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Approaches to characterize chromatin subcompartment organization in the cell nucleus

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Abstract

The mechanism of self-organization of chromatin subcompartments on the 0.1–1 μm scale and their impact on genome-associated activities has long been a key aspect of research on nuclear organization. Understanding the underlying structure-function relationship, however, remains challenging due to the complex hierarchical structure of chromatin and the polymorphic organization of subcompartments that assemble around it. Towards this goal, approaches to measure local properties and compositional dynamics of chromatin in its endogenous cellular environment are instrumental. Here, we discuss recent advancements in studying these features and their functional implications in protein and RNA enrichment and genome accessibility.

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Keywords

Chromatin subcompartment, Nuclear organization, Genome activities, Transcription factor, Phase separation.

Abbreviations

CSC, chromatin subcompartment; TF, transcription factor; LLPS, liquid–liquid phase separation.

Introduction

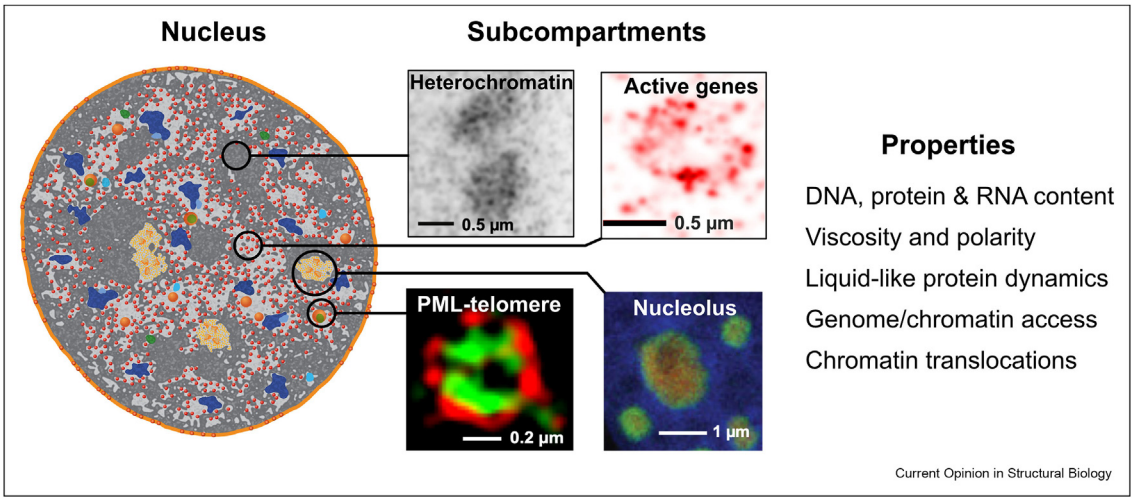
Distinct chromatin states partition the genome to locally enrich associated activities for transcription, DNA replication, repair and recombination in a cell-type-specific manner. Revealing the underlying structure-function relationships is challenging due to the complex hierarchical organization of the nucleosome chain that ranges from clusters of a few nucleosomes to chromosome territories with largely different dynamic properties [1]. Confined translocations of individual nucleosomes with a mass of 240 kDa for a nucleosome core particle occur on the nanometer and second time scale [2••]. In contrast, micrometer-scale translocations of chromatin loci to form CTCF-mediated loops take place on the 10 min to hour scale [3,4]. Translocations of whole chromosomes during interphase are hardly detectable even on the hour scale with human chromosome 1 comprising 0.25 Gb DNA and 1.2 million nucleosomes with a total mass of 520 GDa or 0.87 pg [5]. Thus, the assignment of chromatin material properties and the resulting implications for the enrichment of factors and/or access to the genome will be dependent on the length and time scales studied. Accordingly, the generalized description of chromatin as "liquid" and "solid" is fraught with difficulties as apparent from the ongoing discussions in the field [2••,6–9]. Likewise, identifying subcompartment properties that inform about the contribution of liquid–liquid phase separation (LLPS) for chromatin organization and to distinguish it from alternative mechanisms is critically dependent on the time and length scales studied as discussed previously [10].

Here we review recent approaches to measure features of chromatin subcompartments (CSCs) that have dimensions on the 100 nm to μm scale and involve the assembly of protein/RNA macromolecules around certain chromatin loci. They include for example the nucleolus, constitutive heterochromatin domains, clusters of active RNA polymerase II or complexes of PML bodies at telomeres [10–12] (Figure 1). Measuring the material properties or other features of these CSCs in the endogenous environment of the cell nucleus is challenging but a crucial step to infer structure-function relationships. We discuss recent advancements in the approaches to dissect organizational principles of CSCs and their associated functional implications with respect to the local enrichment of protein and RNA factors as well as regulating access to the genome.

