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CELL SCIENCE AT A GLANCE

SUBJECT COLLECTION: EXPLORING THE NUCLEUS

The liquid nucleome – phase transitions in the nucleus at a glance

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ABSTRACT

Cells organize membrane-less internal compartments through a process called liquid–liquid phase separation (LLPS) to create chemically distinct compartments, referred to as condensates, which emerge from interactions among biological macromolecules. These condensates include various cytoplasmic structures such as P-granules and stress granules. However, an even wider array of condensates subcompartmentalize the cell nucleus, forming liquid-like structures that range from nucleoli and Cajal bodies to nuclear speckles and gems. Phase separation provides a biophysical assembly mechanism underlying this non-covalent form of fluid compartmentalization and functionalization. In this Cell Science at a Glance article and the accompanying poster, we term these phase-separated liquids that organize the nucleus the liquid nucleome; we discuss examples of biological phase transitions in the nucleus, how the cell utilizes biophysical aspects of phase separation to form and regulate condensates, and suggest interpretations for the role of phase separation in nuclear organization and function.

KEY WORDS: Chromatin, Nuclear organization, Nucleolus, Phase separation

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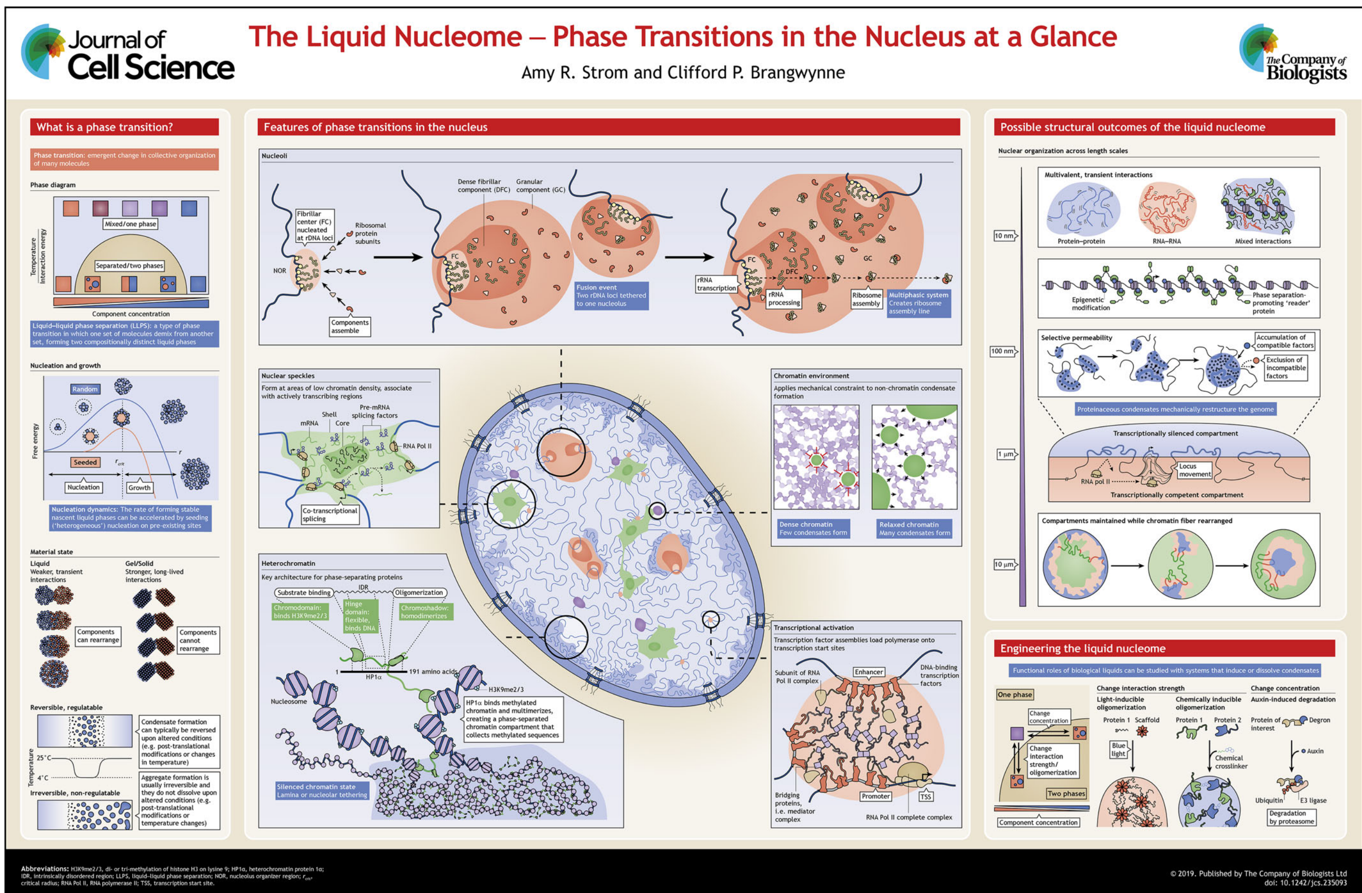
Introduction

