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HYPOTHESES

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May the force be with you: Nuclear condensates function beyond transcription control

Potential nongenetic functions of nuclear condensates in physiological and pathological conditions

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Abstract

Over the past decade, research has revealed biomolecular condensates' relevance in diverse cellular functions. Through a phase separation process, they concentrate macromolecules in subcompartments shaping the cellular organization and physiology. In the nucleus, biomolecular condensates assemble relevant biomolecules that orchestrate gene expression. We here hypothesize that chromatin condensates can also modulate the nongenetic functions of the genome, including the nuclear mechanical properties. The importance of chromatin condensates is supported by the genetic evidence indicating that mutations in their members are causative of a group of rare Mendelian diseases named chromatinopathies (CPs). Despite a broad spectrum of clinical features and the perturbations of the epigenetic machinery characterizing the CPs, recent findings highlighted negligible changes in gene expression. These data argue in favor of possible noncanonical functions of chromatin condensates in regulating the genome's spatial organization and, consequently, the nuclear mechanics. In this review, we discuss how condensates may impact nuclear mechanical properties, thus affecting the cellular response to mechanical cues and, eventually, cell fate and identity.

Chromatin condensates organize macromolecules in the nucleus orchestrating the transcription regulation and mutations in their members are responsible for rare diseases named chromatinopathies. We argue that chromatin condensates, in concert with the nuclear lamina, may also govern the nuclear mechanical properties affecting the cellular response to external cues.

KEYWORDS
chromatin condensates, epigenetics, Kabuki syndrome, mechanobiology, mechanotransduction, MLL4, transcription control

Abbreviations: 3D, three-dimensional; CPs, chromatinopathies; CREs, *cis*-regulatory elements; ECM, extracellular membrane; E-P loops, enhancer–promoter loops; IDRs, intrinsically disordered regions; KS, Kabuki syndrome; LLPS, liquid–liquid phase separation; PcG, Polycomb; PrLDs, prion-like domains; TADs, topologically associating domains; TFs, transcription factors; TrxG, Trithorax.

INTRODUCTION

Hypothesis: chromatin condensates play nongenetic functions in regulating nuclear mechanical properties.

Biomolecular condensates are membrane-less subcompartments in which biochemical cell functions are compartmentalized. Phase separation is now recognized as one of the potential mechanisms for guiding condensate assembly.[1] In this process, a homogenous solution of molecules separates into two coexisting phases. In the nucleus, proteins and nucleic acids demix forming nuclear condensates in which a variety of nuclear processes occur,[2] including transcriptional regulation, which is compartmentalized in transcriptional condensates. The regulation of gene expression is a well-harmonized process that relies on the efficient interplay of transcription factors (TFs), chromatin modifications, and genome spatial organization.[3] A striking example of this is the crosstalk occurring between Polycomb (PcG) and Trithorax (TrxG) proteins. PcG and TrxG are chromatin-modifying factors, acting in concert to maintain a repressive or active chromatin environment, respectively.[4] They regulate the chromatin state introducing covalent histone tail modifications and influencing the three-dimensional (3D) genome organization.

Of importance, besides controlling the local chromatin environment to regulate gene expression, chromatin condensates are also involved in defining the nuclear genome topology. Indeed, the ~2 m of genetic information must be preserved and transmitted while confined within the ~10-μm diameter of the nucleus. To face this challenge, chromatin is organized in multiple layers of 3D organization through multiple levels of genomic contacts.

The interplay between chromatin organization and cis-regulatory elements (CREs) is pivotal for the spatiotemporal control of gene expression during development and tissue homeostasis, and its deregulation is linked to many diseases. Mutations in chromatin regulators can lead to severe disorders known as chromatinopathies (CPs).[5] CPs are a heterogeneous group of rare Mendelian diseases caused by the haploinsufficiency of chromatin regulators involved in chromatin condensate organizations.

The processes described so far occur in the nucleus, the largest and stiffest cell organelle, which can deform and adapt to external mechanical stresses while safeguarding genetic information. Indeed, the nucleus is continuously exposed to biochemical and mechanical inputs modulating nuclear responses in terms of nuclear morphology and gene activity.[6] Nuclear deformation can be the consequence of extrinsic input (i.e., extracellular matrix stiffness) propagating from the cytoplasmatic compartment to the nucleus via the LINC complex and cytoskeleton mechano-transmission.[7] The mechanical signals are then decoded into signaling molecules (mechanotransduction) that define specific cellular functions as well as regulate cell migration.[8] The biological relevance of the nuclear mechanical properties is supported by the observations that several pathological conditions, including muscular dystrophy, cardiomyopathy, and cancer, are associated with altered mechanotransduction.[9]

Besides the external forces, chromatin itself can generate inner forces, given its viscoelastic properties. The genomic contacts exert forces among the chromatin fiber, topological domains, and internal nuclear compartments, which are transmitted to the cytoskeleton through the LINC complex.[10] In addition, the Lamin A/C and B1/B2 filaments are necessary to corroborate nuclear mechanical functions by determining the elastic response of the nuclear lamina. Overall, the contribution of the chromatin organization in determining the nuclear mechanical properties represents an emerging non-genetic function of the genome. Albeit chromatin is mechanically responsive and resistant to various stresses, it behaves locally as a phase-separated system. In this context, chromatin condensates could play an important role in nuclear mechanics. We recently demonstrated how chromatin condensates equilibrium has a fundamental role in regulating the nuclear mechanical state in Kabuki syndrome (KS). KS is a CP caused by mutations in the chromatin modifiers MLL4 (encoded by *KMT2D*) or UTX (encoded by *KMD6A*). By investigating the effects of MLL4 haploinsufficiency, we proved that MLL4 contributes to the assembly of transcriptional condensates, whose impairment caused the strengthening of Polycomb-associated ones. The resulting condensate disequilibrium increases nuclear stiffness and alters nuclear mechanical properties.[11]

Despite these indications, knowledge about the noncanonical functions of chromatin condensates is still limited. In this review, we propose that chromatin condensates, in addition to their potential role in tuning gene expression, may also be involved in defining the mechanical properties of the nucleus, thereby determining the cellular response to external stimuli during development and tissue homeostasis.

