REVIEW

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Blank spots on the map: some current questions on nuclear organization and genome architecture

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Abstract

The past decades have provided remarkable insights into how the eukaryotic cell nucleus and the genome within it are organized. The combined use of imaging, biochemistry and molecular biology approaches has revealed several basic principles of nuclear architecture and function, including the existence of chromatin domains of various sizes, the presence of a large number of non-membranous intranuclear bodies, non-random positioning of genes and chromosomes in 3D space, and a prominent role of the nuclear lamina in organizing genomes. Despite this tremendous progress in elucidating the biological properties of the cell nucleus, many questions remain. Here, we highlight some of the key open areas of investigation in the field of nuclear organization and genome architecture with a particular focus on the mechanisms and principles of higher-order genome organization, the emerging role of liquid phase separation in cellular organization, and the functional role of the nuclear lamina in physiological processes.

Keywords Genome organization · Nuclear architecture · Phase separation · Nuclear lamina

Introduction

The genomic material in each eukaryotic cell is organized in a complex and hierarchical fashion in the cell nucleus (Misteli 2007; Bickmore 2013). DNA, which serves as the primary carrier of genetic information, is wrapped around a histone octamer to form nucleosomes which in turn are arranged in a beads-on-a-string-like manner into higher-order chromatin fibers. Recent observations using electron tomography methods suggest that the chromatin polymer

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exists predominantly as a 5–24 nm disordered fiber (Ou et al. 2017). At a higher level of organization, the fiber folds into ~500 Mb domains, referred to as topologically associating domains (TADs), which are defined biochemically based on the preferential physical interactions of sites within a TAD compared to regions outside of these domains (Dixon et al. 2016). The linear arrangement of TADs ultimately gives rise to chromosome territories which denote the physical volume occupied by a single chromosome in the nucleus (Cremer and Cremer 2001). Intriguingly, the location of individual chromosomes is non-random within the nucleus and a loose correlation between gene activity and proximity to the nuclear periphery has been noted with inactive genome regions in many cell types localized closer to the nuclear edge (Takizawa et al. 2008; van Steensel and Belmont 2017).

The nuclear space is further compartmentalized into distinct bodies (Dundr 2012). These are non-membranous structures that likely form via phase separation of nuclear proteins and nucleic acids (Banani et al. 2017; Shin and Brangwynne 2017). Nuclear bodies often serve as sites of particular nuclear functions such as the transcription of ribosomal genes in the nucleolus or the processing of nascent
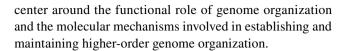
histone transcripts in the histone locus body (Dundr 2012). Alternatively, they may serve as storage sites for functionally related proteins such as splicing speckles, which are enriched in pre-mRNA splicing factors (Dundr 2012; Galganski et al. 2017).

A major landmark, and functionally relevant entity, in metazoan nuclei is the nuclear lamina. This anastomosed network of lamin proteins, which are members of the intermediate filament family, lines the inner nuclear membrane (INM) and protects the genome from mechanical forces that act on the nucleus (Dittmer and Misteli 2011). In addition, the lamina serves as a platform for the association of specific chromatin domains to the nuclear periphery. These lamina-associated domains (LADs) are typically gene poor, structurally condensed and enriched in repressive histone modifications (van Steensel and Belmont 2017), in line with longstanding observations of enrichment of heterochromatin at the nuclear periphery of most cell types (Lemaître and Bickmore 2015). Of relevance to human health, mutations in lamin proteins cause a wide range of tissue-specific diseases, including muscular dystrophies, lipodystrophies, neuropathies, and systemic premature aging syndromes (Gruenbaum and Foisner 2015; Vidak and Foisner 2016).

The past two decades have brought dramatic progress in the detailed description of nuclear structure. Traditional imaging approaches have been refined to provide unprecedented spatial and temporal resolution, and the use of in vivo imaging methods has led to an appreciation for the highly dynamic nature of many nuclear structures and processes. These methods have been complemented and extended by the development of biochemical techniques, so called 3C technologies, to map chromatin–chromatin interactions at a genome-wide scale, leading to a comprehensive description of the chromatin landscape in the cell nucleus (Grob and Cavalli 2018). Furthermore, the recent realization of phase separation as a fundamental organizational principle of nuclear organization has provided a framework for how membraneless suborganelles can exist in the nuclear space (Banani et al. 2017; Shin and Brangwynne 2017), and the description of diseases caused by mutations in lamin genes has highlighted the critical nature of the nuclear periphery (Dittmer and Misteli 2011; Vidak and Foisner 2016). However, many questions regarding the structure and function of the cell nucleus remain. We discuss here a select, yet neither comprehensive nor objective, set of unanswered questions that we consider of high priority in the field.

Genome organization

The fact that genomes are organized in a hierarchical fashion and in non-random patterns is now well established (Misteli 2007). Some of the most relevant questions in the field center around the functional role of genome organization and the molecular mechanisms involved in establishing and maintaining higher-order genome organization.

What is the regulatory function of genome organization?

