Biophysical Perspective

Formation of Chromatin Subcompartments by Phase Separation

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ABSTRACT   Chromatin is partitioned on multiple length scales into subcompartments that differ from each other with respect to their molecular composition and biological function. It is a key question how these compartments can form even though diffusion constantly mixes the nuclear interior and rapidly balances concentration gradients of soluble nuclear components. Different biophysical concepts are currently used to explain the formation of "chromatin bodies" in a self-organizing manner and without consuming energy. They rationalize how soluble protein factors that are dissolved in the liquid nuclear phase, the nucleoplasm, bind and organize transcriptionally active or silenced chromatin domains. In addition to cooperative binding of proteins to a pre-formed chromatin structure, two different mechanisms for the formation of phase-separated chromatin subcompartments have been proposed. One is based on bridging proteins that cross-link polymer segments with particular properties. Bridging can induce a collapse of the nucleosome chain and associated factors into an ordered globular phase. The other mechanism is based on multivalent interactions among soluble molecules that bind to chromatin. These interactions can induce liquid-liquid phase separation, which drives the assembly of liquid-like nuclear bodies around the respective binding sites on chromatin. Both phase separation mechanisms can explain that chromatin bodies are dynamic spherical structures, which can coalesce and are in constant and rapid exchange with the surrounding nucleoplasm. However, they make distinct predictions about how the size, density, and stability of chromatin bodies depends on the concentration and interaction behavior of the molecules involved. Here, we compare the different biophysical mechanisms for the assembly of chromatin bodies and discuss experimental strategies to distinguish them from each other. Furthermore, we outline the implications for the establishment and memory of functional chromatin state patterns.

INTRODUCTION

The cell nucleus and the genome are organized into spatially separated subcompartments that serve distinct functions (1–4). These include DNA-containing "chromatin bodies" such as nucleoli, which are involved in ribosome biogenesis (5), transcription factories associated with active RNA polymerase II (6), as well as Polycomb group bodies (7) and pericentric heterochromatin foci (referred to as chromocenters because of their intense staining with DAPI in fluorescence microscopy images) (8), which contain facultative and constitutive heterochromatin, respectively. Other subcompartments such as PML nuclear bodies (PML-NBs) or paraspeckles are mostly devoid of DNA but can associate with certain genomic loci (9,10) or contain RNA as a structural scaffold (11). Most protein components that define these structures are small enough to rapidly move across the nucleoplasm,

i.e., the liquid phase that fills the nucleus (12,13). Thus, the question of how these nuclear subcompartments are stabilized despite diffusion balancing concentration gradients arises.