MAIN BODY

Chromatin condensates compartmentalize nuclear functions

The nucleus is a crowded environment in which multiple biological processes take place. In this complex environment, the local assembly of functionally related molecules into biomolecular condensates allows to compartmentalize specific biological functions and yet guarantees a dynamic interplay between different biochemical reactions.[1]

Phase separation has been recognized as one of the primary mechanisms underlying biomolecular condensation. In recent years, the concept of phase separation has been considerably expanded beyond the well-studied liquid–liquid phase separation (LLPS) phenomenon and coupled to other phase transitions, such as percolation, formation of hydrogels, and solid amyloid fibril.[12,13] For simplicity, in the text, we will refer to phase separation to encompass all these phenomena.

Protein domains characterized by low structural complexity, such as intrinsically disordered regions (IDRs), are particularly prone to undergo phase separation by mediating a network of weak multivalent protein–protein and protein–nucleic acids interactions. In this respect, the amino-acidic composition of IDRs has an important role in determining their phase behavior. For instance, it was shown that

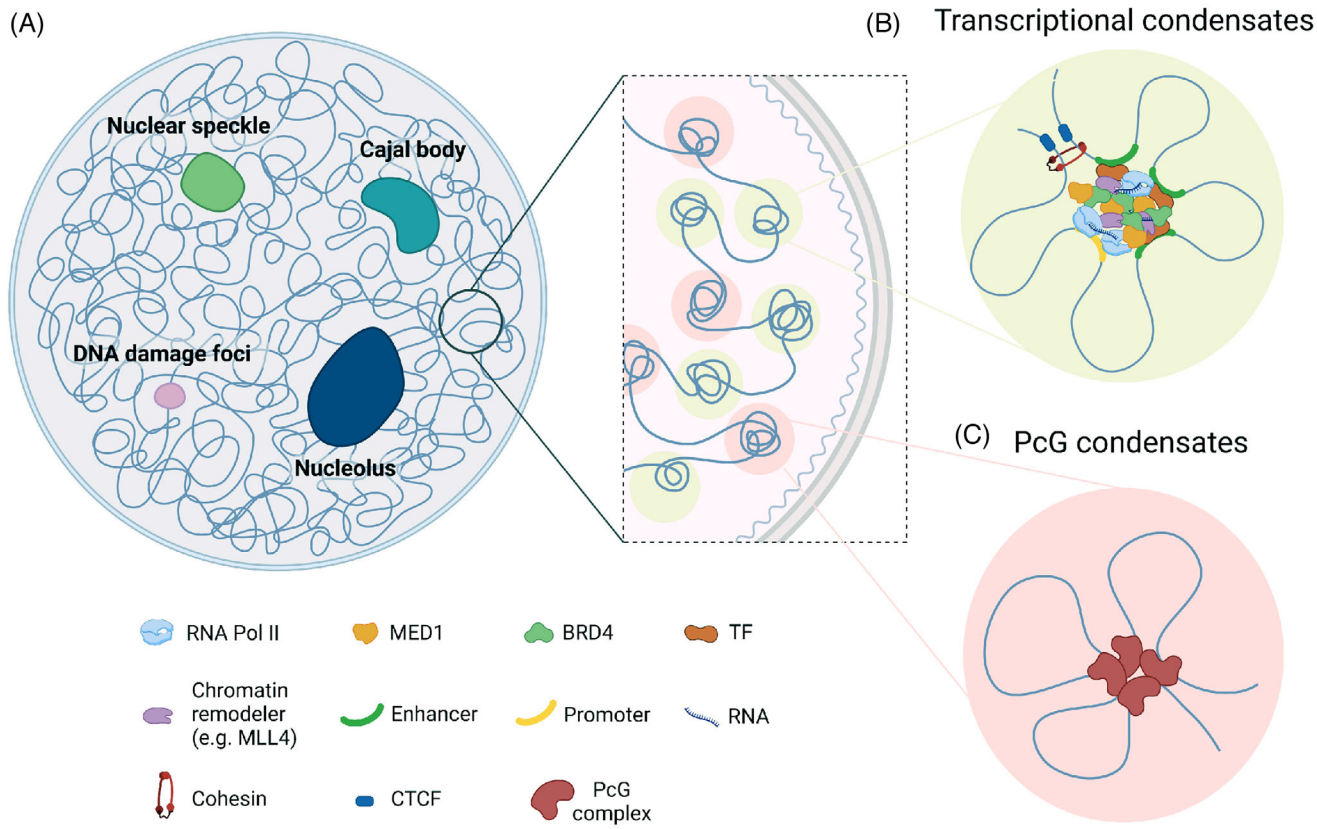

FIGURE 1 Biomolecular condensates in the nucleus. Graphical representation of the nucleus with the main biomolecular condensates (A) and a magnified view of transcriptional (B) and PcG condensates (C).

phase separation of intrinsically disordered prion-like domains (PrLDs) depends on the number and distribution of aromatic residues in the amino-acidic sequences.[14,15]

One still open question is how specific macromolecules are selectively partitioned into specific condensates while others are excluded. It is increasingly clear that disordered domains can carry the information to drive high affinity, specific binding.[16] In some cases, the distribution into blocks of opposite-charged amino acids is a feature shared by proteins clustering in the same condensate.[17] Nevertheless, the molecular grammar underlying this selective partitioning is far from being fully understood.