Genome organization has been shown to be conserved during evolution (Dixon et al. 2012) to be cell-type specific (Dixon et al. 2015) and to be disrupted in disease (Lupiáñez et al. 2015; Valton and Dekker 2016), suggesting a functional role in gene regulation. However, while some features of genome organization, such as the position of a gene relative to the nuclear periphery, have been correlated with transcription, a causative role for these patterns is limited at best (Takizawa et al. 2008), raising the crucial question of whether genome organization is of functional relevance or is simply a reflection of the chromatin state.

With respect to the position of a gene relative to the nuclear periphery, the notion of a repressive role of the nuclear edge has long been supported by the observation that gene-poor regions are often enriched at the nuclear envelope (Bickmore 2013). In addition, association with the nuclear periphery is often correlated with transcriptional repression (Kosak et al. 2002; Dietzel et al. 2004; Zink et al. 2004a). However, this correlation does not apply to all genes; for example, the *PLP* gene is highly expressed and peripherally located in oligodendrocytes (Nielsen 2002) and *T-bet* is silenced without repositioning to the periphery in T helper cells (Hewitt et al. 2004). Furthermore, tethering to the nuclear periphery is sufficient to silence some, but not all, reporter genes (Finlan et al. 2008; Harr et al. 2015). Studies in mice showed that while either decompacting chromatin or independently inducing transcription of an endogenous gene was sufficient to move that gene from the periphery to the center of the nucleus, decompaction was required for repositioning, but transcription was not (Therizols et al. 2014). This observation suggests that peripheral positioning does not provide an independent means of silencing chromatin, but instead is, like transcriptional status, controlled by chromatin state. Similarly, in worms, loss of heterochromatin–lamina association is not sufficient on its own to cause widespread misregulation, although it does decrease the efficiency of differentiation (Gonzalez-Sandoval et al. 2015). It thus appears that positioning at the nuclear periphery is correlated with, but not directly causative of, gene repression.

Similarly, disrupting the boundaries of chromatin domains, particularly TADs, has been shown to sometimes, but not always, result in functional consequences, particularly aberrant interactions between promoters and enhancers (Lupiáñez et al. 2015; Rodríguez-Carballo et al. 2017). In support of a functional role of TADs is the fact that they are evolutionarily conserved (Dixon et al. 2012) and have

been observed to change during differentiation (Fraser et al. 2015a; Ji et al. 2016). However, the interpretation of these correlations is complicated by the fact that the molecular mechanisms underlying TAD formation are unclear. In *Drosophila*, TAD boundaries are marked by active genes (Ulianov et al. 2016), whereas in mammals they are marked by domains enriched for CTCF binding sites (Nora et al. 2012; Tang et al. 2015), although CTCF binding is neither necessary (Barutcu et al. 2018) nor sufficient (Rao et al. 2014) for TAD formation. These observations suggest that rather than being discrete entities with a single conserved mechanism and function, TADs may merely be a recurring shape with limited functional relevance. Fortunately, recent studies identifying several proteins involved in TAD formation in mammalian cells suggest avenues for studying their function on a global level (Wutz et al. 2017). Determining whether the widely observed genome organization patterns, such as the non-random 3D position of a gene or the formation of chromatin domains, has functional consequences is of prime importance and will have profound implications for understanding the biology of the cell nucleus and of genomes.

What are the molecular mechanisms underlying 3D genome organization?

Next generation sequencing (NGS) and 3C technologies have greatly improved our understanding of hierarchical 3D genome organization (Nora et al. 2012; Rao et al. 2014; Bonev and Cavalli 2016). However, the question of what the underlying molecular mechanisms responsible for higher-order genome organization are is only beginning to be explored (Shachar et al. 2015; Guo et al. 2015; Sanborn et al. 2015). Cohesins and CTCF have been identified as two major determinants of higher-order genome structure (Bouwman and de Laat 2015), but it seems likely that other regulators of genome architecture exist. One reason for the scarcity of information is the difficulty in experimental approaches to identify them in an unbiased fashion.

One promising approach to systematically dissect mechanisms of nuclear architecture is the use of imaging-based RNAi screens (Joyce et al. 2012; Shachar et al. 2015; Pegoraro and Misteli 2017) and CRISPR/Cas9 screens (Shalem et al. 2014; Wang et al. 2014; Tan and Martin 2016; Henser-Brownhill et al. 2017; de Groot et al. 2018), in which changes in various nuclear and genome features are assessed, often by imaging after knockdown of specific cellular factors. When compared to RNAi screens, the use of CRISPR/Cas9 is more robust to off-target effects (Evers et al. 2016; Morgens et al. 2016), can be used to scan structural non-coding elements (Gasperini et al. 2017), and can be adapted to knock out or induce the expression of cellular genes (Gilbert et al. 2014).

