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Nuclear bodies: The emerging biophysics of nucleoplasmic phases

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Abstract

The cell nucleus contains a large number of membrane-less bodies that play important roles in the spatiotemporal regulation of gene expression. Recent work suggests that low complexity/disordered protein motifs and repetitive binding domains drive assembly of droplets of nuclear RNA/protein by promoting nucleoplasmic phase separation. Nucleation and maturation of these structures is regulated by, and may in turn affect, factors including post-translational modifications, protein concentration, transcriptional activity, and chromatin state. Here we present a concise review of these exciting recent advances, and discuss current and future challenges in understanding the assembly, regulation, and function of nuclear RNA/protein bodies.

Introduction

Understanding the structure and function of the nucleus is essential for deciphering the computational logic embedded in the genome. It is increasingly apparent that there is an intimate connection between the 3D structure within the nucleus and biological function. However, the strongly non-uniform spatial distribution of molecules within the nucleus is often neglected, even while it is clear that these variations could have a large impact on the molecular interactions governing gene regulation.

Concentration variations within the nucleus are likely to be important due to their effect on reaction rates and diffusive transport. Diffusion is a stochastic, non-directional process, and thus diffusive transport rates depend not on the distance (as for directed transport), but on the distance-squared – e.g. to go twice as far it will take four times as long. Diffusive transport could thus be a rate-limiting step for many nuclear processes, particularly those involving a large number of binding partners. “Active diffusion”, driven by non-equilibrium, ATP-dependent dynamics, could partially address this problem by speeding up dynamics within the nucleus [1],[2]. The three dimensional architecture of the genome itself is likely important, controlling the spatial proximity of co-regulated genes and enhancer elements [3],

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[4]. However, establishing efficient transport and reaction rates remains a potential challenge, particularly given the densely crowded nature of the nucleus [5].

Non-membrane bound nuclear bodies may play an important role in addressing this problem by spatially patterning molecular concentration in the nucleoplasm. Indeed, these structures locally increase the concentrations of molecules involved in chromatin remodeling, transcription initiation, and RNA processing. These structures could also play a role in functioning as biophysical switches, responsive to local signals by assembling only above a threshold concentration. Understanding the biophysical rules governing their assembly and properties thus promises to shed light on the important and still poorly understood aspects of gene regulation in the nucleus.

Nuclear Bodies

The problems of transport and molecular reaction rates in the nucleus are similar to those encountered in the cytoplasm, where organelles play a central role in organizing the spatial and temporal distribution of molecules. Large organelles are sometimes even further sub-compartmentalized, such as in the cristae of mitochondria. It is thus not surprising that as the largest organelle in the cell, the nucleus contains a variety of sub-compartments to organize the nucleoplasm.

The sub-organelles of the nucleus are membrane-less, making their assembly and regulation particularly interesting. These structures are referred to as RNA Granules, RNP bodies, or recently RNP droplets; here we will refer to them simply as nuclear bodies. Examples include Cajal bodies (CBs), speckles, Histone Locus Bodies (HLBs), PML bodies, and nucleoli – the largest and prototypical nuclear body (Figure 1); numerous RNP bodies can also be found in the cytoplasm. Although often exhibiting cell-cycle dependent assembly and disassembly, these structures can remain stable over timescales of minutes to hours, even while their components are in a constant state of dynamic flux with the surrounding nucleoplasm [6]. By concentrating molecules within a small micro-compartment of the nucleus, while allowing these molecules to remain dynamic and mobile, these structures may function to increase reaction rates [7] much like conventional membrane-bound cytoplasmic organelles.

Elucidating biophysical principles underlying nuclear body assembly is a formidable challenge, particularly given the large number of components and dynamic nature of these assemblies. Moreover, research over the last decade suggests that nuclear bodies can be nucleated by a variety of components [8],[9]. This presents an additional conceptual difficulty, since nuclear body assembly may be largely “non-sequential”, which contrasts with the ordered, sequential assembly steps typical of multi-component molecular complexes such as the ribosome [10]. This challenges the well-worn paradigm of a small number of molecules undergoing structurally-defined interactions along ordered assembly pathways. Progress on this problem will require expanding the frontiers of how we think about intracellular organization.

