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Mechanobiology of Chromatin and the Nuclear Interior

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Abstract

The view of the cell nucleus has evolved from an isolated, static organelle to a dynamic structure integrated with other mechanical elements of the cell. Both dynamics and integration appear to contribute to a mechanical regulation of genome expression. Here, we review physical structures inside the nucleus at different length scales and the dynamic reorganization modulated by cellular forces. First, we discuss nuclear organization focusing on self-assembly and disassembly of DNA structures and various nuclear bodies. We then discuss the importance of connections from the chromatin fiber through the nuclear envelope to the rest of the cell as they relate to mechanobiology. Finally, we discuss how cell stimulation, both chemical and physical, can alter nuclear structures and ultimately cellular function in healthy cells and in some model diseases. The view of chromatin and nuclear bodies as mechanical entities integrated with force generation from the cytoskeleton combines polymer physics with cell biology and medicine.

Keywords

Chromatin; Reptation; Genome expression; Self-assembly; Nucleoskeleton; Laminopathy; Nuclear mechanics

INTRODUCTION

Cellular processes are carried out by collections of proteins, each born of a linear sequence of DNA within the genome. Expression of each gene is tightly controlled by transcription and repression factors as well as access to necessary molecules for expression. Genes are also functionally nested within layers of complex, higher-order structural and spatial organization within the nucleus that further regulate their expression.^{48,68,74} Unfortunately only the simplest of DNA and chromatin structures can be recapitulated *in vitro* for biophysical study.^{13,42,65} Conversely, much of our understanding of the physical properties of chromatin organization and positioning and their role in gene expression has emerged

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CONFLICTS OF INTEREST

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ETHICAL STANDARDS

Neither human studies nor animal studies were carried out by the authors for this article.

from imaging and manipulation studies within the nucleus.¹⁰³ A complete understanding of DNA and chromatin as both a biological code and a physical structure requires an intellectual reconciliation and an integration between biology and polymer rheology. For example, structural changes that facilitate loop formation in chromatin are associated with coordinated regulation of transcription and other processes necessary for biological function.²⁸ These changes are driven both by binding of protein complexes as well as physical effects including macromolecular crowding and depletion attraction.³ Further, chromatin mobility is tightly regulated by a delicate balance of driving forces from molecular motors and viscoelastic resistances that govern the physical principles of all polymer reptation in an entangled mesh.^{80,109} Chromatin mobility is critical to evolving functional needs that demand reorganization for genomic processes, and will be described in detail in the later section “Chromatin Dynamics and the Temporal Nature of Genome Function”. Investigation of such dynamic change requires techniques for visualization at the appropriate length and time scales as well as a physical understanding of underlying mechanisms of movement. Thus, developing biophysical techniques capable of measuring these dynamics and bridging the physical underpinnings of chromatin within the biological context becomes critical to discovering genome function in all its complexity.

Here, we review the current knowledge of the genome and its organization with respect to nuclear structure and function. We highlight the physical aspects associated with its dynamic nature and its spatial arrangement within the nucleus. We also demonstrate how the genome structure dynamically evolves to meet new functional needs during physiological changes, and dysfunction arises in disease pathologies associated with nuclear organization. What emerges is a more complete picture of genome function derived in no small part by physical properties that facilitate its organization and dynamic function. The interconnection of structures of chromatin and other subnuclear bodies with biological function of gene expression and how these are both impacted by force are essential aspects of mechanobiology of the nuclear interior.

NUCLEAR ORGANIZATION AND SELF-ASSEMBLY

Within the cell nucleus, DNA is tightly packaged into chromatin to organize and regulate the meters long genome. This packaging consists of DNA wrapped twice around a histone octamer (composed of histone proteins H2A, H2B, H3 and H4) collectively known as a nucleosome, with multiple nucleosomes condensing to form the chromatin fiber. The 100 kDa nucleosome has a diameter measuring approximately 10 nm, giving expanded chromatin the appearance of beads on a string (at least *in vitro*). Most chromatin has laterally-packed nucleosomes with the help of the linker histone, H1, leading to higher-order structures, which are critical to genome function. Based on stoichiometry, there is enough histone protein to bind all of the available DNA within the nucleus, so it is predicted that all DNA within the nucleus is in the form of chromatin rather than as free DNA.⁹¹ Mechanical measurements of the nucleus have shown that the viscoelastic chromatin fiber is the dominant mechanical element of the nuclear interior when a nucleus deforms under high strain.⁷⁷

Chromatin is arranged in the nucleus with loose spatial specificity. This arrangement corresponds to decondensed, gene-rich regions of euchromatin preferentially located at the interior where gene expression is high; condensed, gene-poor regions of heterochromatin are located primarily at the periphery where gene activity is low.^{21,34,37,58} As 98% of human DNA does not code for protein, this noncoding DNA is believed to aid in regulation of the genome through hierarchical organization.⁵³ A variety of epigenetic modifications cause heterochromatin formation including DNA methylation patterns, histone modifications that enhance DNA-histone interactions (and consequently increase condensation)⁶⁸ and the binding of heterochromatin-specific proteins.⁵³ Additionally, regions of heterochromatin commonly bind to the proteins of the nucleoskeleton and nuclear envelope, which aids in repression and organization.^{41,57,80}