Compartmentalization is used throughout all kingdoms of biology to create functionally distinct units within a complex cellular environment. We usually think of these compartments as requiring a lipid bilayer membrane to define the boundary between inside and outside and differentially concentrate certain factors (proteins, metabolites) in the compartment. Alternately, membrane-less compartments can be formed through a process called liquid–liquid phase separation (LLPS), which also underlies oil and water demixing. Condensation and dissolution of phase-separated and compositionally distinct compartments is triggered by interactions



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among the protein, RNA and DNA components (Banani et al., 2017; Shin and Brangwynne, 2017). Preferential interactions among a set of components will lead to condensation of a phase enriched in those components, separated from the rest of the surrounding cytoplasm or nucleoplasm. In materials science, phase diagrams are used to describe the phase behavior of a system as a function of a given set of system parameters, including temperature, concentration and interaction energy (Papon et al., 2007) (see poster). For a fixed interaction strength and temperature, increasing molecular concentration above the saturation concentration results in phase separation, where the uniformly distributed components separate into two phases: a biomolecule-rich phase and more dilute phase. Conversely, at a fixed concentration, phase separation can be driven by changes in the interaction strength of condensate-forming biomolecules, which can be influenced by many factors, including protein or nucleic acid sequence, chemical modifications and ionic strength. Therefore, by controlling the concentration and interaction strength of constituent biomolecules, the cell can alter its position within the phase diagram to form or dissolve condensates, or alter their composition. Here, we discuss the biophysical role of the liquid nucleome, which is encapsulated in three key features: (1) condensate formation and regulation, (2) material state and reversibility, and (3) selective permeability and structure–function relationships. We further highlight the potential functional consequences of the liquid nucleome and discuss emerging tools and techniques to alter and study the role of condensation in the nucleus.

Characteristics of phase transitions

Condensate formation and regulation

Most nuclear bodies are dissolved and reformed every mitosis (Rai et al., 2018), and must therefore be formed reliably in the correct nuclear location during every cell cycle. Formation of a condensate *in vivo* likely occurs through the process of nucleation and growth, in which components that were evenly distributed start to randomly associate, until they form clusters that are large enough to overcome surface tension; beyond this critical radius they become stable and continue to grow (Kashchiev, 2000). Unchecked, such homogenous nucleation leads to phase separation at random locations. One way in which biological systems control location of condensate formation is by seeding nucleation at defined physical locations (Hyman et al., 2014), a process known as heterogeneous nucleation (Kashchiev, 2000) (see poster). For nucleoli, active rRNA transcriptional sites (called nucleolar organizer regions, or NORs) provide the seed for protein components to condense (Shevtsov and Dundr, 2011) and removing rDNA or otherwise inhibiting rRNA transcription leads to asynchronous nucleation at random nuclear locations (Berry et al., 2015; Falahati et al., 2016). Indeed, *in vitro* studies with purified nucleolar components support the thermodynamic preference for condensate formation with rRNA (Berry et al., 2015; Mitrea et al., 2016). Seeded nucleation at specific nuclear loci might be occurring in a variety of structures within the nucleus, including the nucleolus (Grob et al., 2014), Cajal bodies (Kaiser et al., 2008; Sawyer et al., 2016; Wang et al., 2016), and non-nucleolar transcriptional assemblies (Hnisz et al., 2017), and possibly for nuclear speckles, which also tend to colocalize with sites of active transcription (Spector and Lamond, 2011) (see poster). Thus, seeded nucleation appears to be a common mechanism through which the liquid nucleome ensures the proper number and location of important phase-separated nuclear bodies.