Screening approaches will be instrumental to address a variety of questions related to genome organization. In the short term, high-throughput microscopy-based assays, in conjunction with arrayed libraries of CRISPR/Cas9 reagents, and with programmable OligoPaint protocols (Beliveau et al. 2012, 2015), are expected to lead to the identification of molecular pathways involved in the establishment of chromatin domains, which are relatively large and can be readily visualized with fast, diffraction limited microscopy methods. Similarly, this kind of approach may be used to dissect the mechanisms regulating the preferential positioning of certain genomic regions to nuclear landmarks such as the nuclear envelope (NE) (Guelen et al. 2008), speckles, or nucleoli (Quinodoz et al. 2018). In the long term, the adaptation of large single-cell Hi-C protocols (Ramani et al. 2017) to single-cell pooled CRISPR screens (Adamson et al. 2016; Dixit et al. 2016) will potentially lead to the genome-wide measurement of smaller 3D genome structures, such as TADs, in hundreds or thousands of perturbations in parallel. Altogether, these large-scale functional genomics approaches will enable the discovery and understanding of the molecular rules underlying genome organization and its role in regulating other nuclear processes.

What mechanisms underlie the dynamic nature of genome organization?

Assessing genome organization by high-throughput sequencing methods belies the dynamic nature of the genome (Parfrey et al. 2008; Mishra and Hawkins 2017). In live cells, the genome is dynamic at different levels of organization: TADs dissolve during mitosis and reappear at G1, intra-TAD loops change along with expression of the associated genes during differentiation, and gene expression in turn re-organizes nuclear compartments (Denholtz et al. 2013; Smith et al. 2016; Freire-Pritchett et al. 2017; Nagano et al. 2017; Shachar and Misteli 2017). However, the mechanistic basis of these rearrangements is unclear. These changes both reflect and affect changes in gene expression; thus understanding the principles underlying the dynamic organization of genomes is essential for a complete picture of gene regulation (Geyer et al. 2011).

A key question in this area is what factors drive the intra-TAD loops that juxtapose enhancers to cognate promoters. The primary function of intra-TAD loops is thought to bring cognate enhancers and promoters in close proximity in 3D space and enable their interaction (Schoenfelder et al. 2015; Freire-Pritchett et al. 2017). While some of the major drivers of TAD organization have been identified as the chromatin proteins CTCF, cohesin and condensin, little is known about regulators of intra-TAD organization (Bouwman and de Laat 2015). Recent studies show that induction of genes can lead to the formation of pronounced enhancer–promoter
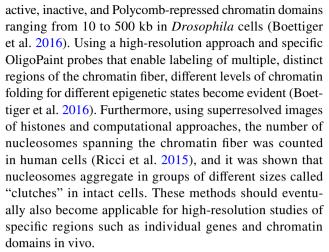
loops or a diffuse increase in intra-TAD contacts (Ron et al. 2017; Vian et al. 2018), suggesting that both the enhancer and promoter loci show dynamic association. However, this leaves us with the question: what cellular factors guide the specificity and the strength of these dynamic interactions?

A further question regarding chromatin dynamics is the issue of what mechanisms relocalize genes to and from the nuclear periphery. In higher eukaryotes, genes associated with the nuclear lamina move to the interior region upon induction (Kind and van Steensel 2010). In yeast, genes often associate with nuclear pores upon activation (Casolari et al. 2004; Takizawa et al. 2008). It is unclear how these directional movements are brought about. In the case of yeast, components of the RNA export pathways are believed to direct the transcribing gene to the nuclear periphery (Steglich et al. 2013). However, such translocations can occur even in the absence of transcription (Brickner et al. 2007). DNA zip codes bound by transcription factors have been implicated in yeast and a close parallel has been reported in human B cells (Ahmed et al. 2010; Zullo et al. 2012). To conclusively resolve mechanisms that directly relocalize genes would require following the movement of native genes in live cells coupled with quick conditional depletion of regulators of chromatin and transcription.

What is the structure of chromatin in the intact cell?

One of the long-standing, and still remarkably poorly understood, fundamental questions in chromatin biology is how the 2 m of DNA is packaged into the ~ 10 μm-sized nucleus. New imaging methods, particularly superresolution and modified electron microscopy methods, have recently provided some insights.

Traditional light imaging methods have a resolution limit of ~ 250 nm, far below what is needed to visualize chromatin fibers. Recent superresolution techniques achieve considerably better resolution, for example structured illumination microscopy (SIM) has a resolution of 100–130 nm and stimulated emission depletion (STED) microscopy ~ 20–70 nm in the XY plane (Yamanaka et al. 2014). However, these methods are still not sufficient to clearly visualize the chromatin fiber which has a diameter in the order of 5–30 nm. One of the most promising approaches to probe chromatin is localization microscopy based on the detection of single molecules using special fluorophores with a capacity for stochastic blinking (Lakadamyali and Cosma 2015). The resolution of photo-activated localization microscopy (PALM) and stochastic optical reconstruction microscopy (STORM) can be as low as 10–30 nm in the XY plane, and, if combined with additional approaches such as optical astigmatism or dual objectives systems, can be reduced down to 10–75 nm in the Z direction (Yamanaka et al. 2014). A notable application of 3D-STORM is the study of the structure of

active, inactive, and Polycomb-repressed chromatin domains ranging from 10 to 500 kb in *Drosophila* cells (Boettiger et al. 2016). Using a high-resolution approach and specific OligoPaint probes that enable labeling of multiple, distinct regions of the chromatin fiber, different levels of chromatin folding for different epigenetic states become evident (Boettiger et al. 2016). Furthermore, using superresolved images of histones and computational approaches, the number of nucleosomes spanning the chromatin fiber was counted in human cells (Ricci et al. 2015), and it was shown that nucleosomes aggregate in groups of different sizes called "clutches" in intact cells. These methods should eventually also become applicable for high-resolution studies of specific regions such as individual genes and chromatin domains in vivo.