The localized assembly of a chromatin body is driven by the presence of specific protein binding sites on the nucleosome chain (Fig. 1 A). A simple mechanism to rationalize its assembly is the (cooperative) binding of soluble factors to the chromatin scaffold and possibly to each other (Fig. 1 B) (12). This may involve sequence-specific interactions that define nucleation sites, as well as feedback-based mechanisms that reinforce a given chromatin state, as discussed, for example, in the context of pericentric heterochromatin (14). Depending on the properties of the molecules that bind to the scaffold, a transition to a phase-separated subcompartment can occur: on the one hand, chromatin-associated proteins that cross-link different chromatin segments can induce a polymer-polymer phase separation (PPPS, Fig. 1 C), which generates an ordered collapsed globule. On the other hand, chromatin-associated proteins that exhibit multivalent interactions with each
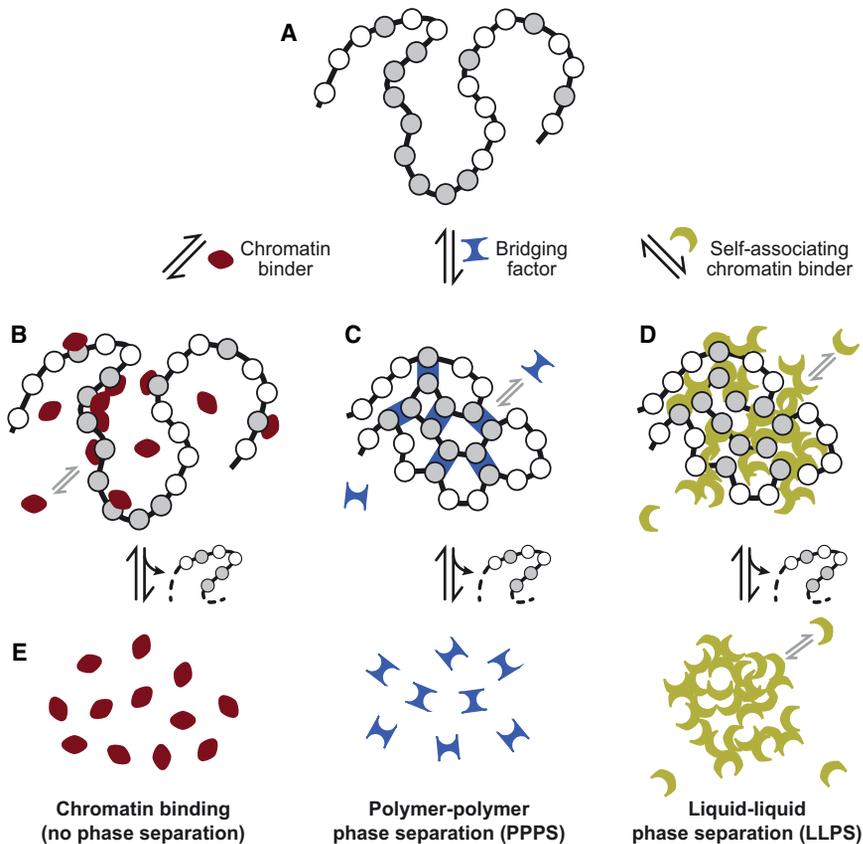
FIGURE 1 Models for the formation of chromatin subcompartments. (*A*) Chromatin regions containing nucleosomes with specific binding sites (*gray*) can form subcompartments by conceptually different mechanisms as shown in the following panels. Intermediate cases are also possible depending on the properties of the proteins involved. (*B*) Protein binding without phase separation is shown. The interacting protein (*dark brown*) will follow the preexisting 3D chromatin conformation. It can yield localized enrichment because of clustering of binding sites, cooperative binding, and/or allostery. (*C*) Polymer-polymer phase separation (PPPS). The formation of an ordered globule is induced via proteins (*blue*) that bridge nucleosomes residing in close spatial proximity to each other. (*D*) Liquid-liquid phase separation (LLPS). A chromatin-associated liquid-like droplet is formed, which is stabilized by proteins (*light green*) that exhibit multivalent interactions among each other. (*E*) Upon removal of the chromatin scaffold, the body will fall apart for simple chromatin binding and PPPS. In case of LLPS, the liquid-like protein droplet is predicted to persist. To see this figure in color, go online.

other can promote a liquid-liquid phase separation (LLPS, Fig. 1 *D*). It results in the formation of liquid-like protein droplets that can also be stable in the absence of the chromatin scaffold (Fig. 1 *E*). All three mechanisms depicted in Fig. 1 explain how cells can establish chromatin subcompartments without investing energy. In the following, we discuss the requirements and implications associated with the formation of chromatin subcompartments by LLPS and PPPS, outline the underlying differences, highlight the functional implications, and propose experiments to identify different phase separation mechanisms in living cells. For a more comprehensive survey of LLPS phenomena in biological systems, the reader is referred to other reviews (15–17).

Phase separation mechanisms for chromatin body formation

Two conceptually different phase-separation mechanisms have been considered to explain the formation of chromatin bodies. The PPPS mechanism is based on the binding of soluble bridging factors that assemble a dynamically cross-linked chromatin scaffold, which resembles a collapsed polymer globule (Fig. 1 *C*) (18–24). PPPS is compatible with only transient chromatin binding of bridging proteins and their constant exchange with those located in the surrounding nucleoplasm, as long as a steady state with a suf-

ficiently high number of bridging interactions is established. The alternative LLPS mechanism is driven by multivalent interactions among soluble components (Fig. 1 *D*). According to this mechanism, a nuclear subcompartment and the surrounding nucleoplasm resemble two liquids that form separate phases, similarly to oil drops in water. LLPS has been used to rationalize the formation of several nuclear bodies (16,17). Many of them can exist independently of a chromatin scaffold and can translocate through the nucleus unless they are too large to penetrate the chromatin network (12,25,26). Recently, the LLPS mechanism has been invoked to describe the assembly of chromatin bodies, including nucleoli, heterochromatin foci, and enhancer clusters (27–30). In the following, we compare both types of phase-separated compartments and discuss the differences that arise from the distinct underlying biophysical mechanisms.