Material state and reversibility

Once assembled, the material state and viscoelasticity of condensates is dictated by the interaction strength between molecular components. In liquid-like condensates, components are driven to phase separate when their interactions are of sufficient strength, but weak enough to allow for rapid internal dynamics characteristic of the liquid state. Weak, multivalent protein interactions indeed appear to be a feature common to many condensates (Hyman and Brangwynne, 2011; Li et al., 2012), and often involve conformationally heterogeneous proteins that are known as intrinsically-disordered proteins (IDPs) or the closely-related low-complexity sequences (LCSs) (Elbaum-Garfinkle et al., 2015; Murray et al., 2017; Nott et al., 2015; Kato et al., 2012). Most condensates of the liquid nucleome appear to be in a dynamic fluid state, with a viscosity higher than that of nucleoplasm (Brangwynne et al., 2011; Feric et al., 2016). This liquid-like state allows for high concentration of components within the condensate while maintaining mobility and reversible association of those components – allowing condensates to be disassembled and reassembled every mitosis, or triggered to form or dissolve upon a cellular signal (see poster). Mutations in many of the same proteins that drive phase transitions can lead to irreversible and often pathological aggregation that is associated with disease states, as seen with transcriptional regulators such as seen with FUS and TAR DNA-binding protein 43 (TDP43; encoded by *TARDBP*), which are found in amyotrophic lateral sclerosis (ALS) inclusions (Patel et al., 2015), and proteins associated with repeat expansion disorders, as occurs in Huntington's disease, among others (DiFiglia et al., 1997; Molliex et al., 2015). Interestingly, whereas much of our understanding of intracellular phase transitions has focused on protein-driven condensation, expanded repeat RNA can also form nuclear foci that have a partially irreversible gel-like character and might play a role in disease progression (Jain and Vale, 2017).

Selective permeability and structure–function relationships

Each condensate in the nucleus represents a complex fluid that has selective permeability for specific biomolecules, which results in proteins partitioning into or out of the compartment, depending on their interactions. This property allows functionalization of condensates; for example, nuclear pores are permeable to importins that can interact with the FG repeats that 'plug' the center of the pore. Proteins not bound to an importin are unable to pass through the pore and enter the nucleus (Frey and Görlich, 2007), resulting in a specific subset of proteins becoming enriched in the nuclear compartment. Coexisting liquids can result in functionally structured partitioning that might enable sorting of specific enzymes or metabolic factors. The nucleolus again provides an instructive example: each of the nucleolar subcompartments has a unique complement of proteins and RNAs that execute its function: ribosomal DNA is transcribed to ribosomal RNA in the fibrillar center (FC), the rRNA is then processed in the dense fibrillar component (DFC), and finally, within the granular component (GC), rRNA is assembled into a mature pre-ribosomal particle (Schwarzacher and Wachtler, 1993) (see poster). The sub-phases of the nucleolus each exhibit liquid-like character, with rapid and nearly complete fluorescence recovery after photobleaching (FRAP) (Phair and Misteli, 2000) and liquid-like coalescence (Feric et al., 2016). The macro-scale structure of the three nucleolar compartments is dictated by emergent properties of the phase-separated liquids, including miscibility and surface tension. When two phases are immiscible, this means that interactions among their