A complementary approach is the use of advanced electron microscopy. A method termed ChromEMT (Chromatin Electron Tomography) uses a photoconvertible DNA-intercalating dye combined with 3D tomography of serial sections to visualize the chromatin fiber in intact cells (Ou et al. 2017). ChromEMT reveals that chromatin exists predominantly as a 5–24 nm fiber and that the difference between heterochromatin and euchromatin is largely due to different packaging densities of the fiber, rather than differences in the folding patterns. Further application and development of this method promises to reveal the fine structure of chromatin in the intact cell.

An overarching critical question in the field is how biochemical 3C methods relate to single-cell imaging methods (Nagano et al. 2013; Dekker and Misteli 2015; Fraser et al. 2015b; Giorgetti and Heard 2016; Stevens et al. 2017). This is a pertinent question since 3C methods report averaged contact frequencies, whereas fluorescent in situ DNA hybridization measures spatial distances in single cells and, even more promisingly, in case of superresolution microscopy, has the potential to reveal the structure of chromatin regions. Superresolution visualization, especially combined with biochemical approaches or CRISPR/Cas9 in vivo tagging, will bring us closer to understanding genome organization in the interphase nucleus in physiological and pathological conditions.

Do post-translational modifications regulate genome organization?

One potential mechanism for modulating chromatin structure and genome organization is post-translational modifications (PTMs). Support for this notion comes from a high-throughput imaging screen to identify factors which affect the position of genes in the human cell nucleus (Shachar et al. 2015). Of 50 factors which altered the position of four tested target genes, 11 were components of post-translational modifying complexes or the modifying enzymes themselves,

and 10 of the 11 identified in the screen targeted histones as substrates (Shachar et al. 2015). These data suggest that specific histone modifications may target genome locations to precise positions within the nucleus. This model is also supported by findings in *C. elegans* where transcriptionally repressed chromatin is targeted to the nuclear periphery via histone H3-lysine 9 mono-, di-, and trimethylation (Towbin et al. 2012; Gonzalez-Sandoval et al. 2015). Furthermore, the histone H3K9-methyl reader CEC-4 was identified and found to independently localize to the nuclear membrane and anchor heterochromatin to the nuclear periphery by binding histones directly in a histone H3 K9 methyl-dependent manner.

An additional, and most likely complementary, possibility is that post-translational modifications of non-histone proteins play a role in genome organization. Such proteins would likely regulate genomic organization through changes in protein–protein interactions caused by the PTMs. A potential set of substrates which could affect gene positioning through the addition of PTMs are the lamin proteins (Simon and Wilson 2013). Lamins A, B1, B2, and C all interact with chromatin and are all extensively modified with PTMs such as farnesylation, acetylation, phosphorylation, ubiquitination, and sumoylation, among others (Simon and Wilson 2013). In addition, a plethora of lamin-binding proteins have already been identified suggesting that these interactions could be regulated by PTMs (Dittmer et al. 2014). Of physiological interest is the fact that many post-translational modifying enzymes and complexes have been implicated in various diseases including cancer (Butler et al. 2012). Identifying how post-translational modifying components regulate gene positioning will lead to a better understanding of how the cell organizes its genome and how these regulatory mechanisms are disrupted in human disease.

How heritable is nuclear organization?

Spatial genome organization is relatively invariable in a given cell type, but can be altered quite dramatically during tissue development, differentiation, and malignant transformation (Krumm and Duan 2018). During interphase, chromatin is constrained in mobility and usually does not move over long distances; however, following cell division during the first 2 h of the G1 phase, global nuclear organization needs to be re-established to allow for diverse nuclear functions (Vazquez et al. 2001; Walter et al. 2003; Thomson et al. 2004; Strickfaden et al. 2010). Evidently, the processes taking place in early G1 have a significant impact on the outcome of nuclear organization, but to date it is unclear what kind of nuclear structures, forces, and factors causally underline these processes. The mechanisms by which chromatin compartments are lost during mitosis and re-established in

G1 or how loci form and lose association with nuclear substructures in daughter nuclei are unknown.

One way to shed light on the players that contribute to re-formation of organization is to use an experimental system that allows tracking of the location of large genomic regions in live cells over multiple cell divisions, similar to the one described for Lamin B1-contacting regions (Kind et al. 2013). This assay could be combined with a CRISPR-based screen to knock out various genes and to discover new organization candidates that function in early G1. Another possible approach to address this question would be to use a well-characterized developing model organism such as the fly or the worm and to generate lines that carry a fluorescent reporter in an endogenous locus, allowing simultaneous visualization of the genomic region and transcriptional outcome at the single cell level (Chen et al. 2018). Such systems are now available and can be used to follow the position of a locus in a developing embryo and to correlate position with gene activity over multiple cell cycles. Combined with genetic screens, factors that affect positioning and/or activity in various stages of development should become apparent. Identification of these mechanisms will shed light on how nuclear memory of genome organization is generated, how it affects transcription and cell fate, and how heritable genome organization is.