PPPS: bridging interactions drive phase separation within polymers

Several elaborate polymer models have been used to explain the multiscale organization of chromosomes as reviewed in (31–36). These models recapitulate many properties of chromatin, including the scaling of spatial distances between genomic loci, the distribution of loop sizes, the dynamics of chromatin segments, and the

response of these features to various perturbations. In the context of these polymer models, compartmentalization can be induced by bridges between nucleosomes that reside in close spatial proximity to each other (Fig. 1 C) (18–24). On the molecular level, bridging and the formation of chromatin loops can be mediated by internucleosomal interactions that, for example, involve histone tails (37), CTCF and YY1 (38), cohesin (39,40), or condensin (41). In addition, hundreds of chromatin-associated proteins and transcription factors contain two or more chromatin/DNA recognition domains or interact directly or indirectly with each other, thus having the potential to bridge different nucleosomes (42–45). In mouse pericentric heterochromatin, for example, these factors include HP1, SUV39H, and methyl-CpG binding proteins, which interact with each other and recognize H3K9-methylated nucleosomes, methylated pericentric DNA, or nascent pericentric transcripts (46–49). In actively transcribed chromatin regions, RNA polymerase molecules and transcription factors have been proposed to act as molecular ties that establish transcription factories (6). Bridging proteins may cluster along the DNA even if they do not interact with each other to minimize bending of the DNA scaffold (50). Furthermore, bridging can drive the transition of the respective chromatin regions into a globular compartment (18–20,51). In this manner, phase separation between chromatin regions that (stably or transiently) interact with different types of bridging factors is induced. The transition might be less distinct if it occurs via the formation of small separated clusters of bridging proteins (52). It can be enhanced by feedback loops that create additional binding sites, for example, via deposition of histone modifications (18,24,53).

Bridging-induced collapse is conceptually similar to the coil-globule transition, which describes the collapse of self-interacting polymers in solution into a polymer-rich globule and a solvent-rich surrounding phase (36,54–56). Collapse is favored by interactions among polymer segments, entropic depletion attraction and spatial confinement (57,58). It has been proposed to proceed via nucleation of the polymer-rich phase and subsequent coalescence (59,60). Dense domains that might represent such collapsed polymer globules have recently been detected by chromosome conformation capture (e.g., (39)) and super-resolution imaging (61,62). It is an important feature of PPPS that it does not require any interactions among bridging proteins (18,24). In PPPS, the fluid that surrounds the chromatin fibers residing in different parts of collapsed globules or in more loosely packaged coils does not phase-separate, and the accessible volume of globules and coils therefore contains the same nucleoplasmic fluid. Accordingly, the concentrations of soluble components dissolved in this fluid are balanced by diffusion across all chromatin bodies that are formed by PPPS. However, the net composition of these bodies, which includes free and chromatin-bound components, is distinct if chromatin in each of them is bound by different factors.

LLPS: multivalent interactions drive liquid-liquid phase separation

Many proteins and RNA molecules in the cell can exhibit multivalent interactions with each other, which frequently involve intrinsically disordered protein domains of low sequence complexity (16,63,64). On the molecular level, such interactions can be mediated by electrostatic attraction between charged residues, dipoles, or aromatic groups, which are also present on the nucleic acid and protein components of chromatin. Multivalent interactions make simultaneous contacts among several partners energetically favorable, thereby promoting the formation of supramolecular clusters. If these interactions are strong enough compared to interactions with solvent molecules but too weak to induce irreversible aggregation, they can induce LLPS: a dense liquid phase is formed and coexists with a more dilute liquid phase (15–17,65). As a result, certain molecules become enriched or depleted in one of the two phases, thereby favoring or inhibiting particular biochemical reactions (16). In everyday life, LLPS is known from emulsions such as oil-water mixtures that contain oil droplets immersed in an aqueous solution. In cells, LLPS has initially been proposed to drive the assembly of several nuclear bodies, e.g., PML-NBs or Cajal bodies (16,17). Although the individual molecules in these bodies can move and exchange with those in the surrounding diluted phase, the net composition of both phases remains invariant because the coexistence of separated liquid phases is energetically favorable.