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components are disfavored, such that they do not mix with one another, and instead form distinct sub-phases. The relative surface tension of these liquids dictates whether and how they contact one another, including which droplet will be engulfed partially or fully within the other (Eckelmann and Lüning, 2012; Zarzar et al., 2015). *In vitro* reconstitution studies of key nucleolar components demonstrate that they are able to phase separate from aqueous solution, forming multiple liquids that each localize key functional proteins and, when mixed, give rise to a liquid-within-liquid structure that recapitulates the endogenous nucleolar structure important for proper ribosomal maturation (Feric et al., 2016). Nuclear speckles also have features reminiscent of this core-shell architecture (Fei et al., 2017), and partially associated bodies, like the Cajal body and snurposome in *Xenopus* oocytes (Handwerger et al., 2002; Nizami et al., 2010) or Cajal body and histone locus body in *Drosophila* and mammalian nuclei (Bongiorno-Borbone et al., 2008; Ghule et al., 2009), likely reflect immiscible co-wetting sub-phases. Indeed, surface tension-associated forces likely play a ubiquitous and centrally important role in sculpting the multiphase architecture, and therefore structure–function relationships, of the liquid nucleome.

Coming together – phase separation in, on and of the genome

The three-dimensional (3D) structure of the genome has important mechanical implications on the nucleation, growth and movement of condensates within the nucleus. The influence of the surrounding environment on condensate formation and movement has been explored in the context of the *X. laevis* oocyte nucleus, where an unusual nuclear actin network stabilizes nucleoli and other nuclear bodies against diffusive coalescence and gravitational forces (Feric et al., 2016). In more typical eukaryotic nuclei, condensate growth and motion are similarly constrained by the mechanical features of the viscoelastic chromatin network (Heo et al., 2015; Poh et al., 2012). Our recent work has characterized these constraints by using a tool (‘CasDrop’) that allows for light-induced initiation of condensate formation targeted to specific genomic loci (Shin et al., 2018). Condensates targeted to specific genomic loci can result in surface-tension-mediated pulling together of the two loci, even while non-targeted genomic elements are mechanically pushed out; the latter effect results in preferential condensate growth in softer and less-dense chromatin regions. These results suggest that seeded nucleation at specific genomic loci and differential chromatin density throughout the nucleus both play a role in determining the number and positioning of liquid condensates (see poster). In addition to affecting nucleation and positioning of proteinaceous liquid condensates, chromatin compartments themselves might be formed and organized through phase separation. For example, constitutive heterochromatin is a nuclear compartment that organizes repetitive sequences into compacted, silenced assemblies usually found near the nuclear periphery or around nucleoli (Clark and Elgin, 1992; Stewart et al., 2005; Almouzni and Probst, 2011). Recent studies suggest that this chromatin compartmentalization is mediated through phase separation of heterochromatin protein 1 HP1α, also known as chromobox protein homolog 5 (CBX5) in humans, an essential and conserved protein important for heterochromatin organization (Larson et al., 2017; Strom et al., 2017) (see poster). Like many proteins that drive phase separation, HP1α has three key domains (Mitrea and Kriwacki, 2016): an oligomerization (dimerization) domain, a flexible intrinsically disordered region (IDR), and a substrate binding domain, which in this case binds methylated chromatin (H3K9me2/3) (Aasland and Stewart, 1995; Paro and Hogness, 1991). Consistent with this common domain architecture, HP1α has been found to readily phase separate into liquid droplets *in vitro*, and is capable of compacting DNA in a model *in vitro* system (Larson et al., 2017).

Taken together, these findings highlight the potential for widespread interplay between chromatin and phase-separated protein condensates, where the two may be closely interwoven. Indeed, differential chromatin compaction and sequence-specific interactions can impact the probability, positioning and timing of condensate formation, and conversely, condensate formation can impact chromatin structure and organization. Selective permeability of each liquid compartment can then specifically functionalize the biological components within, leading to a highly complex nucleoplasm that is organized by numerous distinct liquid condensates (see poster).