Can spatial genome reorganization be used for diagnostic and prognostic purposes in the clinical setting?

Annually, ~ 14.1 million people worldwide are diagnosed with cancer (Torre et al. 2015), yet there are few biomarkers available to aid oncologists in prescribing the most beneficial treatment for a patient or to identify indolent cancers, which in the absence of treatment the patient would remain asymptomatic (Ferté et al. 2010; Welch and Black 2010; Meaburn 2016). Consequently, there is an urgent need for additional clinically relevant cancer biomarkers. While the functional relevance of spatial organization of the genome within an interphase nucleus and the mechanisms that choreograph these positioning patterns currently remain elusive, it is likely that the spatial organization of the genome could be exploited for clinical purposes (Meaburn 2016; Meaburn et al. 2016b).

The rationale for the use of genome organization as a clinical tool is the fact that the non-random organization of various genome features changes in pathological conditions (Meaburn 2016). Specific genes and chromosomes occupy alternative spatial positions in disease, including in epilepsy (Borden and Manuelidis 1988; Walczak et al. 2013), laminopathies (Meaburn et al. 2007; Taimen et al. 2009; Mewborn et al. 2010), Trisomy 21 (Paz et al. 2013, 2015; Kemeny et al. 2018), and cancer (Cremer et al. 2003; Wiech
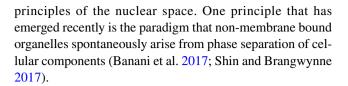
et al. 2009; Meaburn et al. 2016a; Taberlay et al. 2016). An indication for the feasibility of spatial genome organization as a diagnostic tool comes from a set of small-scale retrospective studies in which cancer tissues were accurately identified using spatial positioning patterns (Meaburn et al. 2009, 2016a; Leshner et al. 2016; Meaburn 2016). For example, the *FLI1* gene repositioned in 100% (10/10) of breast cancers (Meaburn et al. 2016a) and 92.9% (13/14) of prostate cancers (Leshner et al. 2016), and *HES5* was repositioned in 100% (13/13) of breast cancers (Meaburn et al. 2009). Moreover, genes that repositioned in a lower frequency of cancers can be used in combination to improve their sensitivity in detecting cancer (Meaburn et al. 2009; Leshner et al. 2016). Critically, positioning biomarkers have high specificity since repositioning rates are low in noncancerous tissues (Meaburn et al. 2009, 2016a; Leshner et al. 2016). The most likely application of spatial genome biomarkers will be in combination with routinely used pathology indicators, such as gross morphology of tissues and nuclei, to refine the current diagnostic methods (Zink et al. 2004b; Meaburn 2016).

Although additional biomarkers for the diagnosis of cancers would be welcome, prognostic biomarkers are in even higher demand. The question remains whether spatial positioning of the genome could yield more nuanced information and be used to sub-type cancers. For example, are there genes, or sets of genes, whose positioning patterns can be used to distinguish aggressive cancers from indolent ones? This remains to be formally addressed, but the fact that there are different spatial positioning signatures between different diseases, including between cancers originating in different organs, suggests a promising potential for the spatial organization of the genome to also provide prognostic biomarkers (Meaburn 2016).

The use of the spatial organization of the genome as a clinical biomarker is a relatively understudied topic and requires further studies on large cohorts of patients. Identification of useful biomarker genes is hindered by a lack of knowledge of what drives the reorganization of the genome, making it currently impossible to predict which genes may be useful spatial biomarkers, meaning large numbers of genes will need to be screened to find promising biomarkers and will require high-throughput imaging and analysis approaches applicable to tissues.

Principles of nuclear organization

The nucleus is like no other cellular organelle in that it is characterized by the presence of not just protein, but large amounts of nucleic acids, and contains a large number of subcompartments which are not defined by membranes (Dundr 2012). These features point to unique organizational principles of the nuclear space. One principle that has emerged recently is the paradigm that non-membrane bound organelles spontaneously arise from phase separation of cellular components (Banani et al. 2017; Shin and Brangwynne 2017).

Is the nucleus an emulsion of phase-separated droplets of nucleic acids and proteins?

Phase separation is likely relevant for nuclear organization. Several nuclear organelles such as nucleoli and nuclear speckles appear as discrete micron-sized bodies that remain compositionally distinct from the surrounding nucleoplasm without the use of a membraned divider (Dundr 2012). Moreover, many of these nuclear bodies share remarkably similar behavior with that of liquid droplets (Brangwynne et al. 2011; Hyman et al. 2014) and their macroscopic properties are governed by specific molecular interactions (Feric et al. 2016). These and other assemblages of nucleic acids and proteins are collectively known as biomolecular condensates (Banani et al. 2017).

