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Sequence-encoded material properties dictate the structure and function of nuclear bodies

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Concomitant with packaging the genome, the cell nucleus must also spatially organize the nucleoplasm. This complex mixture of proteins and nucleic acids partitions into a variety of phase-separated, membraneless organelles called nuclear bodies. Significant progress has been made in understanding the relationship between the material properties of nuclear bodies and their structural and functional consequences. Furthermore, the molecular basis of these condensed phases is beginning to emerge. Here, I review the latest work in this exciting field, highlighting recent advances and new challenges.

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Introduction

Living cells are subdivided into functional compartments called organelles. In contrast to their cytoplasmic counterparts, organelles in the nucleus are not enclosed by a membrane. Instead, these 'nuclear bodies' (NBs) consist of local concentrations of proteins and nucleic acids that rapidly exchange with the surrounding nucleoplasm [1]. NBs participate in a variety of nuclear processes, including ribosome biogenesis, transcriptional regulation and RNA processing. Recent evidence demonstrates that NBs assemble by liquid–liquid phase separation, in which supersaturated NB components condense into dynamic organelles [2]. Over the past two years, great strides have been made in identifying the molecular interactions that govern the material properties of NBs and the consequences for NB structure and function.

Material properties of NBs

In addition to the dynamic exchange of their components, NBs can be characterized by their material properties. Many NBs behave like viscous liquids, such as drops of oil in water. For example, nucleoli, native NBs that produce ribosomal subunits, adopt nearly spherical shapes in *Xenopus* oocytes [3]. Moreover, when two nucleoli meet, they fuse and quickly round up to form larger spherical droplets. Cajal bodies [4], histone-locus bodies (HLBs) [5], DNA damage bodies [6] and nuclear speckles [7] also undergo homotypic fusion and rounding (Table 1). Such liquid-like behaviors arise from the continuous shuffling of intermolecular interactions between NB components (Box 1).

However, not all nuclear subcompartments behave like liquids. Components of the nuclear pore complex form hydrogels *in vitro* and have been proposed to form a solid, sieve-like mesh that serves as a selective permeability barrier between the nucleus and the cytoplasm [8,9]. Nevertheless, recent evidence suggests that nucleoporins behave more like a polymer brush than a hydrogel *in vivo* [10,11]. Amyloid bodies (ABs), or nucleolar detention centers [12,13], which assemble inside nucleoli under stress, also appear solid-like, with immobilized proteins forming insoluble amyloid-like cross-β fibers [14••]. These observations imply that some NBs maintain stable intermolecular interactions that hold constituents in place and prevent them from exchanging with the nucleoplasm (Box 1).

Aromatic and charged amino acids confer distinct material properties to NBs

Pioneering work on cytoplasmic proteins revealed that multivalent domains [15] and low complexity sequences [16] can give rise to phase-separated bodies with liquid-like or solid-like behavior [17]. Low complexity sequences (*i.e.* regions of biased, or repetitive, amino acid content) typically lack a defined three-dimensional structure [18]. Interestingly, these and other intrinsically disordered regions are significantly enriched in DNA- and RNA-binding proteins [19,20], which are often found in the nucleus [21], raising the possibility that they also contribute to the material properties of NBs.

Indeed, recent progress has identified specific residues that drive NB assembly and dictate their physical behavior. For example, Hennig *et al.* [22] performed a yeast two-hybrid screen to generate an interactome for paraspeckles, native NBs involved in regulating gene expression. The authors found an enrichment of proteins containing disordered prion-like domains, including RBM14

Table 1

A summary of the material properties, and supporting evidence, for native and synthetic NBs. Many NBs, though not all, associate with a particular genetic locus.