Multivalent interactions between proteins and nucleic acids can also drive biomolecular condensation. For instance, it has been shown that multivalent DNA molecules promote the formation of phase-separated condensates in vitro and that specific DNA elements mediate the localized assembly of transcriptional condensates at enhancers.[18] In addition, also the RNA promotes phase separation of the Mediator complex (a transcriptional coactivator) regulating the formation and the organization of transcriptional condensates.[19,20] Interestingly, there is strong evidence that RNA has a central role in recruiting and concentrating specific factors into precise nuclear territories.[21]

Nuclear condensates are involved in a variety of biological functions. Some of the better-characterized condensates regulate processes like the transcription of ribosomal RNA (the nucleolus), splicing (nuclear speckles), spliceosomal RNA maturation (Cajal body), and DNA repair (DNA repair foci), to mention a few (Figure 1A).[2]

Here we will focus on the chromatin condensates, which have been classically implicated in the regulation of mRNA transcription, broadly known as transcriptional condensates (Figure 1B). They assemble at specific genomic loci, where the RNA Polymerase II (RNA Pol II) forms phase-separated compartments together with TFs and coactivators, such as the Mediator subunit MED1 and the bromodomain-containing protein BRD4.[22] Several studies demonstrated that transcriptional condensates promote the clustering of distant CREs mediating enhancer–promoters (E-P) contacts, which are instructive for transcriptional regulation.[23,24]

Different evidence have highlighted that architectural proteins are required for the proper formation of transcriptional condensates. For instance, it has been shown that the CCCTC-binding factor (CTCF) is necessary to form phase-separated transcriptional condensates and that CTCF clusters partially co-localize with RNA Pol II clusters.[25] Interestingly, it has been recently suggested that CTCF has a role mainly in establishing E–P contacts rather than in their maintenance.[26] Moreover, some cohesin complex components can phase separate in vitro with DNA.[27]

The assembly of transcriptional condensates is also regulated by chromatin remodelers, which have a role in establishing an open chromatin conformation and in the direct formation of clusters. Among

chromatin remodelers, the members of the TrxG family, such as MLL4 and p300, have a central role in regulating transcriptional condensates.

We recently demonstrated that the H3K4 methyltransferase MLL4 is clustered into condensates and acts as a scaffold for recruiting transcription cofactors, such as BRD4 and MED1.[11] Importantly, MLL4 PrLD is required to adequately form transcriptional condensates.[11] The histone acetyltransferase p300/CREB-binding protein (CBP) was shown to be organized in dynamic condensates together with specific TFs modulating transcriptional activation and bursting.[28] In addition, MLL4 and p300 are reported to co-partition thanks to the direct interaction with the histone demethylase UTX, which is organized in phase-separated clusters as well.[29] Interestingly, UTX condensation depends on its IDR, which has a crucial role in determining condensates properties, such as liquidity and dynamicity, and their proper functionality, such as tumor suppressor activity.[29] Another member of the TrxG family, the chromodomain helicase DNA-binding 7 (CHD7), was reported to associate with active enhancers during cerebellar development and bind genomic regions with a significant overlap with p300-bound regions in human neural crest cells.[30,31]

Conversely, PcG family members have been historically known to functionally antagonize TrxG proteins to maintain a repressed transcriptional state.[4] PcG proteins, which are organized in multiprotein complexes, are involved in the formation of condensates through LLPS (Figure 1C). Several components of the Polycomb Repressive Complex 1 (PRC1) contain IDRs and form phase-separated condensates,[32–34] while the PRC2 component EZH2 is organized in nuclear condensates.[35] Interestingly, it was observed that the formation of phase-separated condensates accelerates the search for target sites of the CBX2 subunit of PRC1.[36]

From an experimental point of view, a lack of tools still limits our ability to investigate chromatin condensates' most relevant features, such as their dynamic behavior. In vitro studies indicate that biomolecular condensates assemble and disassemble quickly and reversibly, suggesting an ever-changing organization and rapid exchange of components among condensates also in vivo. One of the most used methods to study condensate dynamics in living cells is an optogenetic tool recently established by Brangwynne and collaborators.[37] This tool is based on light-dependent stimulation of IDR nucleation to control the assembly of condensates and to follow their evolution over time by fluorescence microscopy. Condensates' in vivo study suggests that their growth relies on a coalescence process while Ostwald ripening has a minor effect due to the dense chromatin environment.[38] Nevertheless, the optogenetic system has some limitations since it relies on the heterologous over-expression of fusion proteins, which might not fully recapitulate physiologically relevant conditions. Moreover, albeit recent technological advances, we are still missing precise information about the nature of interactions between different components of condensates and we are still limited in the spatial resolution of these structures.

Relevance of chromatin factors organizing condensates: The chromatinopathies

Mutations in chromatin regulators can lead to severe disorders that, due to their infrequent occurrence, are commonly referred to as rare CPs.[5,39] CPs represent a group of more than 80 human pathological conditions, which share some clinical features. These clinical evidence suggest that a shared abnormal biological process may be involved, causing common aberrant neurological development and growth abnormalities. Of note, the CPs caused by the haploinsufficiency of chromatin regulators involved in the organization of the transcriptional condensates are characterized by short stature and microcephaly (Figure 2). On the contrary, overgrowth and macrocephaly are associated with mutations affecting repressive chromatin-associated condensates including PcG bodies (Table 1). This indicates that the correct balancing between chromatin condensates plays a central role in regulating cell lineage commitment and differentiation. Supporting this hypothesis, recent studies highlighted the correlation between dysfunctions in biomolecular condensation and pathological conditions.[40,41]

The CPs caused by mutations affecting the organization of the transcriptional condensates include KS, Charge syndrome (CS), Rubinstein–Taybi syndrome (RT), and Cornelia de Lange syndrome (CdLS), while mutations affecting PcG condensates include Weaver syndrome (WS), Cohen–Gibson syndrome (COGIS), and Imagawa–Matsumoto syndrome (IMMAS) (Figure 2 and Table 1).

Recent efforts succeeded in defining the major genetic contributors to these disorders, generally characterized as monogenic diseases in which multiple genetic mutations affect gene functionality. Although the causative genes for CPs have been identified, the consequences of their inactivation at the molecular and functional levels have not been defined. Of importance, each of these Mendelian disorders is driven by a heterogeneous group of mutations such as truncating events (e.g., nonsense, insertion/deletions, duplications, splice-site, and frameshift mutations), but also missense variants whose pathogenicity has not been fully investigated.[39,42] The features of CPs vary widely, and the severity of the disorder can differ even among individuals with the same gene mutation, suggesting that the impact of the haploinsufficiency could depend on the epigenetic state and/or interactions with additional genetic and environmental factors.