Recently, several nuclear proteins, not associated with canonical nuclear bodies, have also been shown to behave as biomolecular condensates. For example, the ability of heterochromatin protein 1a (HP1a) to condense DNA into liquid-like droplets provides a simple, yet robust mechanism for gene silencing of heterochromatin (Narlikar et al. 2017; Strom et al. 2017). By extension, phase separation may play a role in faithfully compartmentalizing chromosomes into discrete territories or sub-compartments within the nucleus (Erdel and Rippe 2018). Furthermore, the clustering of super-enhancer elements and transcription factors along DNA into defined condensates (Hnisz et al. 2017; Chong et al. 2018; Sabari et al. 2018) and the interplay between other liquid-like components, such as the Mediator transcriptional coactivator and RNA polymerase II, may represent a physical means to control gene expression (Cho et al. 2018). Speculatively, other nuclear processes may also involve the phase separation of nucleic acids and proteins into functional interaction hubs, such as the cohesion of proteins to telomeres to prevent the degradation of chromosome ends; the spontaneous formation of DNA damage repair foci in response to damage; and the interaction of intrinsically disordered tails of histone proteins in promoting chromatin compaction.

Together, these results pose an intriguing question: is the genome organized as an emulsion of many phase separated droplets of DNA and protein? If so, what determines the sites and timing of droplet assembly and disassembly? Furthermore, how do the emergent properties of these condensates affect gene expression, and, importantly, how do anomalies in phase separation of the genome contribute to

disease progression, such as in neurodegenerative disorders and cancer?

Answering these questions will require multidisciplinary methods that bridge the physics of phase separation with the specific biological players involved in organizing the nuclear genome. A top-down approach of using high-throughput screening and imaging methods to assay for phenotypic changes in condensate morphology can be complemented by a bottom-up approach of evaluating proteins for phase separation behavior in vitro. Furthermore, systematic mutations and deletions will lead to the identification of the essential sequences and regions that promote formation or partitioning into droplets (Wang et al. 2018). Ultimately, differences in phenomenological properties of biological condensates between disease states may shed light on which molecular interactions therapeutic strategies should target. This future work will provide insight into how information encoded by molecular interactions becomes propagated via phase separation across larger length scales within the cell.

It is intriguing to speculate that phase separation may also have played a role in enabling the origin and evolution of life. In this view, RNA may have acted as a scaffold for the formation of early membrane-less compartments that served to concentrate biochemical reactions by providing the necessary spatial and chemical diversity (Poudyal et al. 2018). This model would explain the large number of non-coding RNAs observed in higher organisms, and the idea is consistent with the prominent role of RNA in early life and the presence of RNA in many phase-separated structures including nuclear paraspeckles (Hennig et al. 2015; Yamazaki et al. 2018) and cytoplasmic ribonucleoprotein granules (Van Treeck and Parker 2018). Furthermore, dysregulation of nuclear RNA-mediated phase separation leads to disease as shown in neuromuscular disorders where repeat expansions of RNA cause aberrant gelation (Aguzzi and Altmeyer 2016; Jain and Vale 2017). To address this model, key phase separating molecules from primitive to complex organisms need to be biochemically dissected to determine the conditions that favor compartmentalization. A prediction from this model is that many non-coding RNAs are indispensable in the context of liquid phase-separated structures in the nucleus.

The nuclear envelope and the lamina

The nuclear lamina is a major structural feature of the nucleus of metazoan cells (Serebryannyy and Misteli 2018). Its primary function has long been assumed to provide physical stability to the nucleus and to protect the genome from mechanical forces transmitted through the cytoplasm. However, recent observations suggest more diverse functions for the lamina, including anchoring specific genome regions to the nuclear periphery, controlling the intranuclear concentration of proteins by their regulated sequestration to the lamina, serving as a platform for nuclear signaling events and possibly other cellular functions (Serebryannyy and Misteli 2018). Numerous important questions regarding the function of the nuclear lamina, particularly with regard to its physiological and pathological functions, remain.

Is the nuclear lamina a site of protein degradation?

The nuclear envelope is a double membrane consisting of the outer nuclear membrane (ONM) and the INM, connected at the sites of nuclear pores (Hetzer 2010; Boban and Foisner 2016). The ONM is continuous with the endoplasmic reticulum (ER). Protein homeostasis in this compartment is ensured by endoplasmic reticulum-associated protein degradation (ERAD) pathways (Khmelinskii et al. 2014); however, little is known about possible protein quality control at the INM.