The formation of nuclear bodies by LLPS is thought to proceed via nucleation and growth of liquid-like droplets (17,66). Formation is favored by multivalent interactions among the respective components and disfavored by the energetic cost for creating an interface between the two phases. The first contribution is proportional to the volume of the droplet, whereas the second is proportional to its surface. Thus, to be stable, droplets need to reach a critical size at which the energetic cost for creating an interface is exceeded by the energy gained from multivalent interactions within the droplet. This step can occur at nucleation sites that recruit the respective components and facilitate droplet formation (66). Once droplets become large enough to be energetically favorable, they grow and are stable in the absence of nucleation sites. Nuclear bodies that show such behavior are PML-NBs, which can be nucleated at telomeres and subsequently detach from them (26), or Cajal bodies, which can form independently of DNA and are only transiently associated with chromatin (67,68). Nucleation of a phase-separated nuclear body requires supersaturating concentrations of the constituting self-interacting molecules (16,66). If the concentration is below this value,

the formation of two liquid phases is energetically unfavorable and molecules will merely bind to nucleation sites, where they might form small clusters but not a phase-separated droplet. In summary, the formation of a chromatin body via LLPS requires multivalent interactions of a certain strength, a sufficiently large concentration of the respective constituents, and the presence of efficient nucleation sites.

Functional differences between chromatin bodies formed via PPPS and LLPS

As described above, the PPPS mechanism is based on bridging interactions that tend to compact the chromatin fiber (Fig. 1 C). These interactions are mediated by molecules that bind to different chromatin segments but do not necessarily bind to each other. In contrast, the LLPS mechanism is independent of bridging interactions between polymer segments because phase separation is driven by multivalent interactions among soluble components (Fig. 1 D). However, bridging interactions might indirectly arise from multivalent binders that interact with each other and with chromatin. Thus, both PPPS and LLPS can, in principle, promote the formation of a compact chromatin compartment, albeit on a different molecular basis. Whereas bodies formed via PPPS strictly rely on the chromatin scaffold and disassemble in the absence of chromatin, bodies formed via LLPS can persist independently of the chromatin scaffold (Fig. 1 E). One functional consequence is that fluctuations of the number of binding sites on chromatin immediately translate into an altered molecular composition of chromatin bodies formed via PPPS because the number of bridging factors scales with the number of binding sites. In contrast, chromatin bodies formed via LLPS are insensitive to such fluctuations as long as the number of binding sites exceeds the threshold above which the bodies are efficiently nucleated and kept at the nucleation site.
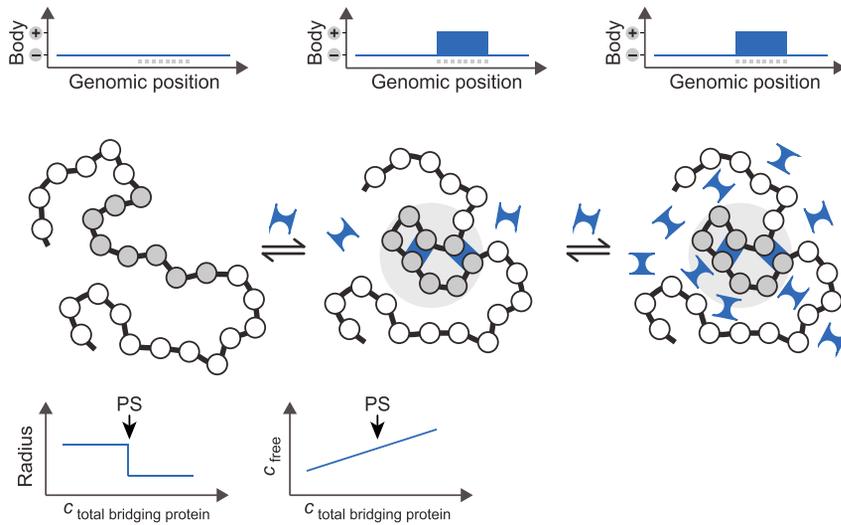
Both mechanisms also differ in their response to concentration fluctuations of the molecules that hold together the chromatin body (Fig. 2). Such fluctuations might, for example, be caused by alterations in gene expression or dilution effects upon cell division. For a chromatin body formed via PPPS, the molecular composition of the fluid that surrounds chromatin within and outside of the compartment is the same, whereas LLPS creates a different liquid composition in the interior of the body. Accordingly, the PPPS mechanism predicts that chromatin within the chromatin body is directly exposed to any changes in the composition of the nucleoplasm. In contrast, LLPS predicts that such changes are buffered because the composition of the liquid droplet is mainly determined by the characteristics of multivalent interactions but not by the total concentration of its constituents in the nucleoplasm. It is therefore decoupled from exterior concentration fluctuations unless the concentration falls below the critical concentration required for phase separation. A second important difference between PPPS and LLPS is the coupling between the size of the body and the composition of the nucleoplasm. The size of chromatin bodies formed via PPPS is independent from the molecular composition of the nucleoplasm as long as the collapsed polymer state is maintained (Fig. 2 A). At very high concentrations of bridging factors, the number of bridges is expected to decrease again because binding sites on chromatin become saturated, and each site binds a separate bridging factor (not depicted in Fig. 2 A). Thus, for moderate concentrations of bridging factors, PPPS robustly separates the chromatin region that resides within the body from neighboring regions, thereby promoting separation of the respective chromatin states. In contrast, increasing or decreasing the concentration of multivalent binders in the nucleus will readily increase or decrease the size of chromatin bodies formed via LLPS (Fig. 2 B). As a consequence, the chromatin region within the droplet either has to change its compaction state to adjust to the new droplet size, or the amount of chromatin within the droplet has to change if chromatin compaction remains unaltered. In the latter case, concentration buffering would directly translate into size fluctuations of the genomic region enclosed in the chromatin body. This process might either lead to spreading of chromatin states due to the incorporation of flanking chromatin into growing bodies, or to contraction of chromatin states due to the exclusion of chromatin from shrinking bodies. To our knowledge, these predictions have not yet been tested experimentally.