Assembling Nuclear Bodies through Phase Transitions

A hypothesis that has gained increasing attention is that nuclear and cytoplasmic bodies assemble through intracellular phase separation [11],[12],[13],[14]. Phase transitions are common in nature and familiar from everyday experiences of dewdrops condensing on blades of grass or water freezing into ice. Phase transitions reflect the thermodynamic forces driving a system to its equilibrium configuration, manifesting in switch-like transitions of large-scale molecular organization. Such behavior is actually well known in biology, from in vitro protein crystallization, where soluble proteins are observed to condense into concentrated liquid phases or crystalline solid phases. In the cell, proteins could thus similarly transition from a dispersed nucleoplasmic state into a localized body, and vice versa. These phase changes can be a strong function of salt, protein concentration, and temperature, parameters that affect the free energy governing interactions between proteins. The hypothesis that phase separation underlies nuclear body assembly is attractive because it provides a well-established physical framework for understanding how the interactions of large numbers of molecules could manifest in their spontaneous, non-sequential self-assembly.

Consistent with the phase separation hypothesis, nuclear bodies often behave like liquid droplets of RNA and protein. For example, it is well known that somatic cell nucleoli often fuse with one another. Using the large and numerous extrachromosomal nucleoli in the nucleus of large *X. laevis* oocytes, the dynamics of coalescence were shown to be quantitatively consistent with coalescence dynamics of simple liquids, such as two oil droplets fusing in water [15]. Interestingly, this study revealed ATP-dependent dynamics, suggesting a possible form of “active diffusion” within the nucleolus itself. Work in *Drosophila* oocytes has revealed that novel nuclear bodies can be induced to nucleate and grow from the nucleoplasm by mechanical perturbation of the egg chamber, which likely changes the nucleoplasmic salt concentration and thus the strength of molecular interactions [16]. These nuclear bodies are highly spherical and can be observed to undergo striking liquid-like coalescence events (Figure 2).

A fascinating recent study by Nott et. al. provides further quantitative support for the role of phase separation in the nucleus [17]. This study utilizes a Ddx4 construct, which when expressed in vitro assembles into liquid phase droplets. The assembly of these droplets depends on protein concentration, salt concentration, and temperature, in a manner that can be quantitatively modeled as a phase transition. When YFP::Ddx4 constructs are expressed in HeLa cells, the protein is transported into the nucleus and assembles into liquid-like synthetic nuclear bodies (Figure 1). The nucleation and growth of these bodies is modeled by the Avrami equation, a common kinetic model describing phase separation; there is a rich literature on this and other mathematical models describing phase separation of synthetic polymers and other non-living materials [18], [19], [20]. This study thus provides strong evidence that phase separation can drive the formation of synthetic bodies that are qualitatively similar to those natively found in the nucleus.

Quantitative tests of the phase separation model in vivo are needed to elucidate the assembly mechanisms of native nuclear bodies. One recent study has provided the first preliminary

mapping of the phase diagram of the nucleolus. Using *C.elegans* embryos and blastomeres of different size, it was found that the size of assembled nucleoli is sensitively dependent on the nucleoplasmic concentration of nucleolar components [21]. The component concentration dependence of nucleolar size could be quantitatively modeled using a simple model that predicts a concentration threshold for nucleolar assembly. Remarkably, by increasing concentration, nucleoli could be induced to form in early blastomeres in which they normally do not, confirming a prediction of this model (Figure 2). These findings are consistent with earlier studies that suggest a concentration dependence of the non-sequential assembly of de novo Cajal Bodies [8]. Analogous dynamics were recently described in prokaryotes, in a study focusing on the ParB-DNA complex, which is responsible for chromosome and plasmid segregation. By developing a thermodynamic model and comparing to quantitative experimental results, both 3D bridging and 1D spreading interactions of the ParB protein along the DNA were found to be important for condensation of ParB protein on specific DNA loci. The authors suggest that the ParB/DNA complex may be a liquid-like assembly whose effective surface tension is important for assembly [22].

Molecular Driving Forces

The non-sequential aspects of nuclear body assembly require molecular players that contribute to assembly through interactions with multiple binding partners. Protein domains containing low sequence complexity, referred to as LCS domains, have emerged as important motifs that could facilitate such interactions. LCS domains tend to be disordered, and work on intrinsically disordered proteins (IDPs) shows they can sample a wide variety of conformations. IDPs thus represent promiscuous and dynamic interaction domains that can contribute to the formation of higher order structures [23],[24],[25], [26] This conformational flexibility of IDPs is what likely makes them ubiquitous in the proteome and reports suggest they are important for assembly of key gene regulatory machinery, including the spliceosome [27].