Whole exome sequencing led to identifying *KMT2D* and *KDM6A* genes as the underlying causes in ~80% of KS cases.[43,44] Most *KMT2D* and *KDM6A* mutations are truncating events that affect the functionality of MLL4 and UTX proteins, respectively, thereby resulting in KS type 1 (MLL4 loss of function) and type 2 (UTX loss-of-function). *EP300* and *CREBBP*, which codify for P300 and CBP, respectively, are mutated in heterozygosity in 60 and 10% of the individuals affected by RT.[45] *CHD7* is mutated or deleted in heterozygosity in 90% of the CS-affected individuals, causing its haploinsufficiency.[46] Most of the mutations are nonsense and frameshift, causing truncation of the CHD7 protein. Mutations in *NIPBL*, *SMC1A*, *HDAC8*, *RAD21*, or *SMC3* cause CdLS by impairing the function of the cohesin complex.[47]

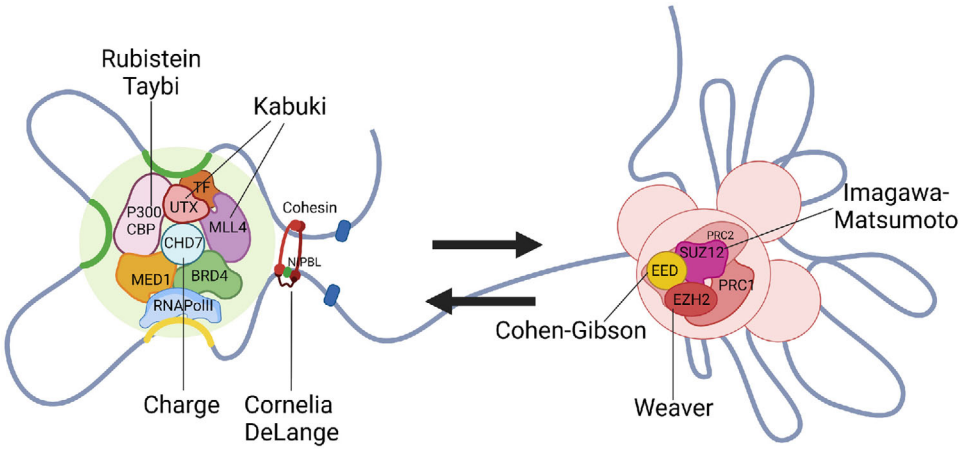

FIGURE 2 Causative factors of CPs. Graphical representation of the chromatin factors organized in biomolecular condensates and mutated in CPs. For each factor, the corresponding CP is indicated. CP, chromatinopathy.

TABLE 1 Table summarizing the chromatinopathies discussed in the text, with the corresponding causative gene(s), the chromatin factor affected by the mutation(s) and the resulting phenotype.

Syndrome	Causative genes	Affected factor	Function of the affected factor	Phenotype	References
Kabuki syndrome (KS)	*KMT2D*	MLL4	Chromatin modifiers	Microcephaly and postnatal growth deficiency	[43,44]
	KDM6A	UTX			
Rubinstein–Taybi syndrome (RT)	*EP300*	p300			[45]
	CREBBP	CBP			
Charge syndrome (CS)	*CHD7*	CHD7			[46]
Cornelia de Lange syndrome (CdLS)	*NIPBL*	NIPBL	Subunits of cohesin complex		[47]
	SMC1A	SMC1A			
	SMC3	SMC3			
	RAD21	RAD21			
	HDAC8	HDAC8	Enzyme involved in cohesin cycle		
Weaver syndrome (WS)	*EZH2*	EZH2	Components of the PRC2	Overgrowth and macrocephaly	[48]
Cohen–Gibson syndrome (COGIS)	EED	EED			[49]
Imagawa–Matsumoto syndrome (IMMAS)	*SUZ12*	SUZ12			[50]

Pathogenic variants in NIPBL were identified as the most frequent (70%) cause of CdLS. Further studies led to the detection of variants in six additional genes causal of CdLS: SMC1A (5%), SMC3 (1%), RAD21 (1%), BRD4 (<1%), HDAC8 (<1%), and ANKRD11 (<1%).[7,14]

The exome era has also allowed us to dissect the spectrum of overgrowth syndromes and distinguish different pathologies with overlapping clinical manifestations. During the last 10 years, *EZH2*, *EED*, and *SUZ12* genes were found to be responsible for the WS, COGIS, and IMMAS syndromes, respectively.[48–50] (Table 1). The three genes encode for core components of the PRC2, and pathogenic mutations result in the loss-of-function of the gene.[51]

Even though CPs are caused by the haploinsufficiency of factors organized in biomolecular condensates, there is no direct evidence of a causal link between CPs onset and alterations in chromatin condensate's function and organization.

The genetic function of chromatin condensates: Orchestrating gene expression and chromatin organization

One of the most outstanding epigenetic regulatory systems involves the antagonistic crosstalk between members of the PcG and TrxG group of proteins. As previously mentioned, PcG and TrxG members are organized in chromatin condensates, and one of the emblematic ways of action to convey their regulation is catalyzing histone

modifications counteracting each other. Briefly, the PcG complex can be subdivided into two complexes, PRC1 and PRC2. PRC2 decorates with three methyl-groups the Lys27 of H3 (H3K27me3) through its SET (Su(var)3-9, Enhancer-of-zeste, Trx) domain. PRC1 recognizes H3K27me3, and in turn, it mono-ubiquitylates Lys119 of H2A (H2AK119) through its RING core, prorogating PcG activity. On the other hand, TrxG contains different methyltransferases (SET1A/B, MLL1/2, and MLL3/4) able to catalyze mono-, di-, and trimethylation on H3K4 at active promoters and H3K4Me1 at enhancers. Furthermore, the KDM6/UTX demethylase removes the repressive H3K27me3 mark favoring its subsequent acetylation by CBP/p300.[4]