Some indications for a role of the INM in protein degradation come from studies in the yeast *Saccharomyces cerevisiae*, where the Asi complex, containing a RING finger (RNF) domain ubiquitin E3 ligases Asi1 and Asi3, has been shown to target integral proteins of the INM for proteasomal degradation (Foresti et al. 2014; Khmelinskii et al. 2014). Although Asi components are not conserved outside of yeast, emerging evidence has suggested the existence of transmembrane RNF-containing E3 ubiquitin ligases in eukaryotic cells (Nakamura 2011). In line with this, the RNF123 and HECW2E3 E3 ubiquitin ligases have been implicated in proteasomal degradation of several nuclear proteins, including lamin B1 and mutants of lamin A/C associated with Emery–Dreifuss muscular dystrophy (EDMD) (Khanna et al. 2018; Krishnamoorthy et al. 2018). Apart from the ubiquitin–proteasome-dependent protein degradation, autophagy-mediated degradation of nuclear envelope proteins has recently been described in mammalian cells, demonstrating that the autophagy protein LC3 interacts with lamin B1 and mediates its clearance by macroautophagy in response to oncogenic stress (Dou et al. 2015). In addition to lamin B1, macroautophagy has been shown to be involved in downregulation of INM proteins emerin, lamin B receptor (LBR), and SUN1 in oncogene-induced senescent cells (Lenain et al. 2015). Although the mechanisms of lamin A/C degradation remain an open question in the field, recent studies suggest that the disease-causing lamin A mutant isoform progerin is partially degraded by macroautophagy (Cao et al. 2011; Cenni et al. 2011; Gabriel et al. 2015; Vidak and Foisner 2016). Progerin is the causative agent in the premature aging disorder Hutchinson–Gilford progeria syndrome (HGPS) and progerin, unlike the wild-type lamin A, is permanently farnesylated and thus tightly associated with the INM (Dechat et al. 2007; Reddy and Comai 2012).

The observed partial degradation of progerin coupled with the observation that wild-type lamin A/C is not degraded by macroautophagy in senescent cells (Lenain et al. 2015) suggests another pathway may be involved in the degradation of lamin A/C in mammalian cells. One possible mechanism is a selective form of autophagy called chaperone-mediated autophagy (CMA) that has recently been implicated in the degradation of nuclear proteins (Park et al. 2015; Tekirdag and Cuervo 2018). One of the distinctive features of CMA is that proteins are selected through a penta-peptide recognition motif in their amino acid sequences, biochemically similar to KFERQ (Cuervo and Wong 2014) present both in wild-type lamin A/C and progerin, making them strong candidates for degradation by CMA. Furthermore, the NFE2L2/NRF2 transcription factor has recently been identified as an important regulator of CMA activity (Pajares et al. 2018). Interestingly, progerin has been shown to sequester NRF2, resulting in impaired NRF2 transcriptional activity (Kubben et al. 2016), allowing for a possible scenario where impaired regulation of NRF2 in progerin-expressing cells could lead to decreased CMA activity and contribute to increased stability and accumulation of proteins at the nuclear periphery. An intriguing possibility is that CMA might represent a missing link to understand pathways involved in the degradation of lamin A/C in health and disease. Taken together, these emerging observations point to an underappreciated role of the nuclear lamina in the control of protein homeostasis.

Does nuclear envelope-associated chromatin maintain cell specificity and differentiation?

Just as the mechanisms that regulate protein homeostasis at the NE remain poorly understood, so are the functional consequences of changes in its protein composition. The NE serves as a selective barrier for the regulation of nuclear import of signaling factors and of mechanical force transduction (Uhler and Shivashankar 2017; Kirby and Lammerding 2018). A combination of microscopy, DNA adenine methyltransferase identification (DamID), chromatin immunoprecipitation (ChIP), and next-generation sequencing approaches have suggested that NE-associated proteins also organize hundreds of genes into transcriptionally silenced domains at the nuclear periphery (van Steensel and Belmont 2017). Although the basic tenets of NE-associated chromatin organization such as the transcriptional repression of genes associated with the nuclear lamina and preferential localization of heterochromatin to the nuclear periphery appear to be conserved (Kind et al. 2015; van Steensel and Belmont 2017), drastic changes in the association of chromatin with the lamina occur upon differentiation and correlate with alterations in histone modifications as well as the tissue-dependent expression of NE-associated proteins

(Peric-Hupkes et al. 2010; Solovei et al. 2013; Swift et al. 2013; Robson et al. 2016; Atlasi and Stunnenberg 2017). These correlations may suggest a role for the nuclear lamina in defining genome organization patterns associated with differentiation. Furthermore, observations in *C. elegans* suggest that the differentiation state may be maintained by the sustained association of chromatin with the NE (Gonzalez-Sandoval et al. 2015), since mutation of the methylated H3K9-binding protein, CEC-4, primarily affects perinuclear gene anchoring and impedes proper cell differentiation but does not alter gene expression (Towbin et al. 2012; Gonzalez-Sandoval et al. 2015). Therefore, delineating the relationship between cell identity and NE-associated chromatin organization will have wide-reaching implications in our understanding of development (Peric-Hupkes et al. 2010) and aging-associated diseases (Berman et al. 2011; Timp and Feinberg 2013; Meaburn 2016; Serebryannyy and Misteli 2018).