Regulation of chromatin body formation by active processes

PPPS and LLPS rationalize the formation of a chromatin body at thermodynamic equilibrium, which implies that its assembly and maintenance does not require energy. However, the steady state concentrations of the factors that drive phase separation can depend on energy-consuming processes. In particular, bridging proteins or multivalent binders that are required for the formation of chromatin bodies might constantly dissipate. This process could involve degradation or export of these proteins from the nucleus or changes of their modification state, which could weaken the interactions that stabilize the chromatin body. A prominent example is the nucleolus, which exhibits liquid-like properties consistent with LLPS (30,69). Interestingly, maintenance of the nucleolus requires active transcription by different polymerases as well as the activity of other enzymes (70,71). At first glance, this dependence seems at odds with the prediction that LLPS does not consume energy. However, the energy dependence might arise from the constant export of nucleolar RNAs into the cytosol, which have to be actively replenished to keep their steady state concentration above the level required for LLPS. Furthermore, cell-cycle-dependent changes in RNA binding properties and posttranslational modifications of

A Polymer-polymer phase separation (PPPS)

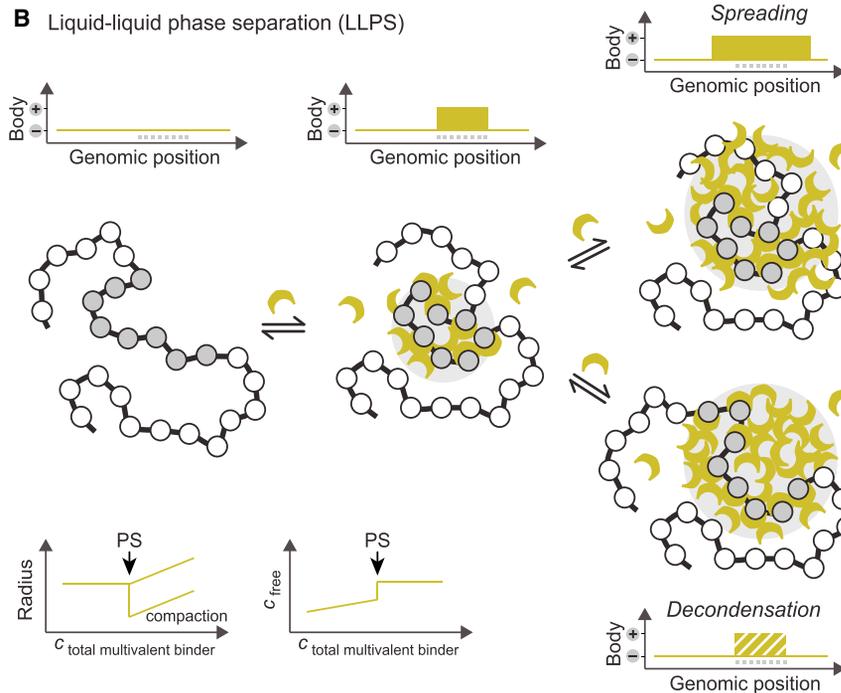**B** Liquid-liquid phase separation (LLPS)