The PcG/TrxG-mediated transcriptional regulation is crucial to defining cell identity, and it occurs in a highly heterogeneous and dynamic chromatin environment. Chromatin is not randomly organized in the 3D space. Multiple evidence showed a correlation between 3D genome folding and gene expression, although the existence of a causal relationship is still debated.[52] Nevertheless, the chromatin topology has been recognized as an additional layer of epigenetic regulation. Imaging techniques and Chromosome Conformation Capture (3C) based methods have greatly increased our knowledge of genome spatial organization.[53] Chromosomes are the basal unit of genome organization and are 3D organized in hierarchical structures at multiple scales (Figure 3). A phase separation process gathers megabase-sized portions of chromosomes in compartments, where homotypic interactions occur.[54] A- and B-compartments can be roughly simplified as euchromatin and heterochromatin, respectively.[54] At the submegabase scale, chromosomes are organized into structures named topologically associating domains (TADs), representing chromatin regions with increased intradomain interactions. TADs are formed through a loop-extrusion process mediated by cohesin that stops at CTCF-marked boundaries.[55] At a finer scale, DNA is organized in nucleosomes, and the chromatin fiber can fold in 3D interactions between CREs forming E–P loops. Large genome topology changes occur during development and cell differentiation or can arise in pathological conditions.[56]

Several indications suggest that proteins belonging to chromatin condensates may have a role in the framework of chromatin organization, conveying their crosstalk also at this stage. As general proof, studies performed in the context of living cells showed that nuclear condensate assembling at specific loci causes the pulling together of distal targeted genomic regions.[57]

Of importance, the proteins belonging to the PcG-condensates favor long-range genomic interactions, shaping the genome in hubs to ensure PcG repressive function.[58,59] Recent studies also strengthen transcriptional condensate members' role in genome folding: RNA Pol II sustains the E–P loops,[60] and UTX controls chromatin looping in a condensation-dependent manner.[9] In the same direction, several TFs and coactivators have been described as instrumental in mediating regulatory elements' proximity.[61,62] Once the 3D contacts are set up, they are consolidated by architectural proteins, including cohesin and CTCF, which are organized in condensates.[25,27] Despite the growing knowledge in the field suggesting the relevance of chromatin condensates in genome organization, numerous open questions remain regarding their relationship with architectural proteins. One promising research indicates that PcG components YY1 and RING1 favor CTCF-phase separation, which controls long-range interactions.[63] Recently, it has also been shown that the E–P loops strongly decrease upon rapid degradation of Mediator, followed by a reduction in cohesin occupancy at enhancers.[64]

Although genome organization and gene expression are in a dynamic relationship, much remains to be understood, particularly in chromatin condensates organization and CPs. The degron system[65] is a powerful tool for addressing the causal relationship between chromatin condensates alteration in CPs, 3D organization, and gene expression. Its specific employment at one of the two alleles coding for a CP-altered protein could resemble the chronic phenotype of the rare syndrome in the study. The onset of degron-induced haploinsufficiency would permit the elucidation of the straight responses, in terms of the genetic and nongenetic functions, mediated by chromatin condensate alterations. Possibly, the degron tool could be coupled with newly developed live-cell imaging techniques to study chromatin contacts.[65]

The nongenetic function of chromatin condensates: Nuclear mechanics

In addition to their transcription-related functions, chromatin condensates could also affect cellular processes by nongenetic means through their physical and structural properties. The nongenetic function of chromatin condensates could lie in their ability to shape nuclear compartments, which in turn exert mechanical forces that affect nuclear architecture. Of importance, the chromatin itself, together with the compartments that characterize it, can be considered a viscoelastic polymer with a mass, volume, and density determined by intrafiber and intra/interchromosomal interactions.[10] It has been recently shown that chromatin behaves like a fluid in the nucleus of living cells, pinpointing a minor contribution of cross-links and topological effect, thus challenging the view that interphase chromatin adopts merely gel-like states.[66]

As a physical entity, the chromatin is also able to exert mechanical forces that are transmitted from the nucleus to the cytoplasm via the LINC complex[10] (Figure 4). Besides them, also the nuclear envelope and the nuclear lamina participate in determining the mechanical properties of the nucleus.[67,68] Of note, chromatin compartments preserve nuclear mechanical stability in concert with the lamins and the cytoskeleton establishing specific viscoelastic responses to applied forces. The timing, magnitude, and equilibrium between the forces that participate in defining this response are determined by the existing interactions between the chromatin polymer and the meshwork of lamin filaments (Lamin A and B1). Specifically, it has been shown that under mechanical load cells respond with two different temporal regimes characterized by distinctive nuclear mechanical responses: at a shorter time scale (≈2 s), the nucleus stretches elastically, while at a longer time scale, it deforms viscously, being controlled predominantly by Lamin-A.[69] Importantly, peripheral heterochromatin,