Several recent papers have focused on the higher order assembly of FUS, a nuclear protein which when mutated is associated with protein aggregation, neurodegeneration [28], and cancer, and which has recently emerged as a potentially important player in nuclear/cytoplasmic body assembly [29]. FUS contains an N-terminal LCS domain, which was found to drive a concentration-dependent phase transition in vitro, resulting in the formation of hydrogels consisting of amyloid-like FUS fibers [30]. This is reminiscent of work on nuclear pore proteins, whose LCS Phenylalanine-Glycine (FG) repeats also form amyloid-like fibers and gels in vitro [31]. Finally, RNA helicases also frequently contain LCS/IDP regions [32], which may facilitate their frequent co-localization with nuclear bodies [33]; This is consistent with the in vivo findings that the LCS domain of helicase Ddx4 drives liquid phase separation in HeLa nuclei [17]. However, these in vivo liquid-like assemblies contrast with the gels that are reported to form in vitro; future progress will address the question of whether these more static fibers/gels play a role in healthy cells [34].

Prion-like domains (PrLD) are another type of protein domain that can drive higher order assembly. PrLD are glutamine(Q)/asparagine(N) enriched, charge-depleted motifs that may be considered a subclass of LCS/IDPs. PrLD play a well-known role in protein aggregation

and neurodegenerative diseases [35]. Recent work has shown that disease mutations in the intrinsically disordered PrLD of hnRNPs strengthen the binding interactions between hnRNPs, and promote aggregation by effectively “zipping up” the amyloid backbone. This altered interaction ultimately leads to the translocation of excess hnRNPs from the nucleus to cytoplasmic stress granules and inclusion bodies in animal models, recapitulating the human aggregation pathology [36].

Proteins containing repeats of individual low-affinity folded interaction domains represent another, related, molecular motif in nuclear body assembly. Two multivalent chains of complementary protein domains have a propensity to phase separate, such that above a critical concentration liquid protein droplets of the two proteins assemble [13]. When the two constructs are expressed in vivo, they co-localized into dynamic, synthetic nuclear bodies. Another novel type of synthetic nuclear body has also been recently formed from multi-valent ferritin cages [37]. Multi-valency is interesting from the standpoint of regulation, as the number of binding sites can be regulated by post-translational modifications (PTMs), thus providing the cell with a mechanism for tuning the degree of higher order protein assembly. Such repetitive interaction domains may thereby promote both non-sequential assembly and dynamic temporal tuning of assembly and disassembly. In reality, the assembly of nuclear bodies is likely often governed by a combination of multivalency and disordered schemes, with PTMs regulating and coordinating features of both.

Regulating Nuclear Body Assembly

A number of post-translational modifications (PTMs) are involved in tuning the interactions driving nuclear body assembly/disassembly. For example, SUMOylation of PML is thought to play an important role in PML body assembly [38],[39]. Poly(ADP-ribosyl)ation is another PTM that could regulate the assembly of nuclear bodies [40] such as nucleoli [41]. PTMs may be particularly effective for LCS/PrLD/IDP motifs, since these are involved in dynamic low-affinity interactions, and their typical conformational flexibility allows for easy access by effector proteins. Indeed, cyclin-dependent phosphorylation sites, SUMOylation sites, and N-glycolysation sites have all been found in IDPs [13].

Phosphorylation, in particular, is an important modification for regulating the binding of LCS domains to LCS bodies. Protein phosphorylation introduces a negative charge at the phosphorylated reside, and can thereby impact molecular interactions. This is consistent with the finding that ionic-strength and changes in the charge distribution within Ddx4 LCS domains strongly impact the phase boundary for droplet assembly. Phosphorylation is a key modification of the C-terminal domain (CTD) of RNA Polymerase II, a repetitive LCS that plays an important role in Pol II transcription initiation and subsequent RNA processing [42]. Recent work has shown that CTD binding to LCS polymeric fibers of FUS, EWS, and TAF15 hydrogels is strongly dependent on CTD phosphorylation [43]. In another paper, Kwon et al showed that Serine-Arginine (SR) domains – a common LCS motif in RNA splicing proteins – bound hydrogel droplets of fibrous polymers of LCS domains in a manner reversible by phosphorylation of the serine residues. In vivo, it was also shown that by changing normally phosphorylatable serine residues in the LCS domain of the splicing