FIGURE 2 Response of chromatin bodies to concentration changes in the nucleoplasm. The axis label "Body + or −" denotes the position of the respective genomic region inside or outside of the chromatin body. The gray dots in the plots represent binding sites corresponding to gray nucleosomes in the cartoon. (*A*) For PPPS, the chromatin region that contains binding sites (*gray*) for bridging factors organizes into a collapsed/ordered chromatin globule when bridging factors are added. The genomic extension of the globule remains invariant if the concentration of bridging proteins is further increased. At very high concentrations, binding sites might become saturated and the size of the body might increase (not depicted in the figure). (*B*) For LLPS, the initial formation of chromatin bodies can occur with or without compaction of the incorporated chromatin (depending on the ability of multivalent binders to bridge chromatin segments). The bodies become larger if the concentration of multivalent binders is increased. This should lead either to the incorporation of adjacent chromatin regions into the chromatin body (*top*) or to decondensation of the chromatin region that is "dissolved" in the chromatin body (*bottom*). At very high concentrations, the dense liquid phase might become a gel or an aggregate (not depicted in the figure). To see this figure in color, go online.

nucleolar proteins might change the respective phase equilibria and thereby actively regulate the (dis)assembly of the nucleolus (72).

In contrast to phase-separated bodies that only indirectly rely on active processes, liquid-like bodies might also form as a direct consequence of active processes. Due to the similarity between both types of bodies, it is difficult to distinguish them in experiments. For example, the RNA molecules transcribed from a reporter gene cluster in U2OS cells remain near this cluster and form a visible speckle (73). The structure rapidly disappears when transcription is stopped (74), which is consistent with a model in which RNA molecules are rapidly produced at the cluster, associate with RNA-binding proteins, and dissipate relatively slowly by diffusion. This series of events can explain a steady-state accumulation of RNA molecules at the cluster without invoking phase separation. Similar observations were made for paraspeckles, which form at the sites of Men ε/β non-coding RNA (ncRNA) transcription but not at the sites where these ncRNAs were artificially tethered (11). These data suggest that active production of ncRNAs is required to maintain the speckle, whereas interactions between ncRNAs and associated proteins are not sufficient to maintain it. In the following section, we will discuss

experimental strategies to identify phase-separated bodies and to distinguish between bodies formed via LLPS or PPPS.

Experimental strategies to dissect the assembly mechanism of chromatin bodies

Chromatin bodies that are formed by different mechanisms have distinct properties that influence their biological functions as outlined above. Therefore, strategies to distinguish between formation mechanisms are highly sought after. In general, an instructive readout to distinguish LLPS from the other mechanisms is the steady-state localization of the chromatin proteins in question (Fig. 3 *A*): if the polymer is not evenly distributed in the dense phase, chromatin binders (Fig. 1 *B*) and bridging proteins (Fig. 1 *C*) should strictly colocalize with the polymer, whereas multivalent binders in LLPS should form a homogeneous liquid phase around the polymer (Fig. 1 *D*). However, the direct structural analysis of chromatin bodies by microscopy-based methods might be complicated due to limited (three-dimensional) resolution. In the following, we will outline alternative strategies that might help distinguish between different mechanisms. We will use heterochromatin foci/chromocenters (8) as an exemplary starting point for which different scenarios are currently discussed. Previous studies have rationalized the formation of chromocenters by binding of heterochromatin protein 1 (HP1) and other heterochromatin proteins to histone H3 trimethylated at lysine 9 (48,49) according to the cooperative binding mechanism depicted in Fig. 1 *B*. Chromocenters in mouse fibroblasts have an ∼2-fold higher nucleosome concentration of ∼230 μM as compared to the nuclear average and an ∼10 μM HP1α/β concentration (46). Furthermore, HP1 dimers can bridge two nucleosomes (49). Thus, it appears conceivable that HP1 bridging promotes chromatin collapse via PPPS (Fig. 1 *C*) as proposed in (18). In this model, chromocenters might be composed of multiple smaller chromatin subdomains such as those described recently (61,62). Alternatively, two studies concluded that the formation of chromocenters is driven by LLPS (27,28), which is inspired by the finding that HP1 undergoes such a transition in vitro.