Potential functional outcomes of phase transitions in the nucleus

The model of HP1α driving phase separation of constitutive heterochromatin provides an explanation for a number of important phenomena, and highlights how the process of chromatin compartmentalization through phase separation might be utilized for the organization of additional aspects of nuclear function. The HP1α-rich heterochromatin domain is depleted for transcription factors and RNA polymerase (Feng and Michaels, 2015), which is a major contributing factor to transcriptional repression of the chromatin within. Regions of H3K9-methylated chromatin that are interspersed along the chromosome arms tend to loop back and interact in 3D space with pericentromeric heterochromatin (Dernburg et al., 1996; Lee and Karpen, 2017), which is consistent with liquid-like fusion of multiple condensates into one compartment. The chromatin fiber can be viewed as a long polymer with many epigenetic states, each of which imparts an interaction signature to define compartment identity of the locus (Erdel and Rippe, 2018). Recent models suggest the utility of this framework for understanding large-scale chromatin organization (Jost et al., 2014). Rod cells of nocturnal animals concentrate their heterochromatin in one large mass in the center of the nucleus by downregulating lamin B receptor, the protein that links heterochromatin to the nuclear envelope in this cell type (Solovei et al., 2009). Without this additional interaction, which tethers the phase-separated heterochromatic compartment to the periphery, heterochromatic sequences instead congregate in a single mass in the center of the nucleus. Such large-scale localization changes (inversion) of heterochromatin reorganize the chromatin fiber on the nuclear scale (Falk et al., 2019). In a similar way, differential interactions between chromatin compartments might be responsible for the organization and relative positioning of many flavors of chromatin blocks (see poster). Hi-C experiments read out 3D interactions of the chromatin fiber and have previously been found to show preference for regions to exist in one of two compartments types, called A and B (Lieberman-Aiden et al, 2009). These compartments have preferential self-interaction that is not dependent on the topologically associating domain (TAD) structure within them – this level of organization provides another example of the emergent collective biomolecular behavior, at the core of the concept of a phase transition (Fortin and Hansen, 2015; Lieberman-Aiden et al., 2009; Nuebler et al., 2018; Schwarzer et al., 2017). Large-scale nuclear organization might thus be fruitfully modeled as the consequence of an effective free energy minimization-driven demixing of multiple liquid phases that each include proteins, RNAs and select blocks of the chromatin fiber (Lohse and Hadjichristidis,

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1997) (see poster); however, further investigation is necessary to verify that this is indeed the case *in vivo*.

Phase separation is potentially also at play at smaller genomic length scales. Within a chromatin compartment, the functions of single loci can be regulated by relocating them to different functional compartments. For example, double-strand breaks in repetitive DNA actively move out of the constitutive heterochromatin compartment to complete repair (Chiolo et al., 2011) in order to prevent accidental recombination with similar repetitive sequences. Interestingly, polyADP-ribosylation (PARylation) at DNA damage repair foci promotes liquid-like properties (Altmeyer et al., 2015; Duan et al., 2019) and might allow for transient functionalization of a small compartment in order to complete repair. Additionally, transcriptionally repressed loci are often physically separated from actively transcribing loci (Brickner and Brickner, 2012; Lieberman-Aiden et al., 2009; Quinodoz et al., 2018; Takizawa et al., 2008), suggesting that movement of a locus from a transcriptionally repressed to a transcriptionally competent compartment is important for its functional state (Chambeyron and Bickmore, 2004). If chromatin compartments are organized by phase transitions and functionalized by selective accumulation of specific factors, moving a locus into a different compartment could expose it to a new environment and therefore change its functional state. Consistent with this picture, when transcriptionally competent loci are forced to interact with the nuclear lamina, a transcriptionally silencing compartment, they become epigenetically modified, similar to lamin-associated domains (LADs), and become less transcriptionally active (Peric-Hupkes et al., 2010). Moreover, when a sequence normally in a LAD is forced into a transcriptionally active state, it loses repressive marks and moves away from the lamina (Robson et al., 2016) (see poster). However, notwithstanding the explosion of interest in phase separation throughout the cell, phase separation-based models likely do not yield quantitatively accurate predictions of genomic organization in all cases and on all length scales (kb, Mb etc.), and more subtly textured non-equilibrium models may be required (see Box 1).