factor (SRSF2) to glycine, CLK1 was not able to phosphorylate the mutated domains, giving rise to a hypophosphorylated form of the protein that could not be released from nucleoli. This suggests that phosphorylation plays an important role in regulating the engagement/disengagement of factors with the nucleoli [44]. Finally, a recent paper has also shown that the assembly and disassembly of non-membrane bound, cytoplasmic P granules are regulated by the phosphorylation of IDP components [45]. Taken together, this suggests that the protein charge state, modifiable by a variety of PTMs, plays an important role in regulating the composition and assembly/disassembly of nuclear bodies.

In addition to tuning molecular interactions, another mechanism for regulating nuclear body assembly is controlling the concentration of protein components themselves. Indeed, protein concentration is reported as an important parameter in a large number of studies [21],[17],[30],[13]. This is likely because in phase separating systems, assembly only occurs for concentrations that exceed a critical concentration, defining the phase boundary for the system [21],[8],[17]. The absolute and relative nuclear concentration of a set of proteins could tune the nuclear body's composition and material properties, such as viscosity and surface tension, which ultimately would affect diffusion and reaction rates in the system. Within the nucleus, the concentration of specific components is controlled by both cytoplasmic protein synthesis/degradation rates and the balance of nuclear import and export. As a result, nuclear trafficking should have a strong effect on the assembly, biophysical properties, and function of the nuclear bodies. This is supported by the existence of neurodegenerative, aggregation pathologies associated with dysregulated nuclear/cytoplasmic partitioning [46],[47],[48].

The Link Between Assembly and Function

Function and assembly appear to be tightly linked, with disruptions in nuclear body assembly leading to disruptions in function, and vice versa. For example, the nucleolus functions primarily to process nascent ribosomal RNA (rRNA) transcripts. When rRNA transcription is inhibited, the composition [33] and structure [49] of nucleoli are strongly affected. In turn, mutations in the nucleolar LCS phosphoprotein, Nopp140/Dao-5, were recently shown to alter rDNA transcription in *C.elegans* [50]. A recent study adds an interesting twist to this feedback between nucleolar assembly and function, by the discovery of a new class of histone modification, glutamine (Q) methylation, by the conserved nucleolar protein Fibrillarin. Glutamine methylation of histone H2A is restricted to the nucleolus, and directly increases transcription of rRNA at the rDNA locus. This suggests that Fibrillarin condensation drives rDNA accessibility, and thereby promotes rRNA transcription in the nucleolus [51].

The links connecting chromatin compaction state, transcription, and nuclear body assembly are further elucidated in several recent papers that used arrays of DNA repeats to nucleate synthetic nuclear bodies in vivo. Grob et al demonstrate that synthetic nucleoli can be nucleated at pseudo nucleolar organization regions (NORs) formed by arrays of repetitive upstream binding factor (UBF) binding sites [52]. The formation of these synthetic nucleoli is strictly UBF-dependent, and this combined with the compaction of endogenous rDNA repeats when UBF is depleted, suggests a strong link between maturation of nucleoli at the

NORs and chromatin architecture. Similarly, Salzler et al showed that promoters are necessary and sufficient for the nucleation of histone locus bodies (HLBs). Interestingly, however, this work suggests that the maturation of proto-HLBs in the region of the histone gene promoters depends on the promoters being active [32]. Maturation of HLBs may thus depend on transcription initiation, while nucleation does not. These studies [52],[53] as well as a similar study demonstrating the necessity of non-coding RNA (ncRNA) in nucleating paraspeckles [54] suggest that nuclear body formation can involve some aspects of sequential, ordered assembly.

