization of the recruitment of paraspeckle proteins [6]. Upon induction of Men ϵ/β ncRNAs transcription, all four paraspeckle proteins tested (PSP1, p54nrb, PSF and PSP2) were efficiently recruited to the transcription sites. Multiple criteria were applied to conclude that the newly formed structures are *bona fide* functional paraspeckles. First, all three core paraspeckle proteins exhibited indistinguishable kinetics in *de novo* formed and endogenous paraspeckles. Second, *de novo* formed paraspeckles had similar assembly/disassembly dynamics through the cell cycle compared with their endogenous counterparts. Third, *de novo* formed paraspeckles were functional as they had the ability to retain A-to-I hyperedited mRNAs. Furthermore, it was demonstrated that it is the active transcription of the *Men \epsilon/\beta* gene, together with the ncRNAs, that regulates paraspeckle maintenance and dynamics. Finally, FRAP analyses revealed a significant longer $t_{1/2}$ of Men ϵ/β ncRNAs than protein components in paraspeckles, consistent with a mechanism wherein Men ϵ/β ncRNAs serve as the seeding molecules to assemble paraspeckles.

These results have identified a hierarchical difference between ncRNA and protein components and established that nascent Men ϵ/β transcripts act as a seed to recruit nucleoplasmic proteins to assemble paraspeckles. The subsequent assembly steps after the initial RNA seeding event could be either stochastic or ordered, which needs to be distinguished by future live cell imaging studies. In this seeding model, one initial component is sufficient and necessary to initiate the nuclear body assembly process (Figure

2b). This concept emphasizes the initial determinant as the pivotal and essential factor, and studies aimed at signaling pathways that control the availability of seeding molecules will expand our understanding of how such nuclear bodies are dynamically regulated.

Nuclear body organization is highly regulated

The RNA seeding model wherein newly transcribed RNAs provide platforms to assemble nuclear bodies sheds light on the regulatory mechanism of controlling the location, size, and number of nuclear bodies. It was found that paraspeckles form and localize in close proximity to the *Men ε/β* gene loci, corroborating that *Men ε/β* ncRNAs continuously serve as a nucleator and active transcription is required to maintain paraspeckles. We also found that the size of paraspeckles correlates with the amount of *Men ε/β* ncRNAs being transcribed. However, there is apparently a size limit and once the size of the paraspeckle exceeds this limitation, fission/splitting/budding events occur to generate a cluster of paraspeckles that remain in the vicinity of the *Men ε/β* gene locus. Indeed, only two paraspeckles or two clusters of paraspeckles are observed adjacent to two *Men ε/β* gene loci in normal diploid mammalian tissue (unpublished data), whereas many more are observed next to multiple *Men ε/β* gene loci in aneuploid cultured cells [6].

The advantage of the seeding model is to provide means to rapidly regulate nuclear bodies by modulating the level of seeds in the nuclei in response to environmental stimuli, differentiation states, and metabolic conditions. For example, human embryonic stem cells (hESCs) lack the expression of *Men ε/β* ncRNAs and paraspeckle structures [14]. Upon hESC differentiation, the expression of *Men ε/β* ncRNAs increases, resulting in the formation of paraspeckles which retain A-to-I hyperedited mRNAs in the nuclei of differentiated cells [14]. hESCs lacking paraspeckles efficiently release hyperedited *Lin28* mRNAs to the cytoplasm which contributes to pluripotency [19]. The increase of *Men ε/β* ncRNAs expression has also been demonstrated upon muscle differentiation and viral infection, although the biological functions in these processes are yet to be identified [17, 20].

RNA seeding serves to assemble multiple nuclear bodies—The paradigm of utilizing the RNA seeds to nucleate and organize nuclear bodies also applies to other nuclear bodies. Histone locus bodies (HLBs) are enriched in histone pre-mRNA 3' end processing components and are known to be involved in processing of histone pre-mRNAs [21]. By artificially tethering histone H2b pre-mRNAs, which are tagged with bacteriophage-derived MS2 stem-loop structures, to a specific engineered site in the HeLa cell genome, NPAT, a HLB component, was recruited to the tethering site implying that histone pre-mRNA seeds the formation of HLBs [7]. However, the assembly process *in vivo* might be more complex. Histone pre-mRNA transcription and processing are limited to S phase. While in *Drosophila*, HLBs are found throughout the cell cycle [21]. Similarly, tethering human satellite (Sat) III transcripts can recruit components of nuclear stress bodies (nSBs) indicating the formation of nSBs even in the absence of stress [7]. nSBs are unique nuclear structures which form upon heat shock and other cellular stress. Upon heat shock, HSF1 binds to human pericentric heterochromatin on chromosome 9 q11-12 region composed of long tandem arrays of Sat III repeats [22, 23]. HSF1 redistribution initiates a series of events including the recruitment of CBP/p300, chromatin remodeling, the recruitment of RNA polymerase (Pol) II, and the transcriptional activation of pericentric heterochromatin. Nascent Sat III RNA transcripts then act as seeds to assemble nSBs, which could function as a molecular trap for transcription and splicing factors contributing to global shut down of transcription and splicing alteration. Alternatively, these transcripts may play roles in heterochromatin assembly and maintenance, or affect the organization of the cell nucleus in response to stress [22, 23].