Measuring intrinsic CSC features

A number of recent studies introduce novel approaches to provide information on intrinsic properties of CSCs in the cell nucleus (Table 1).

Figure 1



Organization of the cell nucleus into distinct chromatin domains and mobile nuclear bodies that locally enrich protein and RNA factors. The scheme illustrates what is seen by high-resolution fluorescence microscopy upon staining marker proteins and DNA with exemplary chromatin subcompartments shown on the magnified images. CSC properties as listed on the right side can be largely different, which has a number of functional implications for the genome-associated activities that are enriched in these CSCs.

Frequently, the local composition of a CSC with respect to its DNA, protein and RNA content is not well defined with large differences in the relative abundance of the different types of macromolecules. For example, the nucleolus is filled with protein and ribosomal RNAs but has a low density of DNA. In contrast, heterochromatin domains are enriched in DNA but mostly devoid of RNA. Both have implications on the mechanism that underlie their assembly [10]. By introducing fluorescent tags into 1310 genes in human embryonic kidney (HEK) 293T cell lines, the intracellular location, abundance and interactions of the corresponding proteins were measured with combination of live cell confocal fluorescence microscopy and mass spectrometry [13•]. With respect to the local DNA content/density, it is important to note that this value will depend on the spatial resolution as differences are averaged upon increasing the observation volume. Recent high-resolution measurements by single-molecule localization microscopy show large variations of the local DNA density ranging from <5 to >300 Mb/µm^3 in human and mouse cell nuclei [14•]. This is in line with previous findings from high-resolution microscopy that revealed chromatin density variations due to its organization into nucleosome clutches of variable size and density [49]. The local chromatin compaction and crowding can also be probed by using molecular sensors that employ crowding-sensitive linkers [17] or by directly detecting structural changes in CSCs via measurement of fluorescently labeled marker proteins [15•,16•]. In this manner, compaction of heterochromatic regions in embryonic stem cells was shown to be lower than expected and dependent on differentiation state and a balance of heterochromatin protein 1 (HP1) isoforms [15•]. Finally, methodological advancements to identify RNAs associated with specific CSCs by sequencing methods make it possible to simultaneously map interacting DNA loci and associated RNAs [18,19].

The different macromolecular content and density of CSCs are also reflected in their viscosity. A systematic analysis of the length-scale-dependent intracellular viscosity was conducted with a set of nanoprobes from 1 to 150 nm diameter in the cytoplasm that yielded a liquid-like behavior for length scales below 100 nm and more gel-like properties for larger length scales [21]. This approach can also be exploited to detect local intracellular viscosity differences. However, a higher spatial resolution is provided by polarization-dependent fluorescence correlation spectroscopy [20]. It was applied to study the local viscosity experienced by HP1 inside and outside heterochromatin compartments. Another approach to test CSC material properties is to employ environment-sensing nanoprobes attached to peptides [22]. This was demonstrated for a polarity-sensing photoactivatable probe targeted to HP1-marked domains.

To measure protein dynamics in CSCs in comparison to the surrounding nucleoplasm, fluorescence recovery after photobleaching (FRAP) is a well-established method. However, as discussed previously, the absolute values of measured recovery times and apparent diffusion coefficient or kinetic dissociation rates are not sufficient to determine whether a protein of interest has liquid-like protein dynamics within a CSC as expected

Table 1

Approaches to measure intrinsic features of CSCs and perturbing them.

Intrinsic CSC properties Property	Techniques	References
DNA, protein and RNA content	Mass spectrometry; fluorescence microscopy; sequencing	[13•,14•,15•,16•,17,18,19]
Viscosity and polarity	Polarization-dependent fluorescence correlation spectroscopy; nanoprobes	[20–22]
Liquid-like protein dynamics	Half-bleach FRAP	[20•,23••]
Genome/chromatin access	Single particle tracking	[24•,24,25]
Chromatin translocations	Tracking of fluorescently labeled chromatin loci over time	[2••,3,4,26,27,28,29]
Perturbances of CSCs **Targeted feature**	**Techniques**	**References**
Concentration-dependent assembly and associated activity	Phase separation propensity analysis; transcription response below/above the concentration of droplet formation	[30,31••,32••,33].
IDR mediated protein interactions	IDR engineering, exchange/addition of IDR or introducing changes of amino acid patterns in IDR	[31,32,34–39]
Protein condensates	dCas9-targeted, (light-)induced increase in multivalency of fusion proteins; ligand mediated disassembly; 1,6-hexanediol treatment	[23,31••,40–46]
Interactions between nucleosome chain segments	Applying external point force or capillary forces; CRISPR-mediated chromatin looping	[47••,48•]