As LLPS creates a sharp boundary between the exterior and the interior liquid phase, a change of a tracer protein's mobility at the boundary could be informative, although PPPS can also create a boundary that separates polymer phases with different density and substructure (Fig. 3 *B*). Previous single-particle tracking experiments did not observe significant mobility changes of "inert" tracers in different intramolecular environments, including chromocenters and the nucleolus, which have been proposed to form via LLPS (75). In contrast, the measurements reported in (28) argue in favor of a reduced mobility at the boundary of chromocenters. There might be several caveats when in-terpreting and comparing such experiments: 1) chromatin bodies are dynamic and diffusion barriers constantly change their configuration, as shown, for example, for chromocenters in (76), which makes it difficult to measure transport across the same barrier or compartment boundary over time; 2) the chemical nature of a given tracer particle will affect its mobility when translocating across compartment boundaries, which might lead to differences among different tracers even if they are considered "inert"; and 3) both PPPS and LLPS generate two coexisting phases that resemble viscoelastic polymer solutions, with transport coefficients being affected by both the viscous and the elastic contribution of the medium (77,78). As changes in the concentration and distribution of the polymer (PPPS and LLPS) as well as in the strength of multivalent interactions among soluble molecules (LLPS) will affect these properties of the medium, there might be several PPPS- or LLPS-based scenarios for which a given mobility profile is obtained. Thus, it appears to be difficult to identify the mechanism of chromatin body formation based on transport properties of soluble components, although such experiments provide valuable insight into the dynamic organization of chromatin bodies.

An alternative approach for distinguishing LLPS from PPPS is to follow the (dis)assembly process of nuclear bodies over time. A hallmark feature of LLPS is its persistence in the absence of nucleation sites (Figs. 1 *E* and 3 *C*). Thus, following the fate of nuclear bodies upon removal of endogenous nucleation sites could be a helpful test for LLPS. Alternatively, introduction of artificial nucleation sites that can subsequently be removed appears to be a viable strategy to assess whether nuclear bodies are held together by multivalent interactions or whether they require persistent nucleation. The growth law associated with the assembly process might also be instructive to distinguish between PPPS and LLPS. For LLPS, the size of the body increases over time with a power of one-third if growth is mainly driven by diffusion (79). In contrast, PPPS-driven formation occurs via clusters organized in a beads-on-a-string-like manner with a different time dependence (52,80). Furthermore, as outlined in Fig. 2, the expected response of chromatin bodies to concentration changes of constituting protein factors in the nucleoplasm are different between PPPS and LLPS. For PPPS, no size increase is expected when the concentration of bridging factors is increased, and the concentration of bridging factors should increase both inside and outside of the body (Fig. 3 *D*). For very large concentrations of bridging factors, each binding site will become saturated with its own bridging factor, thereby reducing the number of bridges and destabilizing the ordered and collapsed globule. For LLPS, chromatin bodies are expected to continuously increase in size with increasing external concentrations of multivalent binders, whereas the concentration of multivalent binders inside the body should remain invariant.