Proteins can also act to seed nuclear bodies—In addition to RNA seeding nuclear bodies, certain proteins could also serve as the seed to assemble some nuclear bodies. The tumor suppressor protein promyelocytic leukemia (PML), a major component of the PML-nuclear body (PML-NB) (a.k.a. nuclear domain 10, ND10), is essential for PML-NB formation since human leukemia and solid tumors cells in which the *PML* gene is inactivated and *pml*^{-/-} mouse cells fail to assemble PML-NBs [24]. On the other hand, tethering PML initiates the *de novo* formation of PML-NBs (as defined morphologically by recruiting another component, SP100) [5]. In addition, PML and other PML-NBs components, such as SP100 and DAXX, can be post-translationally modified by small ubiquitin-like modifier (SUMO). PML and DAXX both contain SUMO-interacting motifs (SIMs) allowing PML to interact with itself and other proteins through sumoylation and SIMs. A PML mutant that cannot be modified by SUMO fails to recruit SP100 and DAXX, and de-sumoylation of PML during mitosis results in the disassembly of PML-NBs [24]. CK2 kinase-mediated phosphorylation within SIM of DAXX has recently been demonstrated to increase its binding affinity to SUMO, therefore facilitating DAXX and PML interaction and DAXX targeting to PML-NBs [25]. Finally, under stress such as viral infection and senescence, PML is upregulated and the size and number of PML-NBs increase [26]. Together, these lines of evidence indicate that PML, presumably the sumoylated form, works as a seed to assemble and regulate PML-NBs.

In summary, the interactions among the protein and RNA components of nuclear bodies determine their structural integrity. However, the rapidly exchanging pools of components and the transient nature of their interactions make these steady-state macromolecular complexes highly dynamic, flexible, and prepared for change in response to various forms of stress and different signaling events. Additional investigations are needed to assess the mechanisms by which other nuclear bodies are formed and maintained. In addition, future studies focusing on how cells regulate the assembly process of nuclear bodies in the context of cell differentiation and human diseases at the tissue level will significantly advance our understanding of the precise physiological functions of nuclear bodies.

Nuclear bodies exert diverse and important biological functions

The genome is physically and functionally organized into complex higher-order structures. Nuclear bodies provide a variety of specific functionally compartmentalized microenvironments within the three-dimensional nuclear volume (Figure 1). By concentrating proteins and/or RNAs required for specific biological processes, nuclear bodies can serve as reaction sites to efficiently facilitate these processes (Figure 3a). In addition, by providing unique compartmentalized microenvironments, nuclear bodies can act as hubs to regulate the expression of recruited gene loci (Figure 3b). Nuclear bodies can also function as storage/modification sites to recycle and modify RNAs and proteins (Figure 3c). Notably, one nuclear body is very likely to combine different themes to execute diverse functions and accommodate different processes at the same time. In the following sections, we focus on the functional properties of four prominent nuclear bodies: nucleoli, CBs, nuclear speckles, and Polycomb bodies. Using these bodies as examples we describe their connections to higher-order genomic organization, epigenetic modifications and gene expression, present their relevance for physiological and pathological processes, and summarize common themes of how nuclear bodies function.

Nucleolus

rRNA biogenesis—The nucleolus is organized around the multiple tandemly-arrayed rDNA clusters known as nucleolus organizer regions (NORs), and it is the site of rDNA transcription, pre-rRNA processing and modification, and initial steps of pre-ribosome assembly and maturation [27]. Nucleoli in mammals and birds typically contain three

morphologically distinct subregions termed fibrillar centers (FCs), dense fibrillar component (DFC) and granular component (GC) (Figure 1). The tripartite architecture of the nucleolus reflects its function as rDNAs are localized in the FCs; transcription occurs on the border between the FCs and the DFC; fibrillarin and small nucleolar ribonucleoproteins (snoRNPs) are in the DFC where pre-rRNAs are processed; and pre-ribosomal subunits accumulate in the GC where they are assembled [28]. Nucleolar organization changes when ribosome biogenesis is altered. Low doses of actinomycin D which inhibit RNA Pol I transcription, and many forms of stress which affect rDNA transcription have been shown to induce nucleolar reorganization [29, 30].

Mammalian rDNA clusters are characterized by multiple alternating modules of a long intergenic spacer (IGS) of approximately 30 kb and a pre-rRNA coding region of approximately 14 kb. The level of rDNA transcription in eukaryotic cells is tightly regulated according to the protein synthesis requirements of the cell. It has been estimated that approximately half of the several hundred copies of rDNA repeats are transcriptionally active. Generally, transcriptionally active rDNAs exhibit an open euchromatic structure with histone H4 acetylation and histone H3 lysine 4 (H3K4) methylation. Transcriptionally silent rDNA repeats display a more condensed heterochromatic structure with H4 hypoacetylation and H3K9, H3K27, and H4K20 methylation [31].

Recent studies have demonstrated profound and complex roles of long noncoding RNAs (lncRNAs) (Box 3) in regulating rDNA expression. lncRNAs extending 150-250 nucleotides, termed pRNAs, have been found to originate from a RNA Pol I promoter located within the IGS. A specific stem-loop structure found in pRNAs is conserved in several mammalian species and involved in binding with and the recruitment of the nucleolar remodeling complex (NoRC) to rDNA [32]. NoRC, a chromatin remodeling complex, then shifts the positions of rDNA promoter-bound nucleosomes and acts as a scaffold coordinating the activities of other enzymes to rewrite histone modifications, methylate DNA, and establish heterochromatin [33]. pRNA could also form a DNA:RNA triplex structure that is specifically recognized by the DNA methyltransferase DNMT3b to achieve *de novo* CpG methylation at the rDNA promoter region [34]. Moreover, two nucleolar proteins PHF8, a member of JmjC family of histone demethylases, and KDM2B, a histone demethylase, have also been shown to play roles in regulating the epigenetic state of rDNAs [35-37]. These mechanisms seem to operate at the level of the promoter within each individual rDNA repeat. However, nucleolar dominance (Box 4) has clearly demonstrated that an entire NOR can be repressed as a unit.