To elucidate the role of the NE in differentiation, it will be important to comprehensively identify NE-associated protein candidates and changes in chromatin conformation during differentiation processes and mutant proteins must then be experimentally reintroduced to test their effect on cell fate and plasticity. Identifying NE-associated proteins responsible for cell identity may be complicated by the large number of proteins localized to the NE (Korfali et al. 2012), the changes in chromatin organization mediated by mechanoregulation of the nucleus (Uhler and Shivashankar 2017), and the functional redundancy between NE-associated proteins (Amendola and van Steensel 2015). However, unbiased screening approaches to identify functional proteins (Shachar et al. 2015) or analysis of RNA and protein expression differences between cell types may narrow the pool of candidates (Robson et al. 2016). Further clues may be ascertained by studying chromatin reorganization in the presence of disease-causing nuclear envelope protein mutants or loss of NE-associated proteins (Dittmer and Misteli 2011; Chen et al. 2014; Elzeneini and Wickström 2017; Briand et al. 2018; Whitton et al. 2018). To determine if changes in organization have functional implications, inducible protein dimerization systems or protein targeting domains may be used to relocate proteins within the cell (Stanton et al. 2018), whereas artificial tethering arrays, specific targeting sequences, and dCas9 can be employed to reposition chromatin within the nucleus (Reddy et al. 2008; Harr et al. 2015; Morgan et al. 2017). These types of detailed investigations into the functional role of chromatin compartmentalization to the nuclear envelope should reveal general principles of genome regulation and their likely relevance to differentiation and cell fate decisions.

Is disruption of the nuclear lamina architecture a prerequisite for the development of aging-associated diseases?

Nearly half of human deaths are caused by chronic aging-associated diseases, including cancer, diabetes, cardiovascular and neurodegenerative pathologies, as well as chronic lung and kidney diseases (Lopez et al. 2006). At the tissue level, all of these diseases are characterized by an increased accumulation of aged cells (Kubben and Misteli 2017). The functional decline of aged cells has been attributed to the manifestation of various cellular defects, so-called hallmarks of aging, including a disruption of the nuclear lamina architecture, and loss of (epi)genomic, proteostatic and metabolic integrity (López-Otín et al. 2013). While disruption of the nuclear lamina architecture is known to give rise to other hallmarks of cellular aging and to be overall sufficient to trigger cellular aging and several aging-associated pathologies, it remains unknown whether loss of the nuclear lamina structural integrity is a prerequisite for cellular aging and the development of aging-associated diseases (Kubben et al. 2016; Kubben and Misteli 2017). Establishing that disruption of the lamina structure is essential in aging-associated diseases etiology would have major therapeutic implications for the discovery and targeting of cellular pathways that serve to protect the nuclear lamina architecture in the treatment of most leading aging diseases.

A cellular hallmark of aging is the increased lobulation and blebbing of the nuclear lamina, which has been observed across cells of various tissues and these architectural malformations have been observed with increased frequency in various aging-associated diseases, including cancer, Parkinson's disease, and atherosclerosis (Olive et al. 2010; Thaller and Patrick Lusk 2018). No studies have been performed to date to systematically quantify the degree of structural disruption of the nuclear lamina across prevalent aging-associated diseases or to determine the timing of their occurrence in relation to other pathological and clinical hallmarks of these diseases (Kubben and Misteli 2017). It is anticipated that if structural disruption is a requirement for the development of aging-associated diseases, lobulations and nuclear blebbing occur relatively early in the disease process and that the degree of nuclear disruption has predictive power for the severity of pathological developments.

Mouse models provide a powerful complementary experimental strategy to directly assess the role of nuclear lamina architecture in aging-associated disease (Stewart et al. 2007). Age-related nuclear lamina deformities have been attributed to the accumulation of the alternatively spliced lamin A protein progerin in the premature aging disorder HGPS (Scaffidi and Misteli 2006) which is characterized by numerous phenotypes that recapitulate physiological aging pathologies, including atherosclerotic cardiovascular defects (De Sandre-Giovannoli et al. 2003; Eriksson et al. 2003). It would be important to generate a novel mouse model in which the ability to express progerin is eliminated through mutating its alternative splice site, and to determine whether the loss of progerin is sufficient to improve life- and health span and delays the onset and severity of aging-associated pathologies, when crossed with aging-disease specific mouse models.

Regardless of disease relevance, more insight is needed regarding the pathways that prevent or repair structural damage to the nuclear lamina. A potential strategy to identify such pathways is to perform genome-wide CRISPR screens for genes that modulate nuclear shape across various cell types as well as prevent the formation of aging defects and nuclear dysmorphology upon expression of nuclear structure-disrupting proteins such as progerin. Identified druggable pathways that strengthen the nuclear architecture may represent an attractive new class of therapeutics for many aging-associated diseases.

Conclusions

The questions of how the cell nucleus and its genome are organized and the corresponding functional implications are of fundamental importance. We have seen remarkable progress in this still relatively new area of cell biology. As expected for a developing field, much of the effort over the past years has been on describing the structural features of the nucleus and of genomes at various levels using novel imaging and biochemical mapping methods. The future of the field will be in the exploration of how the structural features of the nucleus and genomes relate to their functions. Of particular relevance is the question of what the functional role of the most prominent nuclear features is. Furthermore, while numerous patterns of protein and chromatin organization have been described in the nucleus, the molecular mechanisms that create them are in most cases unknown. Finally, and possibly most importantly, the relevance of nuclear architecture and genome organization to physiological and pathological events needs to be fully elucidated. As is often the case when surveying a field of research, one marvels at the wealth of tantalizing insight that has been generated in recent years from efforts to understand nuclear structure and genome organization, and at the same time one is humbled by how much still remains to be discovered.

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