A Internal structure

Colocalization
with chromatin

Homogeneous
liquid phase

B Molecular transport

Size-dependent
exclusion

Exclusion based on
chemical properties

C Stability without chromatin

Dissolution

Stable droplet

D Concentration/size regulation

Concentration
increase

Size
increase
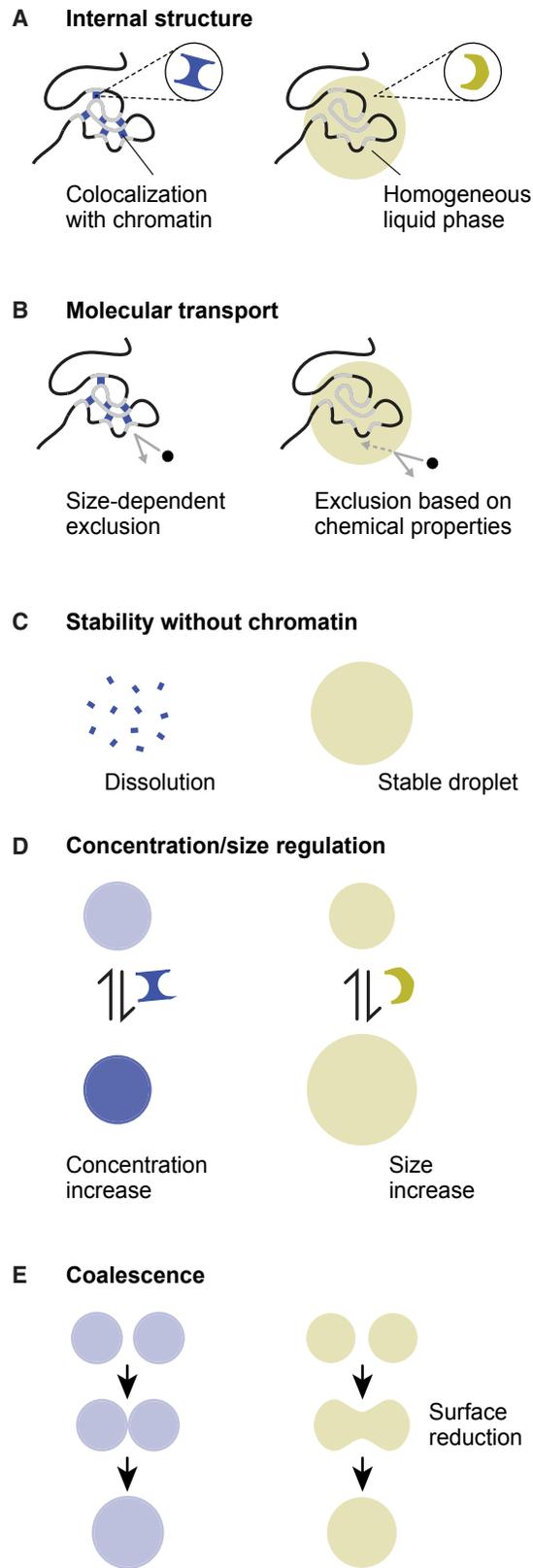
E Coalescence

Surface
reduction

FIGURE 3 Properties of chromatin bodies that are informative about the underlying assembly mechanism. Chromatin bodies formed by PPPS and LLPS are shown in blue and beige colors, respectively. (A) PPPS predicts strict colocalization of bridging proteins and chromatin, whereas LLPS establishes a homogeneous liquid-like phase around the chromatin fiber. (B)

Finally, chromatin bodies formed via either PPPS or LLPS are predicted to coalesce when they come in contact with each other because bridging proteins and multivalent binders cannot distinguish between chromatin segments that are identical on the molecular level but initially reside in different bodies (59,60,81). However, the surface tension that is present between the liquid phases in LLPS might accelerate coalescence and lead to different droplet geometries during fusion as compared to the PPPS mechanism (Fig. 3 E). Thus, monitoring the geometry of fusion intermediates at high resolution could help to elucidate the underlying assembly mechanism.

Conclusion

It is a fundamental question how cells stably establish intricate three-dimensional chromatin subcompartments along the genome scaffold without internal membrane boundaries. The resulting chromatin state patterns are instrumental to implement hundreds of different cell-type-specific gene expression programs. Notably, the vast majority of these subcompartments that we refer to here as chromatin bodies (nucleoli, chromocenters, Polycomb group bodies, enhancer clusters, etc.) disassemble during cell division. Accordingly, they need to be reestablished in a reliable and faithful manner. Self-organizing phase separation mechanisms with local chromatin features acting as nucleation sites can give rise to these systems properties. They lead to well-defined spatial boundaries to target activities to the "correct" part of the genome without the need for energy consumption or a complex assembly machinery. Within the parameter space that induces different phases, they are stable structures that are robustly maintained, although the exchange of soluble factors with the nucleoplasm is highly dynamic. Furthermore, either the molecular composition (LLPS) or the size (PPPS) of chromatin subcompartments can be decoupled from fluctuations of the nuclear environment. Despite the elegance of these models, we are only beginning to understand how chromatin bodies can form, and the distinction between different mechanisms will require the rigorous assessment of their distinct predictions

Both models are compatible with dynamic exchange of constituents. Transport across the phase boundaries is regulated by their different molecular properties. (C) Once nucleated, phase-separated droplets formed via LLPS should be stable independently of the chromatin scaffold. (D) Increasing the concentration of the constituting factors in the nucleus can have different effects on phase-separated chromatin bodies. For PPPS, the size of collapsed chromatin bodies remains constant, whereas the concentration of chromatin binders in the body increases. Chromatin bodies formed by LLPS show the opposite behavior, i.e., their size increases but the concentration of chromatin binders in the body is not changed. (E) Coalescence upon contact between two chromatin bodies is shown. The geometry of the intermediate state and the coalescence rate should differ in PPPS and LLPS because of the surface tension at the liquid-liquid interface in LLPS. To see this figure in color, go online.

in living cells. One future challenge will be to define a set of critical features that can experimentally be examined to accomplish this task. We hope that this article will stimulate the discussion about strategies to tackle this issue.

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