for LLPS [50]. This issue is addressed by FRAP experiments in which only one half of a CSC is bleached [20,23••]. This approach separates the contributions of fluorescence recovery arising from exchange with molecules in the unbleached half of the CSC from recovery by molecules from the nucleoplasm surrounding the CSC that cross the CSC boundary and diffuse into its interior. The resulting ratio of fluorescence recovery from internal mixing vs. exchange across the CSC boundary can then be used to distinguish LLPS, which is characterized by preferential internal mixing, from other mechanisms like chromatin binding to clustered sites.

A crucial functional feature that arises from CSC properties is their regulation of genome access that could be determined via the chemical nature of a given factor or its size [10]. Genome accessibility is particularly relevant for regulatory proteins like transcription factors (TFs) that activate or repress transcription. It can be probed by single particle tracking to reveal nuclear regions of confined particle mobility that could locally accelerate the target search process. This type of transition between different mobility modes was shown for the p53 tumor-suppressor protein, which alternates between rapid diffusion in nuclear regions devoid of chromatin and confined mobility when interrogating chromatin-dense regions [24•]. Furthermore, several other studies established that the interactions of intrinsically disordered regions (IDRs) found in many TF activation domains significantly contribute to the target search and binding process [51–53]. In addition, it is noteworthy that access to chromatin is in many instances unrestricted even for relatively large complexes and dense heterochromatic regions. A recent example is the finding that RNA polymerase II is not physically excluded from the inactive X chromosome by phase separation or other physical barriers but can freely access the territory delineated by the Xist RNA [25•].

While the assembly of CSCs around certain genomic loci leads to some confinement of their nuclear location, they are also subject to chromatin movements. Local chromatin motions over the entire nucleus can be revealed from the analysis of fluorescent microscopy image series of stained DNA with a high-resolution diffusion mapping approach [27]. In addition, locus-specific chromatin labeling methods were developed to study the viscoelastic properties of chromatin [2••,26,29]. They include dual color chromatin labeling of separated sites to reveal correlated motions and compaction levels from the length scale of individual nucleosomes [2••] to large chromosomal domains that carry labeled sites on the same chromosome [29]. Furthermore, a combination of conventional fluorescence and super-resolution photoactivated localization microscopy (PALM) was introduced to resolve differences in the mobility and translocation confinement of telomeres in relation to their compaction state [28].

Probing CSCs by targeted perturbances

Experiments that locally or globally perturb chromatin organization provide further insight into the links between CSC properties and their biological activities. Strategies that apply targeted perturbations and functional readouts with high temporal and spatial precision allow it to dissect the events that underlie the formation or state transition of a CSC (Table 1).

Comparing the activity of nuclear factors below and above the critical concentration for the formation of (liquid-like) condensates allows to assess the functional consequences of non-stoichiometric protein assembly. By fine-tuning the multivalency of transcription factors, a recent study linked a higher propensity for multivalent interactions to the TFs activation potential [31••]. This relation, however, was independent of TF phase separation since condensate formation at sufficiently high concentrations had a neutral or inhibitory effect on transcription. Similar results were obtained by Chong et al. who studied the IDR-containing oncogenic fusion transcription factor EWS::FLI1 [32••,33]. Here, increasing the number of IDR−IDR self-interactions via the expression level led to an optimum at endogenous concentration while above this level phase separation and repressed transcription was observed. These findings are in line with another study conducted for the Gal4 TF in yeast. It showed that Gal4 self-interaction can aid in DNA target search. However, indirect binding of additional TF molecules at the target site did not enhance productive transcription but rather had a negative effect [54]. Another study looking at oncogenic mutations in the histone acetylation reader ENL revealed structural changes that facilitate condensation and oncogenic gene activation [16]. However, overexpression of these ENL mutants can lead to the formation of non-functional condensates. Thus, there appears to be an optimal concentration of TFs and IDR-mediated TF−TF interactions to form active assemblies around the chromatin template during transcription initiation.