While RNA is an essential functional output of the nucleolus and a key functional substrate for Cajal bodies/splicing bodies, it also plays a highly active role in assembling the nuclear body machinery that operates on it. Shevtsov and Dundr have shown that tandem repeats of RNA can nucleate RNA splicing bodies in vivo [9]. This builds on the work from the McKnight group showing that in addition to LCS proteins, b-isoxazole precipitated hundreds of RNA binding proteins that are typically constituents of RNA granules, suggesting that RNA is an important component of relevant nuclear bodies [30]. Furthermore, the Cech group has shown that RNA binding both nucleates the assembly of FUS RNP hydrogels and mediates its binding to the CTD of RNA polymerase II [55]. Taken together, this work links the assembly of nuclear bodies to their function, by suggesting that the nucleation and maturation of nuclear bodies is heavily influenced by the presence of their functional substrates.

The connection between nuclear body assembly and chromatin architecture is interesting given the concept of mechanical regulation of chromatin by nuclear lamins and possibly nuclear actin. Nuclear lamins are structural components of the nuclear envelope that play a role in tethering transcriptionally silent chromatin to the nuclear periphery; with artificial tethering repressing the transcription of some genes [56]. Indeed, several B-type lamins are associated with chromatin binding [56]. While the role of nuclear actin remains unclear, it could play a dual role, in both nuclear architecture as well as in regulating transcription [57–59]. For example, actin and nuclear myosin I are distributed within the nucleolus in a transcription-dependent manner, with nuclear actin present in transcriptionally active complexes of the nucleolus [58]. Interestingly, a nuclear actin network was recently found to structurally stabilize the emulsion of nuclear bodies found in the *X. laevis* oocyte nucleus; actin disruption lead to gravitational sedimentation, large-scale coalescence, and the formation of a single, large, spherical nucleolus [57].

Conclusion/Outlook

The assembly and function of nuclear bodies has been an important problem in cell biology for almost two centuries. The last several years has witnessed the emergence of a number of themes that suggest we are at a critical point in our understanding of the biophysics of nuclear body assembly. These include: 1. The non-sequential aspects of nuclear body assembly, regulated by specific RNA and/or DNA. 2. The important role of disordered and/or multivalent domains in driving assembly. 3. Molecular regulation, including PTMs and protein concentration, which can tune assembly. And 4. The concept of phase transitions in the nucleus, which provides a conceptual physical framework for understanding how

higher-order assemblies condense from solutions of many interacting molecules. A key question is how dynamic low-affinity interactions can give rise to compositionally specified bodies. Why do some proteins localize to only the nucleolus, while others can be found in both the nucleolus and Cajal bodies? Can we begin to construct multi-dimensional phase diagrams that specify the molecular concentrations and degree of PTM that promote assembly of various types of nuclear bodies, as shown schematically in Figure 3? How is the logic of molecular specificity encoded in the promiscuous interactions of intrinsically disordered proteins? We believe that the recent elucidation of important molecular motifs, and their role in driving nucleoplasmic phase transitions, provide a conceptual foundation for making steady progress in understanding the problem of assembly.

These recent advances in the molecular biophysics of nuclear body assembly are also likely to shed light on the major outstanding questions concerning nuclear body function. While it is clear that by co-localizing reactants within a small microcompartment can increase transport efficiency and reaction rates, for example in RNA splicing reactions, quantitative studies demonstrating such function are needed. Combined with the threshold concentration dependence of phase transitions, these facilitated functions could allow for switch-like gene regulation. A central challenge is to elucidate what increasingly appears to be an intimate, but still poorly understood, feedback between nuclear body assembly/disassembly, chromatin compaction state, transcriptional activity, and RNA processing. The emergence and refinement of quantitative, predictive biophysical models for assembly promises to lay the groundwork for dissecting this functional feedback, and building a comprehensive understanding of the role of nuclear bodies in the 4-dimensional regulation of the genome.

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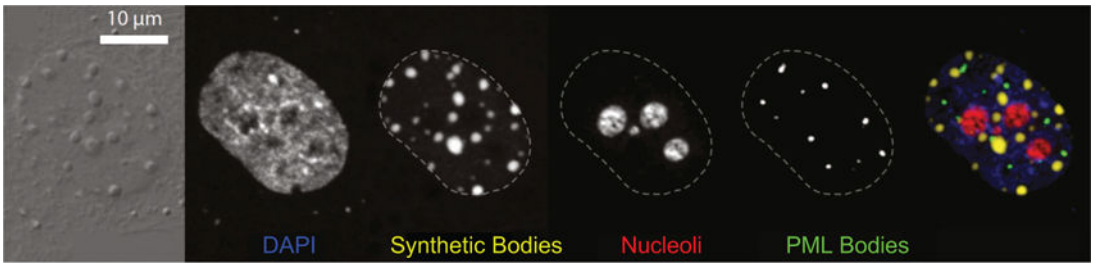