Box 3

Long Noncoding RNAs (lncRNAs)

The majority of mammalian and other eukaryotic genomes are transcribed into ncRNAs [117]. lncRNAs are arbitrarily considered longer than 200 nucleotides in length. The expression of some lncRNAs exhibits development- and tissue-specific patterns and the sequence of some lncRNAs is evolutionary constrained, suggesting that they are not merely transcriptional noise [118]. An emerging body of evidence has demonstrated that they play important biological functions, such as in transcriptional interference, epigenetic regulation, dosage compensation, parental imprinting, and nuclear organization, therefore contributing to development and pathogenesis [119-122].

Box 4**Nucleolar Dominance**

Nucleolar dominance is an epigenetic phenomenon observed in interspecific hybrid cells in which the expression of rDNAs is inherited from one parental species due to the silencing of those derived from the other parent. It occurs in both plant and animal kingdoms and provides an excellent system to study how genes are chosen to be and maintained silent epigenetically and how this process is established and regulated [123]. Species-specificity of the Pol I transcription machinery and enhancer-imbalance (the dominant NOR is that containing the greater number of enhancer repeats within the IGS) hypotheses can explain this phenomenon in human-mouse somatic cell hybrids and *Xenopus* hybrids, respectively [124]. However, the molecular basis of NOR selection and details of its repression remain unclear in plants. Short interfering RNAs (siRNAs) and RNAi pathway components have been shown to be involved in this intriguing process [125].

Other functions of the nucleolus—The nucleolus has been shown to be the site of RNA processing and modifications and assembly of multiple ribonucleoprotein (RNP) complexes, such as the telomerase RNP, RNase P RNA, the signal recognition particle RNA, and microRNAs [27, 38, 39]. The nucleolus can also regulate post-translational modifications such as sumoylation and phosphorylation of some nuclear proteins, and sequester specific proteins to inhibit their activities during the cell cycle to coordinate and regulate multiple aspects of cell cycle progression [27]. In addition, the nucleolus is a target of many viruses. Many virally encoded proteins are detected in the nucleolus and the localization could potentially contribute to virus replication and export of viral RNAs [40]. Finally, the nucleolus plays essential roles coordinating multiple functional aspects in response to many forms of stress. One prominent example is the critical role of the nucleolus in regulating the level of the tumor suppressor protein p53 under stress and its implication to human disease, which has been comprehensively and systematically reviewed [29].

The perinucleolar region—The nucleolus is generally surrounded by a shell of highly compact heterochromatin, known as the perinucleolar heterochromatin (Figure 1). It contains satellite DNA that surrounds NORs, and silent rDNA clusters [4, 31]. Condensation of a part of the rDNA repeats into the perinucleolar heterochromatin could be a general strategy against recombination of these highly repeated rDNAs, thereby preserving rDNA stability [41]. Live cell imaging analysis of chromatin indicated that the perinucleolar association constrains the movement of DNA sequences at different sites in multiple chromosomes [42]. In fact, the perinucleolar region has been demonstrated to function in the maintenance of silencing of non-ribosomal genomic regions. During X-chromosome inactivation, the X-inactivation center targets the inactive X chromosome (Xi) to the perinucleolar region and it has been proposed that the Xi must continuously visit this region during mid-to-late S phase, when the Xi is replicated, to duplicate its epigenetic state and stably repress resident genes. The association of the Xi with the perinucleolar region is dependent on a lncRNA, Xist [43]. By serial-section transmission electron microscopic analysis, a subsequent study confirmed this association and further revealed the extensive contacts of the Xi with the nuclear envelope [44]. A system has recently been developed in ES cells to visualize the X-inactivation center and pairing prior to X-chromosome inactivation [45]. Future studies, including live cell imaging analysis using this system, are essential to determine if, how, and why the Xi continuously visits the perinucleolar region and to characterize the involvement of the nuclear envelope during X chromosome inactivation. Separate studies have found that another lncRNA, Kcnqlot1 antisense ncRNA,

mediates the relocalization of an imprinted chromatin region to the perinucleolar region in mid S phase which correlates with subsequent bi-directional imprinting [46, 47]. In *Saccharomyces cerevisiae*, tRNA-mediated gene silencing, in which tRNA genes transcribed by RNA Pol III can silence nearby Pol II promoters, requires perinucleolar localization as well [48]. Collectively, the temporal and spatial compartmentalization of various chromosomal regions to the perinucleolar region may provide a mechanism to replicate and maintain repressive epigenetic chromatin states of the Xi, imprinted chromatin regions, and other heterochromatin (Figure 3b). Further studies are required to dissect the precise roles that lncRNAs, such as Xist and Kcnq1ot1, play in perinucleolar localization and to analyze their functional relationships with histone modifying enzymes, chromatin remodeling complexes, and the DNA replication machinery.

The perinucleolar region also harbors nuclear bodies with unknown functions, such as the perinucleolar compartment (PNC) [49, 50] and the Sam68 nuclear body (SNB) [51]. The PNC contains nascent Pol III transcripts and RNA-binding proteins and the SNB contains various RNA-binding proteins. It seems unlikely that the interaction of Xi or imprinted region occurs in the PNC or SNB since both bodies are primarily found in transformed cells [52]. Notably, PNC prevalence is positively correlated with the metastatic capacity of many types of cancer and has been proposed to serve as a marker for tumor diagnosis and anti-tumor drug selection [53]. However, the specific function of the PNC in malignancy remains to be determined.