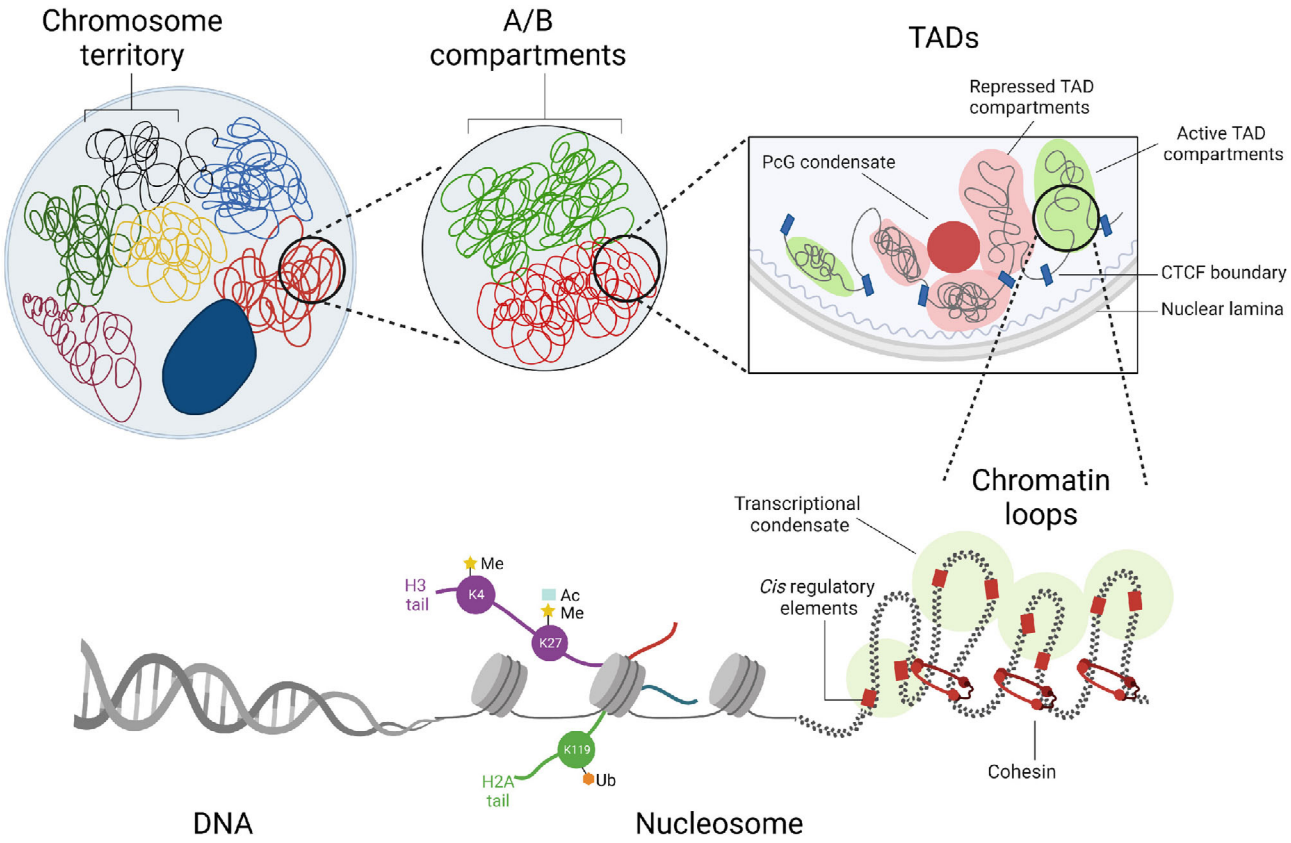

FIGURE 3 Three-dimensional genome organization. Graphical representation of the different levels of the 3D chromatin organization. In the interphase nucleus, chromosomes occupy distinct territories. Within each territory, the chromatin is organized in A (accessible) and B (repressive) compartments. Topologically associated domains (TADs) organize chromatin with homotypic interactions. TADs are defined by CTCF boundaries and can be subdivided into repressed- and active- TADs. Several repressed TAD compartments can be brought into proximity by PcG condensates, forming long-range genomic interactions. At higher resolution, CREs can be organized into chromatin loops, sustained by transcriptional condensates and cohesin contribution. Finally, DNA is packaged around histones whose tails are decorated with covalent modifications. H3K27Me3 and H2AK199Ub are deposed by PcG, and commonly associate with heterochromatin. H3K27Ac and H3K4Me1 positively correlate with gene expression and are deposited by TrxG proteins. CRE, cis-regulatory element; CTCF, CCCTC-binding factor; TAD, topologically associating domain.

which establishes physical contacts with the inner nuclear membrane, also contributes in orchestrating the cellular response to mechanical stimuli.[70] Considering these observations, it remains difficult to properly uncouple the contribution of lamins and chromatin at the nuclear periphery, as their physical connections contribute to the nuclear stiffness and rigidity.[69,70]

In this framework, biomolecular condensates have recently emerged as regulators of chromatin structure, suggesting a novel function of chromatin condensates in contributing to nuclear mechanics. One of the first demonstrations of the structural function of biomolecular condensates in shaping nuclear architecture comes from the finding that heterochromatin formation is mediated by LLPS.[71] Of note, several studies show that peripheral heterochromatin provides the structural robustness needed to withstand the mechanical insults that cells physiologically encounter in their tissue of origin or during migration.[68,70] For instance, it has been shown that the passage of cells through narrow openings is facilitated by chromatin condensation.[72] Furthermore, a more recent work pinpoints a novel role of heterochromatin in altering nuclear stiffness to maintain genome integrity in response to stretch-driven deformation.[73] Although it has been demonstrated that constitutive heterochromatin is formed through LLPS, other forces could play a role in shaping its organization across the cell cycle. Indeed, heterochromatin is stable during cell division and therefore its organization may not be guided solely by LLPS-related processes. Notably, the phase separation of chromatin itself, driven by histone tails, together with one of other chromatin-binding proteins, enables the establishment and maintenance of chromatin subcompartments.[74] Other chromatin players have been shown to promote chromatin condensation by forming multicomponent condensates. For instance, the PRC1 PcG subunits participate in biomolecular condensates to induce the writing of repressive histone marks, which subsequently drive chromatin compaction.[32] In our recent work, we provided evidence that balancing PcG-mediated and transcriptional condensates is essential for preserving nuclear mechanical properties in KS.[11] Indeed, MSCs harboring the *KMT2D* truncating mutation presented increased PcG clustering, which impairs nuclear structure and mechanics, resulting in aberrant nuclear morphology