Fig 1. Examples of Nuclear Bodies

Examples of nucleoli, PML bodies, and synthetic Ddx4YFP bodies assembled in HeLa cells. DNA is stained with DAPI. Adapted with permission from [17]

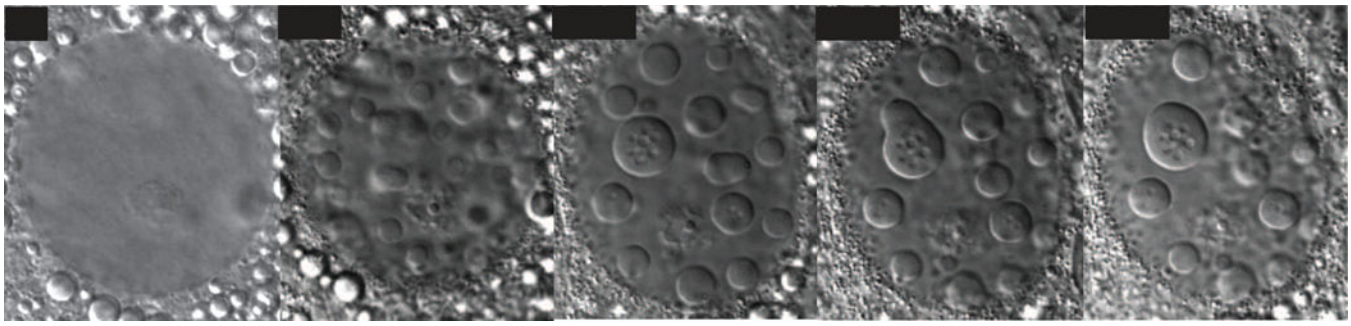

Fig 2. Nucleation, Coarsening and Coalescence of Induced Nuclear Bodies

A novel type of nuclear body first described in ref [16] is induced to nucleate and grow upon gentle mechanical pressure applied to the nucleus of drosophila oocytes (t=0s). Large liquid-like nuclear bodies grow and coalesce upon contact with one another (courtesy Brangwynne, unpublished). The nucleus is approximately 40 microns in diameter.