In summary, the concept of the nucleolus being “plurifunctional” was first raised in 1998 [38] and is now widely accepted [27, 29]. As the site of rRNA synthesis, the nucleolus provides a perfect system to study Pol I transcription, gene silencing, and heterochromatin metabolism. Moreover, the nucleolus is the modification/assembly site for many other RNAs/RNPs, regulates proteins important for cell cycle progression, and directs cellular responses to many forms of stress. Finally, the perinucleolar region provides a unique compartment for the establishment and maintenance of gene silencing.

Cajal body

snRNPs assembly/modification—The CB contains a variety of proteins and RNAs involved in the assembly and modification of small nuclear RNP (snRNPs) and snoRNPs [21]. For example, spliceosomal snRNAs are first transcribed in the nucleus, exported to the cytoplasm, each assembled into a complex with seven conserved Sm proteins, and hypermethylated at their 5' end. The newly assembled snRNPs are subsequently imported back into the nucleus. They concentrate first in CBs, later travel to speckles, and eventually move to active genes where they play essential roles in pre-mRNA splicing [54]. CBs have been suggested to play roles in promoting some final steps of snRNP maturation and/or facilitating the interaction of individual snRNPs to form higher-order complexes [21]. However, *Arabidopsis thaliana* and *Drosophila melanogaster* lacking CBs, due to the deficiency of a conserved CB protein coilin, are fully viable and develop normally [55, 56]. Coilin knockout mouse lines, which do not form CBs, display semilethality with about 50% dying in the gestation stage. The surviving adults show fertility and fecundity defects [57]. In these three organisms studied, coilin or the CB is not essential for viability. However, a recent study of the function of coilin and the CB during embryogenesis in the zebrafish *Danio rerio* suggested otherwise [58]. Morpholino-mediated knockdown of coilin resulted in the loss of CBs and the dispersal of snRNPs, and led to developmental arrest at the 15- to 16-somite stage in the embryo, presumably because of increased intron retention and reduced mRNA production. Remarkably, the developmental defect could be rescued by the injection of pre-assembled mature human snRNPs, but not the snRNAs or snRNP proteins

alone, suggesting that coilin and possibly CBs are essential for the efficient macromolecular assembly of snRNPs in zebrafish [58].

The CB also harbors a class of CB-specific RNAs (scaRNAs) that are involved in the post-transcriptional modification of snRNAs. Studies in cultured cells demonstrated that scaRNAs in the CB are important for methylation and pseudouridylation of snRNAs [59]. Since all of the snRNAs are correctly modified in coilin-null flies, which lack CBs but have normal levels of scaRNAs, it has been suggested that these scaRNA-mediated snRNA modifications can occur in the absence of CBs [21]. Thus, the role of the CB *per se* might be locally concentrating the reactants thereby promoting the efficiency of snRNPs assembly and modification (Figure 3a). If any snRNPs maturation step concentrated in the CB becomes rate-limiting due to an altered metabolic requirement (for example, embryogenesis in zebrafish discussed earlier), cells lacking coilin and subsequently CBs might exhibit apparent sensitivity and a notable disadvantage. This presents challenges to identify the appropriate environmental and developmental cues to reveal the physiological functions of the CB. These challenges might apply to other nuclear bodies as well, such as the paraspeckle [18].

Telomerase RNP assembly—The CB also has a role in telomerase assembly and telomere length homeostasis. Telomerase RNP enzyme is composed of telomerase RNA (TR) and telomerase reverse transcriptase (TERT) and is responsible for maintaining telomeric DNA at the ends of eukaryotic chromosomes. The 3' end of TR is closely related to box H/ACA motif-containing snoRNAs and scaRNAs [60]. TR and TERT have been shown to localize in CBs in human cancer cell lines, suggesting that the CB may function in some aspects of telomerase RNP assembly and maturation [61, 62]. During S phase, when telomeres are elongated, TR-containing CBs were observed to move and make transient associations with telomeres indicating that specific interactions occur between CBs and telomeres [63-65]. Together, these results imply a fascinating hypothesis that the CB mediates the assembly of telomerase RNP and the delivery of telomerase to a subset of telomeres during S phase to regulate telomere elongation [66].

In summary, the CB promotes the efficiency and specificity of snRNPs modification/assembly by enriching the relevant components, and might not be essential under “normal” conditions but become indispensable under certain stress. It is important to determine the physiological or pathological conditions under which the CB is essential. The possibility that CB localization/delivery licenses telomerase RNP for telomere elongation also merits further investigation.

Nuclear speckle

Storage/modification sites of the splicing machinery—Nuclear speckles (a.k.a. interchromatin granule clusters) are generally regarded as the storage/modification compartment of pre-mRNA splicing factors, including snRNPs and serine/arginine-rich (SR) proteins. Several kinases and phosphatases that can phosphorylate and dephosphorylate components of the splicing machinery respectively have been found localized in speckles, supporting its role in regulating the post-translational modification of splicing factors [67] (Figure 3c). Speckles have long been recognized to contain a population of poly(A)⁺ RNAs [68-70] and a recent report suggested that a lncRNA, Malat1, is part of these long-sought after poly(A)⁺ RNAs enriched in speckles [71]. Knockdown of Malat1 results in a reduction in the recruitment of SR family splicing factors to the transcription site of a transgene array, suggesting that Malat1 plays an important role in splicing factor dynamics [72]. The contribution of Malat1 to pre-mRNA splicing/processing has also been demonstrated in regard to various endogenous genes [72-74]. A recent study examining alternative splicing

patterns after Malat1 knockdown provides some insights into this process [75]. Malat1 depletion in HeLa cells has been shown to significantly decrease the level of phosphorylated SR proteins, which affects the alternative splicing of many genes thereby resulting in aberrant mitosis and increased cell death [75]. However, it remains to be determined if the altered alternative splicing pattern is the direct consequence of Malat1 depletion or a secondary effect because of cell death and mitosis defects, what kinases/phosphatases are responsible for the phosphorylation change of SR proteins, and how these enzymes are modulated by Malat1.