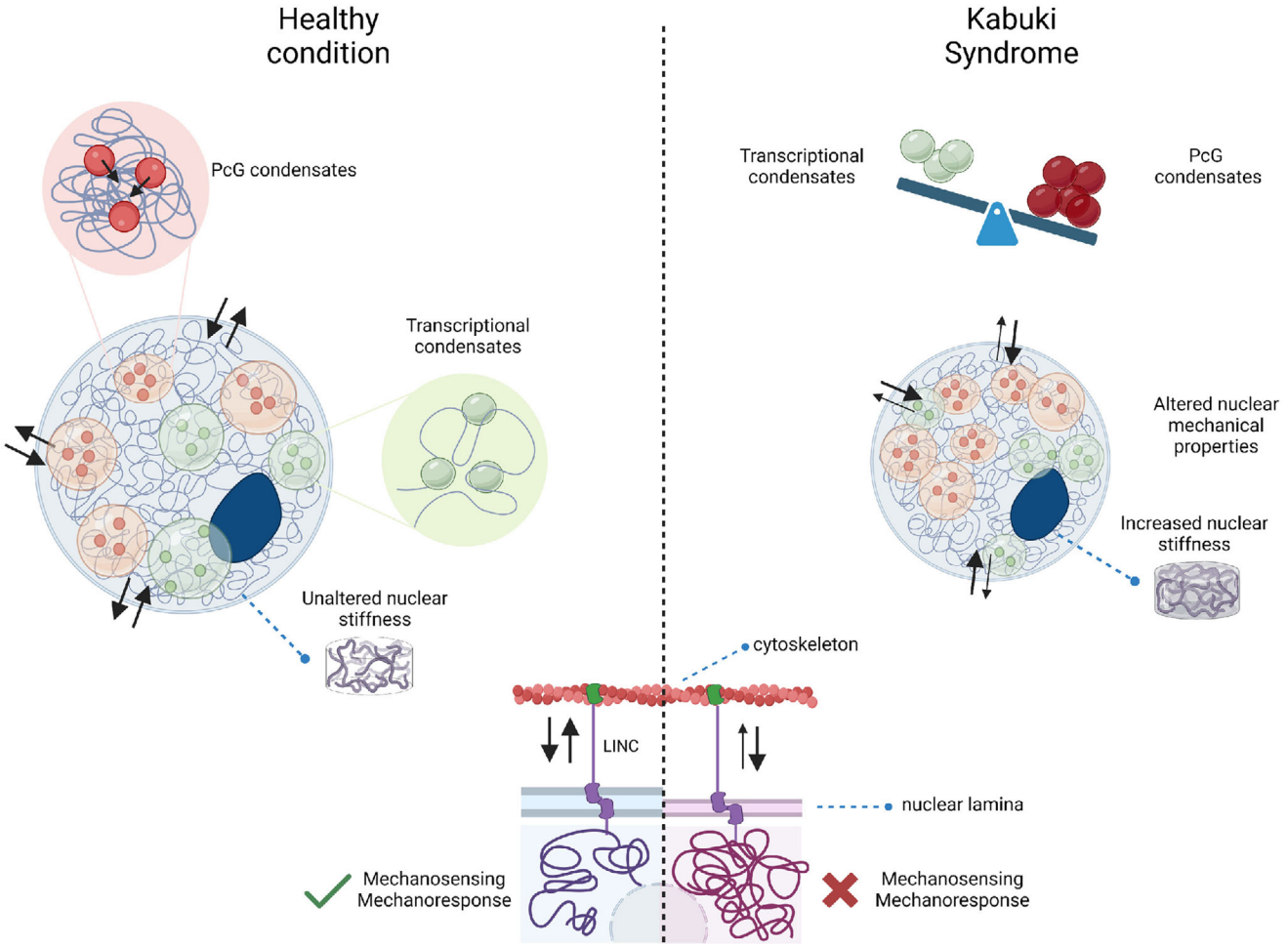

FIGURE 4 Role of chromatin condensates in affecting nuclear mechanics in physiology and disease. In healthy conditions (left side), pulling forces between chromatin domains are generated by nuclear condensates (HP1, PcG, red spheres), which compact chromatin. In addition, LLPS of chromatin and chromatin-binding proteins (i.e., Transcriptional condensates, green spheres) determine chromatin subcompartments. The sum of the forces established within the nucleus and the peripheral nuclear lamina defines nuclear mechanical properties. In healthy conditions, the inward nuclear forces generated by the nucleus balance the outward forces coming from the extracellular matrix (black arrows), which are transmitted through the LINC complex. The equilibrium between these forces ensures a correct mechano-response. In KS (right side), the unbalancing between Transcriptional and PcG condensates alters the equilibrium between inward and outward forces, thus affecting the mechanical properties of the nucleus and consequently impairing nuclear mechanotransduction. KS, Kabuki syndrome.

characterized by smaller, stiffer nuclei with respect to the healthy condition (Figure 4). Of importance, these altered nuclear mechanical functions were restored upon inhibition of the mechanosensor ATR (Ataxia Telangiectasia and Rad3-related protein). Although the magnitude and the type of physical forces within nuclear condensates which are implicated in shaping nuclear architecture, compaction, and stiffness via LLPS have yet to be investigated, we could speculate that this process could benefit from surface tension forces, which allow to establish mechanical tension at the boundary between phases.[75] In addition, we cannot exclude that the high density of histone modifications (i.e., H3K27me3, H2AK119ub1) in the compact chromatin could participate in determining the saturation concentration necessary to elicit LLPS.

Altogether this evidence shows that the nongenetic function of chromatin condensates could rely on their role in affecting nuclear morphology and cellular response to mechanical forces. Even if multiple studies suggest that nuclear condensates structurally shape chromatin compartments, how they specifically contribute to regulating nuclear mechanical properties remains elusive.

The function of nuclear mechano-response in tissue homeostasis and pathology

Elasticity, viscosity, and stiffness of the cells determine how they respond to forces and external cues. Atomic force microscope (AFM)[76] measurements revealed that the elastic module of mammalian cells spans from 1 to 100 kPa.[77] Inside cells, cellular organelles have different mechanical properties. For example, mithocondria are large organelles able to move and go through fusion and fission events. When subjected to 15-nN AFM-mediated force, mitochondria undergo fissions to resolve the accumulated mechanical stress.[78] Another

example of large organelle is the nucleus, where the elastic module of the dsDNA here accommodated is of 1200 pN.[79] The nucleus has been described as the stiffest organelle being 2–10 times stiffer (1–10 kPa)[80] than the surrounding cytoplasm whose effective module is 0.5–4 kPa.[81,82] In the face of this, the nucleus needs to physically adapt to external stimuli to ensure the ability of cells to sustain mechanical stress and survival.[83] Nuclear stiffness is commonly fluctuating under physiological events such as differentiation processes (six-fold increase)[84] and cell divisions (five-fold changes).[85] Similarly, in pathological conditions (e.g., cancer), the nuclear structure is altered, and the nuclear stiffness tends to be reduced.[86] For this reason, the mechanical properties of the nucleus and its component are finely controlled within tissues.