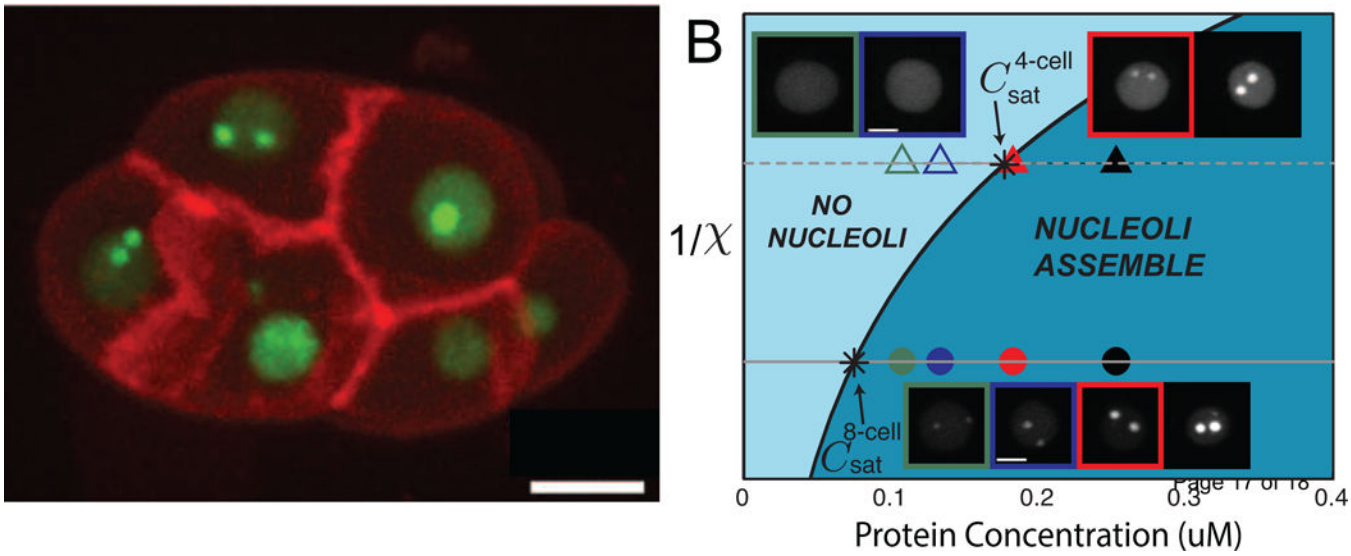

Fig 3. Phase Transition of Nucleoli in *C. elegans* Embryos

(A) *C. elegans* embryo in the 8-cell stage. The cell membrane is labeled with mCherry (red) while the nucleolus is labeled with GFP tagged Fibrillian (green). (B) Phase diagram for nucleoli in the *C.elegans* embryo, adapted from Weber et al [21]. The vertical axis represents interaction parameters that are hypothesized to govern the phase boundary. The horizontal axis represents the concentration of Fibrillian (proportional to other nucleolar proteins) in the nucleoplasm. The saturating concentration, C_{sat}, is the concentration below which nucleoli do not assemble, defining the phase boundary; beyond this concentration nucleoli assemble and are increasingly large.

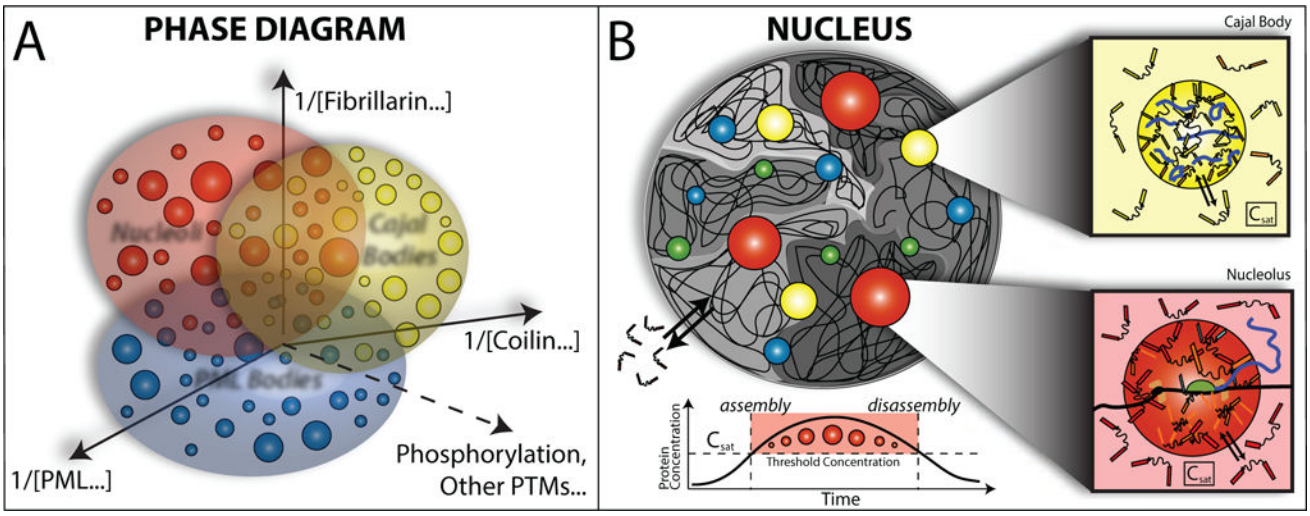

Fig 4. Conceptual View of Nuclear Body Assembly

(A) Schematic multi-dimensional phase diagram for nuclear bodies including nucleoli, Cajal bodies, and PML bodies. Each axis represents the concentration of key proteins promoting assembly, or phosphorylation state or other PTMs. The transparent ovals represent sets of parameter values where particular bodies will form; the boundaries of these ovals are phase boundaries beyond which the body does not form. (B) The concentrations in the nucleus are typically such that multiple types of nuclear bodies can coexist, which would correspond to overlapping regions in the diagram in (A). However, molecular concentration can be regulated by import and export of protein from the nucleus, as well as various PTMs, thus tuning nuclear body assembly and disassembly.