Hubs for gene activation—Previous findings have demonstrated that speckles are in close proximity to many active genes [67]. Multiple specific genes have been shown to cluster around a common nuclear speckle. Therefore, nuclear speckles have been proposed to serve as hubs of enhanced mRNA metabolic activity involved in mRNA maturation and export [76-81]. A recent study has indicated that the association of the *Hsp70* gene with speckles is mediated by its promoter and dependent on active transcription, but does not correlate with the level of nascent transcript accumulation [82]. Furthermore, given that the *Hsp70* transcript does not contain introns, the functional implication of this association remains to be determined. However, proteins other than pre-mRNA splicing factors, such as some transcription factors, serine 2 phosphorylated RNA Pol II, and components of the transcription elongation complex have also been shown to localize in speckles [67].

Based on these findings, one can envisage the possibility of speckles serving as “hubs” to link active transcription sites (Figure 3b) as has been suggested for other nuclear regions such as “transcription factories” [83, 84]. Indeed, two recent reports have revealed interchromosomal interaction events induced by hormones, which could be potentially mediated by nuclear speckles [85, 86]. In MCF7 breast cancer and human mammary epithelial cells, estradiol triggers the juxtapositioning of two estrogen receptor α (ER α)–responsive genes (*TFF1* and *GREB1*) at nuclear speckles, accompanied by the long-distance movement and interaction of their respective chromosomes 21 and 2 [85]. This association is dependent on ER α and its co-activator CBP/p300. It remains to be determined by live cell imaging studies if the highly active genes move together and recruit a large amount of splicing factors thereby cytologically resembling a speckle or if the genes actually move to an existing speckle [87]. Moreover, this rapid and long-range movement is implied to be mediated by actin and nuclear myosin 1, which have been suggested to play a role in several aspects of transcription [88-90]. Interestingly, nuclear speckles have also been shown to contain actin, a critical actin regulator–phosphatidylinositol 4,5-bisphosphate, and the α isoform of phosphatidylinositol 4-phosphate 5-kinase [91]. The precise roles of actin and myosin in mediating gene movements and the potential function of speckles in this process remain to be deciphered. Depletion of the histone lysine demethylase LSD1, that is essential for the activation of ER α –responsive genes, has no effect on the *TFF1* and *GREB1* association, but prevents the localization of both loci within speckles and attenuates the transcriptional activation of both genes [85]. These results reveal an essential role of LSD1 in targeting loci to speckles, and the potential function of the speckle in coordinating transcriptional regulation and co-transcriptional processing [85].

Likewise, in LNCaP prostate cancer cells, androgen induces specific intra- and interchromosomal interactions to generate spatial proximity for the androgen receptor (AR)–responsive gene *TMPRSS2* and two other genes (*ERG* and *ETV1*) at speckles [86]. AR then promotes site-specific DNA double strand breaks (DSBs) by recruiting activation-induced cytidine deaminase and the LINE-1 repeat-encoded ORF2 endonuclease to the interacting loci at speckles. DSBs are later ligated by non-homologous end joining (NHEJ) to create non-random chromosomal translocations in prostate tumors [86]. The potential involvement

of speckles in recruiting and assisting the DSB and NHEJ machineries remains to be elucidated.

AR-induced gene loci interactions were also observed in an independent study [92]. However, ER-induced interactions could not be detected in MCF7 and human mammary epithelial cells by a different group [93]. Although the same cell lines were used and the same conditions were followed, such long-range chromosome movement and rapid gene interaction could not be replicated. Moreover, while the initial study suggested a diploid representation of chromosomes 21 and 2 which harbor the *TFF1* and *GREB1* genes respectively [85], the MCF7 cells used in this study are from hypertriploid to hypotetraploid with 4-6 *TFF1* or *GREB1* gene loci [93]. Thus, further investigation is required to resolve this discrepancy [94].

In summary, splicing factors are stored and modified at nuclear speckles and are recruited to active genes that are at nuclear sites away from speckles or reside on the periphery of speckles. This proximity to speckles may increase the efficiency of splicing of some genes. Future studies are needed to assess whether speckles may act as hubs to directly influence gene expression.

Polycomb body

Hubs for gene repression—Polycomb group (PcG) proteins are gene-silencing proteins that regulate the expression of a variety of genes. PcG proteins form at least two classes of complexes designated Polycomb repressive complexes 1 and 2 (PRC1 and PRC2) that are thought to collaborate to repress gene transcription [95]. Examination of the localization of PcG proteins has revealed that they are organized into distinct nuclear domains called Polycomb or PcG bodies, which are often localized close to pericentromeric heterochromatin [96]. In *Drosophila melanogaster*, PcG proteins silence *Hox* genes through binding to *cis*-regulatory DNA modules, called Polycomb response elements (PREs) [97]. *Drosophila* PREs are responsible for inducing long-distance pairing/clustering of multiple endogenous *Hox* genes, and a transgene containing *Fab-7*, a well-characterized PRE-containing element. The pairing events occur specifically within PcG bodies [98, 99] (Figure 3b). Intriguingly, three components of the RNA interference (RNAi) machinery, Dicer2, PIWI, and Argonaute1, also localize to distinct nuclear foci and a subset of these foci in *Drosophila* are frequently colocalized with PcG bodies [98]. After gene pairing is established in PcG bodies, bi-directional transcription in the vicinity of the PRE is stimulated and the RNAi machinery associated with the PcG body cleaves dsRNA to produce siRNA, which is proposed to participate in stabilizing the chromosomal pairing and maintaining gene silencing [98]. In addition, the loss of association of one *Hox* gene locus with PcG bodies remarkably de-represses the expression of other *Hox* gene loci, suggesting that specific spatial nuclear organization of *Hox* gene pairing within PcG bodies reinforces PcG-mediated gene silencing [100]. Strikingly, once long-distance pairing is abolished by removal of endogenous *Fab-7*, the de-repressed chromatin state induced at the transgene locus can be transmitted through meiosis into a large fraction of the progeny [101].