In addition to modulating TF assembly into condensates via the protein concentration, ongoing efforts aim at systematically identifying sequence features of IDRs that determine their activity like targeting proteins into certain types of assemblies [37,55,56]. Here, we refer to these approaches as IDR engineering. In their simplest form, they involve exchanging/adding IDRs or deleting IDR parts from a protein construct to assess the effect on genome activities like TF binding/activity [31,32,38,39], transcription initiation [34] or heterochromatin assembly [20]. A recently developed system combined different approaches to target IDR-driven TF activation domain assembly via LLPS to specific genomic loci. It displayed efficient transcription activation of various endogenous model genes with some differences between the IDRs of FUS, TAF15 and DDX4 [42]. To reveal the sequence-based "grammar" that governs IDR function, several current studies evaluated their amino acid composition. For the Msn2 TF, more than 100 IDR mutants were studied with respect to the effects on its genomic target sites [35]. This analysis revealed an important role of multivalent interactions of hydrophobic residues for promoter recognition. Furthermore, another study showed an IDR-mediated selective partitioning into MED1 condensates for gene activation [36••]. The patterning of charged amino acids in the IDR was found to be critical for specific partitioning. By designing synthetic IDRs and fusing them to functional proteins, property engineering approaches have been implemented that modulate cellular functions through condensate formation to sequester plasmids and regulate transcription [37].

The approach to assemble light-induced droplets targeted to certain genomic loci [57] has been applied and further developed in several studies. It was adapted to target transcription factors to a reporter locus by various dCas9-based approaches [31••]. Combining dCas9-targeting with proximity biotinylation enables subsequent purification and further analysis of CSCs by proteomics and chromatin conformation capture [41]. This allows to assess changes in the folding of the nucleosome chain and interacting proteins that arise upon induced condensate formation. Lastly, light-induced condensate formation was also applied to exert capillary forces on DNA loci to measure the material properties of chromatin [48•]. Here, two loci are connected by the formation of a condensate between them, which is subsequently dissociated and pulls the two loci closer together. The latter also provides insight into how similarity in IDR sequences influences the interaction strength between two condensate surfaces. Complementing these approaches is the introduction of a method which enables rapid disassembly of user-specified condensates [40•].

Perturbing CSC structure by treatment of cells with 1,6-hexanediol is frequently used to conclude from the induction of CSC disassembly that they form via a LLPS mechanism. However, it is noted that 1,6-hexanediol treatment globally weakens hydrophobic interactions that are not specific to LLPS [23••]. Furthermore, it has a variety of confounding effects on nuclear components. These include the loss of selective permeability of nuclear pores and nuclear transport receptors [58], an increase in nucleosome density [43], impairing kinases, phosphatases and DNA polymerases [44], interfering with enhancer-promoter interactions, organization into A or B compartments and TAD insulation [45•] and suppressing chromatin mobility while leading to its hyper-condensation [43,44,45•,46]. Thus, it appears questionable whether 1,6-hexanediol treatment provides tangible insight into the assembly mechanisms of CSCs.

A novel approach to assess chromatin domain properties is the targeted micromanipulation of genomic loci in living cells with controlled and quantifiable force. Keizer et al. used magnetic force to move a ferritin-labeled reporter locus to the nuclear periphery [47••]. The study revealed micrometer chromatin locus displacements within minutes in response to near-piconewton forces without large-scale changes in DNA density. These

findings are consistent with a Rouse polymer model for an ideal chain with relatively weak additional constraints from the local environment. The approach of viscoelastic chromatin tethering via light-inducible condensates mentioned above suggests that the material state of chromatin is best described by a viscoelastic fluid-like Jeffreys model with a high degree of heterogeneity at the length scale studied [48•]. This view is also supported by tracking the mobility of nucleosomes and chromatin loci [2••,26]. It will be interesting to see how different endogenous loci behave in point-force micromanipulation experiments and whether there might be differences in the forces needed to translocate different functional chromatin subdomains like heterochromatic regions or transcription hubs.

Conclusions

It is becoming clear that CSCs are highly diverse with respect to their composition, dynamics, material properties and functional roles in the cell. As reviewed here, an increasing number of approaches become available that sheds light on these features. However, these methods are frequently applied in isolation to address specific aspects of a given system. To move forward, it will be important to define sets of complementary readouts that can be applied to map informative CSC features and to develop a common coordinate system for their systematic characterization. The goal of such an approach would be to capture both the differences between CSCs and, at the same time, also identify shared principles according to which structure-function relationships can be assigned.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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