Phase separation has also been invoked in clustering distal regulatory sequences with promoters during transcriptional activation (Cho et al., 2018; Cisse et al., 2013; Hnisz et al., 2017) (see poster). Transcription factors bind regulatory sequences of DNA and have IDRs that can interact with the low complexity C-terminal domain (CTD) of RNA polymerase (Pol) II to help stabilize its binding at transcriptional start sites and promote transcription initiation (Boehning et al., 2018; Hnisz et al., 2017; Kwon et al., 2013). These regulatory sequences (enhancers) can be linearly distant from the promoters they regulate, but tend to come into close proximity in 3D space during transcriptional activation (Fukaya et al., 2016; Nolis et al., 2009). Phase separation-based transcriptional condensates provide an explanation for the observation that a single enhancer can simultaneously co-activate multiple distinct promoters (Fukaya et al., 2016), as well as explaining the process of transvection, in which an enhancer on one chromosome can initiate transcription at a promoter on a different chromosome (Lim et al., 2018). Transcriptional compartments are also important in viral infection, where many viruses form 'replication compartments', which localize RNA polymerase molecules to maximize viral production. These replication bodies resemble condensates in many ways, and might represent phase-separated compartments (Heinrich et al., 2018). However, other non-phase separation mechanisms may be at play, for example through preferential interactions with non-nucleosomal viral DNA (McSwiggen et al., 2019).

Phase separation in the nucleus thus appears to underlie the creation of diverse functional compartments, like the nucleolus, that can complete their essential roles due to condensation of specific factors into a liquid-like domain. Phase separation can also organize chromatin compartments like constitutive heterochromatin and helps to properly position these regions within the nucleus. Further investigations of the liquid nucleome will be important to determine how phase transitions in the nucleus are regulated by the cell and which, if any, other compartments or functions they may contribute to (see Box 1).

Box 1. Engineering condensates in the nucleus.

Uncovering the biophysical basis of functional nuclear compartmentalization has been enabled by the development and application of new tools and techniques to control phase separation in the nucleus (see poster). Altering concentration or effective interaction strength of condensate-forming proteins allows experimental control over condensate formation and dissolution by moving through space on a phase diagram. Tight control over protein concentration by overexpression or, for example, auxin-inducible degradation (Nishimura et al., 2009), can adjust protein concentration to be above or below the critical concentration that is necessary for condensate formation and allow study of the differential functions of a protein in dilute and condensed states. Switch-like changes in protein function across this critical concentration threshold might be attributable to phase separation-related functions. Alternatively, interaction strength and/or entropy can be altered by controlling oligomerization, for example through optogenetic or chemical means (Aonbangkhen et al., 2018; Bracha et al., 2018; Dine et al., 2018; Shin et al., 2018, 2017). In these systems, oligomerization of weakly interacting protein domains upon an experimental signal (addition of light or chemical) triggers condensate formation and allows study of differential functions in dilute and condensed phases. Combing these tools with CRISPR/Cas9-based genome targeting approaches (Shin et al., 2018; Wang et al., 2018) enables linking of biophysical studies with precise gene-specific functional studies. Additionally, experimentally controllable genome targeting introduces the ability to investigate the role of 'seeding' location, sequence specificity and epigenetic state. Together with our rapidly increasing understanding of the physical properties of biomolecular phase separation, these tools promise new and exciting future discoveries that will shed critical light on nuclear organization and function.

Conclusions and perspectives

Here, we have focused on mesoscale nuclear organization into non-covalent biomolecular assemblies that comprise the liquid nucleome. These compartments include both relatively chromatin-poor nuclear subcompartments such as nucleoli, Cajal bodies and speckles, and also chromatin-rich compartments, like constitutive heterochromatin, all of whose assembly is increasingly thought to reflect underlying biomolecular phase transitions, in particular LLPS. We note that the structures we discuss here are certainly not an exhaustive list of nuclear bodies and compartments, many of which also likely reflect underlying biomolecular phase transitions; chromosome territories and Barr bodies (Cerase et al., 2019) are also likely candidates, among others (Lu et al., 2016; da Rocha and Heard, 2017). Phase transitions play a role in other nuclear structures and functions as well; in mitosis, a surfactant layer of the Ki-67 protein buffers space between condensed chromosomes and aids in their proper segregation (Cuylen et al., 2016) and, during meiosis, the synaptonemal complex that holds homologous chromosomes as pairs for faithful genome propagation can exist in a condensed liquid crystalline state (Rog et al., 2017). Throughout the nucleus, collective

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self-assembly through biomolecular phase transitions is centrally at play. New tools enabling this focused biophysical lens (see Box 1) promise to greatly accelerate our understanding of the rich structure and function of the liquid nucleome.

Competing interests
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