Sumoylation centers—PcG bodies have also been found in human cells and they appear to directly associate with pericentromeric heterochromatin [102]. Recently, Polycomb-dependent regulatory regions have been identified in vertebrate genomes [103, 104]. However, how PcG bodies function in mammalian systems remains to be determined. The human PcG protein Pc2 has been demonstrated to act as a SUMO E3 ligase by bringing the SUMO E2 (Ubc9) and the substrates (CtBP and CTCF) together, thereby making PcG bodies sumoylation centers [105, 106] (Figure 3c). Moreover, the *Caenorhabditis elegans*-specific PcG protein SOP-2 is sumoylated, and contains RNA binding motifs which are

capable of binding small RNAs and are evolutionarily conserved in vertebrate PcG proteins [107, 108]. Furthermore, recent studies have shown CTCF/cohesin to be implicated in long-range interchromosomal interactions in many systems [109]. Therefore, it is tempting to speculate that PcG-dependent sumoylation may functionally relate to RNAi and CTCF-mediated chromosomal interaction and repression.

In summary, over the past several years, we have gained significant insights into the functions of PcG bodies and PcG-mediated gene pairing, but several important questions remain to be answered. The pairing has been suggested to occur in a locus- and tissue-specific manner [110]. Therefore, identification of tissue-specific factors and characterization of intrinsic properties of specific gene loci are required to fully understand the molecular mechanism underlying this process. How this long-range pairing is accomplished also remains largely unknown. It has been postulated that PRE-containing PcG target genes dynamically localize to PcG bodies, such that genes localized within one PcG body may only linger for a certain time and then leave to incorporate into another PcG body [101]. This PcG body-hopping process prevents PcG target genes from diffusing away randomly in the nucleoplasm, but at the same time, allows these genes to explore parts of the nucleus and to stay in the vicinity of other genes via PcG bodies. Once proximity is achieved, a strong association might be established by regulatory components either on chromatin or from other protein machineries [101]. Live cell imaging with high temporal resolution is crucial to verify the PcG body-hopping hypothesis. PcG target genes are distributed throughout all chromosomes. How the interactions of these genes are re-established after each round of cell division and what roles PcG bodies play during meiotic/mitotic inheritance also warrant further investigation.

Concluding remarks

Nuclear bodies are highly dynamic structures involved in the modulation of numerous nuclear activities. Studies of their biogenesis have provided important mechanistic and molecular insights into their organization and function. While certain nuclear bodies form *de novo* in a stochastic fashion, others are organized via a seeding model whereby RNA or protein molecules serve as the nucleator to initiate nuclear body assembly. The seeding model provides cells with means to rapidly regulate the localization, number, size, and activity of nuclear bodies by modulating the availability of seeds, under normal physiological conditions or in response to various forms of stress, altered metabolism, and differential signaling. This ability allows for the potential regulation of gene expression at multiple levels.

The conceptual framework of the biological functions of nuclear bodies is just beginning to be uncovered (Figure 3), molecular and mechanistic details still remain largely incomplete. A comprehensive understanding of the three-dimensional organization of the genome in the context of the nuclear body landscape is crucial to fully appreciate the control and regulation of gene expression in normal cellular processes and the implication in human diseases. Future studies combining cell biology, biochemistry, and genetic approaches are likely to provide exciting insights into this rapidly evolving field.