Nuclear body	Material property	Evidence	Protein marker	Locus	Refs.
Native					
Nucleolus (GC)	Viscous liquid	FRAP, time-lapse imaging microrheology	NPM1	rDNA	[3,5,34••]
Nucleolus (DFC)	Viscoelastic	FRAP, time-lapse imaging microrheology	FIB1	rDNA	[34••]
Nucleolus (FC)	Viscous liquid	Time-lapse imaging	RNA polymerase I	rDNA	[34••]
Histone locus body	Viscous liquid	Time-lapse imaging	FLASH	Histone genes (H3/H4)	[5]
Cajal body	Viscous liquid	Time-lapse imaging	Coilin	N/A	[4]
DNA damage	Viscous liquid	Time-lapse imaging	FUS, EWS, TAF15	PAR at sites of DNA damage	[6]
Nuclear speckles	Viscous liquid	FRAP, time-lapse imaging	SC-35	N/A	[7]
PML body	Viscous liquid	FRAP	PML	N/A	[69]
Sam68	Viscoelastic or elastic solid	FRAP	Sam68	N/A	[45,70]
Paraspeckles (shell)	Viscous liquid	Imaging	TARDBP (TDP43)	NEAT1	[25]
Paraspeckles (patch)	Viscoelastic or elastic solid	Imaging	RBM14	NEAT1	[25]
Paraspeckles (core)	Viscoelastic	FRAP	FUS	NEAT1	[25,43]
Nuclear pore	Elastic solid	FRAP (purified proteins *in vitro*)	Nsp1, Nup98	N/A	[8,9]
Amyloid body	Elastic solid	FRAP	VHL	N/A	[14••]
Synthetic					
NICD	Viscous liquid	FRAP, time-lapse imaging	NICD	N/A	[26•]
Ddx4	Viscous liquid	FRAP, time-lapse imaging	Ddx4	N/A	[31]
TDP43	Viscous liquid (CR replacement is elastic; M337V mutant is viscoelastic)	FRAP, time-lapse imaging	TDP43 (TARDBP)	N/A	[39]

and FUS, which are both essential for paraspeckle formation [23]. Interestingly, purified RBM14 forms a hydrogel with features of amyloid-like cross-β structure, as observed previously for FUS [16] and the nucleoporin Nsp1 [24]. This solid-like behavior *in vitro* is consistent with the morphology of paraspeckles *in vivo*. Rather than spherical, paraspeckles appear amorphous with punctate [22] or sausage-like shapes [25]. Furthermore, mutation of tyrosine residues to serine renders both $RBM14^{Y \rightarrow S}$ and $FUS^{Y \rightarrow S}$ incapable of forming hydrogels *in vitro* or of rescuing paraspeckle formation *in vivo*. Similarly, substitution of phenylalanines in Nsp1 with serine, but not tyrosine, prevents gelation and genetic rescue, suggesting that nucleoporin hydrogels are crosslinked by π–π stacking between aromatic residues [8]. Together, these findings suggest that hydrophobic interactions, particularly between aromatic residues, confer solid-like behavior to paraspeckles and nuclear pores.

In contrast, charged residues appear to play an important role in assembling liquid-like NBs. Pak *et al.* [26•] found that the nephrin intracellular domain (NICD), which is negatively-charged and intrinsically disordered, forms synthetic NBs when ectopically expressed. NICD rapidly exchanges between these NBs and the nucleoplasm, and the NBs fuse to form large spherical droplets. Furthermore, assembly of these NBs is concentration-dependent, as expected for liquid–liquid phase separation [2]. However, the partition coefficient, or the ratio of NICD concentration in the NB and the nucleoplasm, is not constant. Rather, it decreases as expression increases. This observation suggests that NICD condenses through complex coacervation with a positively-charged counterion rather than through simple coacervation by itself.

Coacervation refers to a specific type of liquid–liquid phase separation in which a homogenous polymer solution demixes to form a polymer-rich phase and a polymer-poor phase. Simple coacervation is driven by homotypic interactions of a single polymeric species, while complex coacervation is driven by heterotypic interactions between oppositely-charged polyelectrolytes [27]. Since complex coacervation depends on complex formation between two polymeric species, as the concentration of one species increases, the other species becomes limiting, resulting in a decreasing partition coefficient. Consistent with this mechanism, purified NICD is soluble on its own, but phase separates into liquid-like droplets when mixed with positively-charged variants of GFP. Finally, by