One example of how the non-genetic property of the genome can affect the biological functions of an entire tissue is seen in the physiology of vision in nocturnal animals.[87] In rod cells of nocturnal animals, heterochromatin mainly localizes in the nuclear interior instead of the nuclear periphery. This reverse pattern of heterochromatin localization is evolutionarily convenient since it leads to an increased refractive index in the nucleus center, reducing the scattering of light and improving the quality of night vision.

Physiologically, several tissues (e.g., cardiac tissue and skin) are subjected to constant mechanical stresses, thus their nuclei must withstand and dissipate a high load of mechanical stress to preserve tissue homeostasis. It has been shown that an architectural chromatin protein, HMGN5 (High Mobility Group Nucleosome Binding Domain 5), controls chromatin compaction and nuclear rigidity during heart contraction.[88] Indeed, mice overexpressing HMGN5 develop hypertrophic hearts with cardiomyocytes having deformed nuclei and disrupted lamina.

During development, mechanical forces affect tissue morphogenesis by controlling cell shape, number, position, gene expression, and differentiation. Therefore, the coordination between long-range force transmission and cell mechanosensing within tissues is crucial to control organ growth and morphogenesis.[89] Changes in the mechanical properties of the ECM of cardiovascular tissues are associated with pathological conditions (e.g., acute trauma, genetic predisposition, hypertrophy) and reduced cardiac performance. A recently published work demonstrated that mechanical cues, such as ECM stiffness, guide cell fate determination during heart development by affecting the organization of heterochromatin through nuclear mechanosensing.[90] Interestingly, in the context of epidermis homeostasis, mechanical strain causes global chromatin rearrangements through PcG-mediated transcriptional repression, which controls lineage progression during epidermal morphogenesis.[91] Perturbing this mechanosensory pathway leads to precocious lineage commitment, compromising tissue morphogenesis. Some years later, Nava et al. demonstrated that mechanical stretch induces a rapid loss of constitutive heterochromatin and subsequent nuclear softening as a protection mechanism to dissipate mechanical stress and preserve the genome from DNA damage.[73]

Other mechanically active tissues include the bone and cartilaginous tissues. Interestingly, these tissues present abnormalities in different CPs, including KS.[92] This condition is characterized by an impairment of nuclear mechanics, leading to failed differentiation towards osteocytes and chondrocytes.[11] Although an altered mechanical response for KS has been found to drive its pathogenesis, the link between impairment of nuclear mechanics and other CPs remains to be addressed.

One characteristic shared among mechanically active tissues is the lamina expression levels, which are high in stiff tissues subjected to high mechanical stress.[67] Although nuclear lamina and chromatin have a role in defining nuclear mechanical properties and response, their single contribution to these functions is difficult to assess due to technical limitations. The development of new technologies could shed light on this matter. Of importance, Brillouin microscopy, an optical technique that combines Brillouin spectroscopy with Confocal microscopy, is considered an emerging tool in the field of mechanobiology.[93] This technique provides a noncontact and label-free readout of the mechanical properties of cells and has been recently applied to follow changes in nuclear stiffness during cancer cell migration.[94] Furthermore, thanks to Brillouin microscopy, we demonstrated in our KS disease model that increased PcG condensates affect the stiffness of the nucleus despite decreased lamina levels[92] (Figure 4). Overall, these studies indicate that nuclear mechano-sensing and response play important roles in safeguarding tissue homeostasis and that their dysregulation leads to several pathological conditions.

CONCLUSIONS

Throughout the review, we have described the biological relevance of chromatin condensates in physiological and pathological conditions, with a special focus on CPs. We stressed chromatin condensates' genetic and nongenetic functions, arguing their possible contribution to modulating nuclear mechanical functions.

The genetic function of chromatin condensates in modulating gene expression is generally accepted and proved by recent publications.[95] However, other indications question this linear relationship. In this direction, it has recently been suggested that E–P loop strength and related microcompartments are not linearly connected with the transcriptional outputs.[96,97] Also transcription activation is independent of TFs' phase separation process.[98] Last, the employment of degron tools highlighted that depletion of key architectural proteins perturbs chromatin organization with minor effects on gene expression.[26,99,100]

Nevertheless, the role of chromatin condensates is central for cell functions as mutations in their members cause rare genetic disorders showing minor changes in global gene expression,[11] yet sharing severe phenotypic traits. This opens a knowledge gap in the field, suggesting that other nongenetic roles of chromatin condensates may be instructive in determining chromatin function.

Condensates are highly dynamic entities, able to condense or dissolve under numerous stimuli, so they may impact nuclear mechanical properties, thus affecting the cellular response to mechanical cues during the development and tissue homeostasis. Indeed,

it has been shown that the nucleation of chromatin condensates can generate local mechanical forces that remodel the genome compartmentalization.[101] In addition, external forces propagate through the cytoskeleton and the LINC complex to the nucleus, causing a reversible chromatin relaxation.[102] However, it is still unknown the mechanism by which condensates perceive the mechanical load and how this information is decoded bringing about changes in the chromatin condensation and possibly 3D organization. We propose a possible crosstalk between the nuclear lamina and chromatin condensates in tuning the nuclear mechanical response. It has been shown that the loaded forces at the nuclear lamina are sensed and transmitted by the Lamin A/C proteins and Emerin through reversible conformation changes. Indeed, tensile forces cause phosphorylation-dependent responses through the Lamin A/C disassembly and the exposure of hidden surfaces of Emerin.[103] Similarly, some chromatin architectural proteins (i.e., condensin and cohesin) harbor an IDR's similar domain (HEAT domain), which may function as a mechanosensor undergoing conformational changes in response to external forces[104] to mediate cellular responses.[105]

All the examples discussed so far provide evidence that chromatin condensate members holding mechano-responsive domains can change their conformation in response to mechanical forces. These indications reinforce our hypothesis that chromatin condensates may be implicated in nuclear mechanical functions.

AUTHOR CONTRIBUTIONS

Maria L. Negri and Alessio Zippo conceived the manuscript; Maria L. Negri, Sarah D'Annunzio, and Giulia Vitali wrote the manuscript; and Alessio Zippo finalized and edited it. Maria L. Negri, Sarah D'Annunzio, and Giulia Vitali prepared the figures.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the proposed hypothesis are openly available and presented in the cited papers.

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