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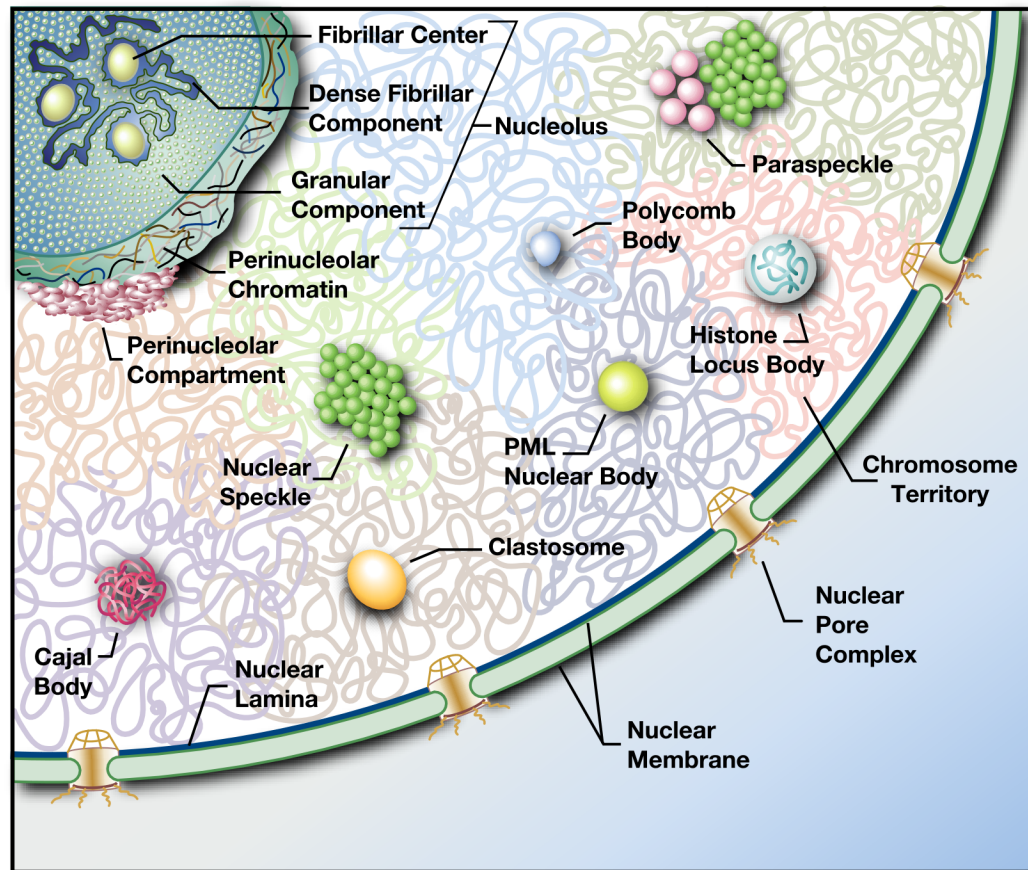


Figure 1.

Diversity of nuclear bodies. The cartoon represents the landscape of an interphase mammalian cell nucleus. The nucleus is enclosed by a double membrane structure called the nuclear envelope, which is contiguous with the rough endoplasmic reticulum and serve as a physical barrier to separate nuclear contents from cytoplasm. The nuclear envelope is interrupted in places by nuclear pore complexes controlling the nucleocytoplasmic transport. Under the inner face of the nuclear envelope, the nuclear lamina provides mechanical support and participates in chromatin organization. The nucleus contains the vast majority of the cell's genetic material organized as multiple chromosomes. Interphase chromosomes occupy distinct chromosome territories. Chromatin fibers and loops from the same chromosome territory and from neighboring chromosome territories can make contact and intermingle in *cis* and *trans*. The interchromatin space is very organized, highly dynamic, and harbors multiple nuclear bodies, such as Cajal bodies, clastosomes, histone locus bodies, nuclear speckles, nucleoli, paraspeckles, perinucleolar compartments, PML-nuclear bodies, and Polycomb bodies. The nucleolus is composed of fibrillar centers, dense fibrillar component and granular components and is surrounded by perinucleolar heterochromatin.

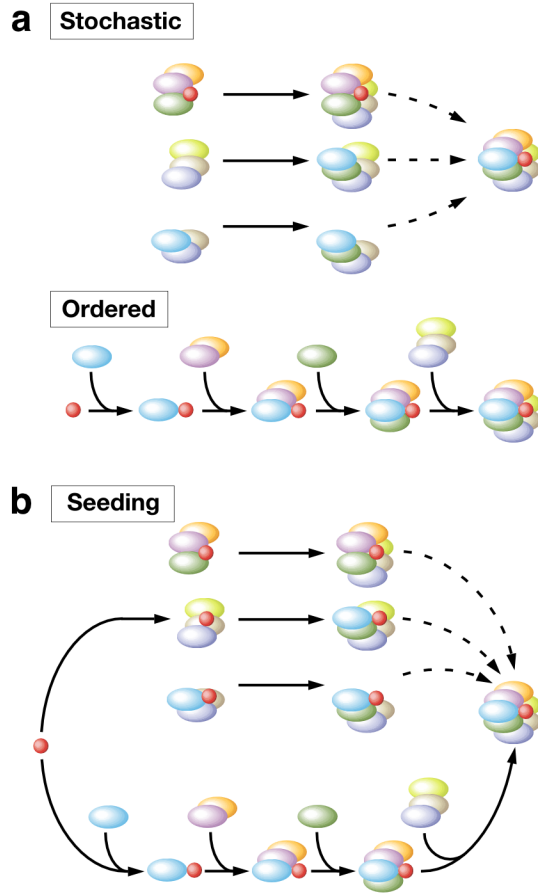


Figure 2.

Mechanisms of nuclear body assembly. **(a)** The formation of a nuclear body can occur via a stochastic assembly mechanism in which multiple pathways can be followed to assemble a nuclear body (top), or a hierarchically ordered mechanism in which only one or limited number of pathways are followed to assemble a nuclear body (bottom). **(b)** The seeding model of nuclear body assembly. RNAs or proteins serve as seeds (shown in red) to nucleate the formation of a nuclear body. The subsequent steps after the initial seeding event could be either stochastic or ordered. If they are completely random (top), the seeding model still differs from the stochastic model because the seeding component is hierarchically different from the other components. If they are tightly ordered (bottom), the seeding model becomes an ordered mechanism.

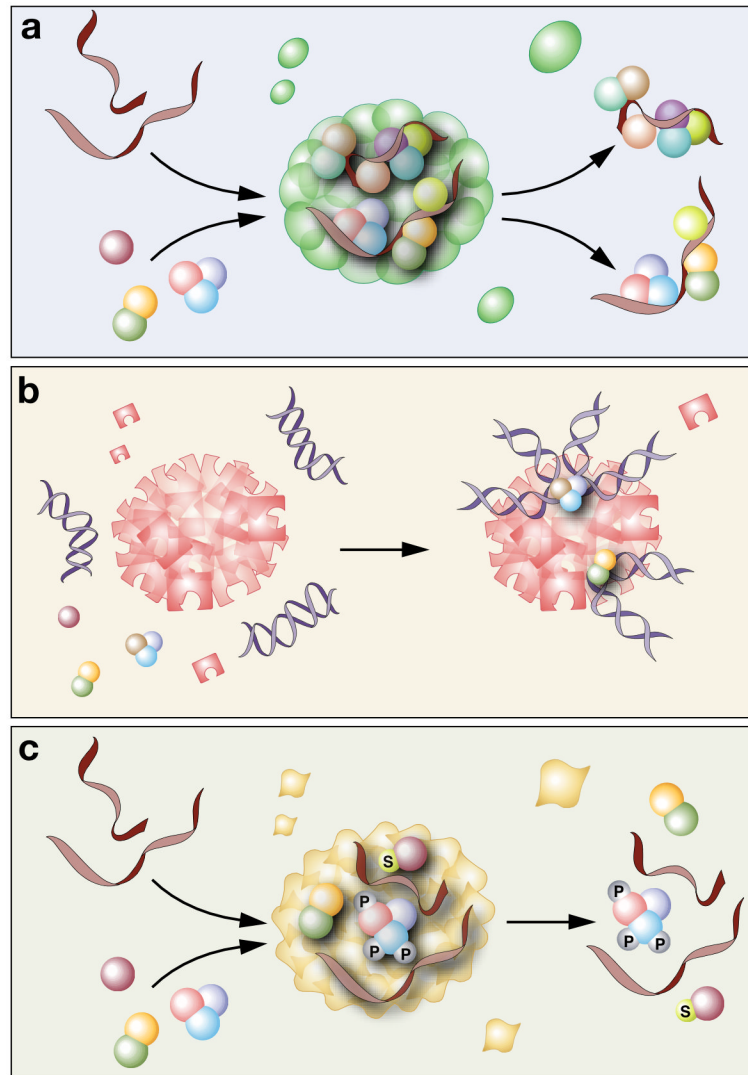


Figure 3.

Conceptual frameworks of how nuclear bodies function. Here, we summarize the common themes of how nuclear bodies may function in the nucleus. However, many nuclear bodies combine multiple mechanisms to perform their diverse cellular functions. **(a)** Reaction site. Nuclear bodies can concentrate the substrates and enzymes in a confined volume, thereby enhancing the specificity and efficiency of biological reactions. For example, nucleoli serve as pre-rRNA synthesis and processing factories and CBs promote the modification of snRNAs and the assembly of snRNPs, by enriching RNAs and proteins required for these processes. **(b)** Hub. Nuclear bodies can act as hot spots to activate or repress gene expression by recruiting gene loci. For example, the perinucleolar heterochromatin recruits Xi and imprinted regions to establish and maintain their epigenetic heterochromatin state. Nuclear bodies can also recruit multiple gene loci to stabilize their interactions and subsequently regulate their expression. For example, in *Drosophila* PcG bodies harbor *Hox* genes interactions. **(c)** Storage/modification site. Nuclear bodies can store and recycle proteins and RNAs. For example, paraspeckles retain some A-to-I hyperedited mRNAs. Nuclear bodies can also modify the components being stored. For example, a nuclear

speckle-associated kinase can regulate the phosphorylation state of SR proteins and PcG bodies act as sumoylation centers for several nuclear proteins.

Table 1
Basic characteristics of several nuclear bodies

Body Name	No./Cell	Typical Size (μm)	Defining components	(Putative) Functions
Cajal body	0-10	0.1-2.0	coilin, SMN	Involved in snRNAs and snoRNAs modification, and assembly and trafficking of snRNPs and snoRNPs. Also plays a role in telomerase assembly and telomere length regulation.
Clastosome	0-3	0.2-1.2	19S, 20S proteasome	Contains 20S and 19S proteasomes, ubiquitin conjugates, and protein substrates of the proteasome. Forms in response to stimuli that activate proteasome-dependent proteolysis.
Histone locus body	2-4	0.2-1.2	NPAT, FLASH	Involved in the transcription and processing of histone pre-mRNAs.
Nuclear speckle	25-50	0.8-1.8	SRSF2, SRSF1, Malat1	Involved in the storage, assembly, and modification of pre-mRNA splicing factors.
Nuclear stress body	2-10	0.3-3.0	HSF1, HAP	Contains satellite III ncRNAs and is a part of the general response to stress. Precise function not yet determined.
Nucleolus	1-4	0.5-8.0	RNA Pol I machinery	Involved in the transcription and processing of rRNA and the assembly of ribosomal subunits. Plays roles in the modification and assembly of other nuclear RNAs and RNPs. Regulates cell cycle progression by sequestering and modifying many proteins.
Paraspeckle	10-20	0.5	PSP1, p54nrb, Men e/ β (Neat1)	Involved in nuclear retention of some A-to-I hyperedited mRNAs.
Perinucleolar compartment	1-4	0.2-1.0	PTB, CUGBP	Precise functions are unknown but its prevalence positively correlates with metastatic capacity.
PML-nuclear body	10-30	0.3-1.0	PML	Involved in response to many forms of stress, viral defense, and genome stability by the sequestration, modification, and degradation of many partner proteins.
Polycomb body	12-16	0.3-1.0	Bmi1, Pc2	Involved in Polycomb proteins-mediated gene silencing and silencing in <i>Drosophila</i> . Precise function in mammalian cells remains to be determined.