This hierarchical organization of DNA into varying levels of condensation serves many functions. By virtue of being less condensed, DNA in euchromatin is more readily transcribed with accessible binding sites to transcription factors and other proteins⁶⁶ as well as having heightened mobility.^{17,69} Accessible DNA can serve to nucleate *de novo* formation of functional sites or nuclear bodies, and these multi-molecular assemblies form at the initiation of associated activities,^{29,86} leading to the idea that the nucleus is a self-organizing system.^{48,74} The most heavily studied example is the formation of the nucleolus, where ribosomal biogenesis occurs.⁹⁰ Nucleolar disassembly and assembly during cell division mechanistically depends on the suspension and re-initiation of ribosomal biogenesis, respectively.^{60,75,96} The formation of nucleoli occurs *via* the coalescing of necessary proteins and ribosomal genes, from the five different pairs of homologous chromosomes containing them,⁷⁵ by complex mechanisms that are likely facilitated by physical properties of chromatin in addition to protein binding. Nucleolar specific proteins (e.g., fibrillarin and nucleophosmin) form liquid-like assemblies within the nucleus and *in vitro*.¹⁰⁸ Nucleolar assembly can be induced by extrachromosomal ribosomal DNA⁴⁷ and disassembly by inhibition of ribosomal gene transcription.⁹⁵ Self-organization is further supported by the fact that nucleolar proteins are continuously exchanged with the nucleoplasm and that nucleolar size is correlated with ribosomal production.⁷³ The nucleolus is the best understood nuclear body, but others are believed to function similarly. The most analogous example is evidence pointing to the emergence of specific transcription hubs, called “transcription factories”, that may act to service other genes and function much the way the nucleolus does for ribosomal gene transcription and biogenesis.¹⁸ We will describe transcription factories in detail later.

The formation of nuclear bodies emerges as a dominant feature of nuclear organization, which is central to nuclear function. While more compact than euchromatin, heterochromatin remains accessible to diffusing proteins and protein complexes.^{5,6} This leaves a reservoir of available components upon the initiation of any nuclear process. As observed in the nucleolus, nuclear bodies experience a constant flux of protein components, continuously evolving in response to functional needs. Thus, rapid turnover in function is possible because the nucleus maintains this capacity for dynamic change rather than functions at equilibrium or steady-state. Consequently, gene expression is not simply an on and off process, but one of varying degrees. This is most evident in comparing the stochastic

single cell gene expression profiles³² with the population, exposing the “myth of the average cell”.⁶²

Part of the stochastic nature of nuclear functions arise from the assembly of processing complexes. This occurs in a complementary fashion if all of the necessary factors are locally available for binding within the available space within the appropriate time (i.e., the residence time), implying the need of a spatial limitation for transit. The protein flux into and out of nuclear bodies serves to take advantage of the available reservoir. As this is an inherently inefficient process, recurring and continuous processes often keep the machinery largely intact. The inefficiency of *in vitro* transcriptional complex assembly¹¹ has been a hypothesis put forth for the possible presence of mostly intact transcription factories within the nucleus¹⁸ to allow quick changes in gene expression. These potential transcription factories may contain several active polymerases simultaneously transcribing multiple genes.¹⁸ Each factory, by virtue of a distinct protein composition, would confer unique environments that help regulate the expression of genes in shared factories.¹⁸ In this way, the cell tailors expression in a manner unique to genes and co-regulated gene groups. Thus, the possible existence of transcription factories ameliorates the otherwise relatively low probability of a transcription factor entering the nucleus, diffusing throughout the genome, localizing to the gene of interest, binding and simultaneously coupling with the other factors required for transcription on the DNA.

THE NUCLEUS AS AN INTEGRATED MECHANICAL SYSTEM

The nucleus is the largest and stiffest organelle in the cell,^{22,84} but it is not an isolated structure.^{23,94} The nucleus has been shown to play a role in balancing the contractile forces inside a cell associated with cell adhesion and motility.^{2,10} The actin filaments of the cytoskeleton play a prominent role in these processes.^{10,52} Cytoskeletal filaments are linked to the nucleus through a series of proteins that span the inner and outer nuclear membranes and are collectively called the Linker of Nucleoskeleton and Cytoskeleton, or LINC complex. Nesprin and SUN-domain families of proteins connect the cytoskeleton to the lamina nucleoskeleton. Nesprins 1 and 2 bind actin, nesprin 3 binds plectin (which associates with intermediate filaments),^{19,51} and nesprin four interacts with microtubules in specialized cells.⁸⁹ The C-terminus of nesprin proteins contains a KASH domain, which binds SUN 1 and SUN2, and SUNs traverse the nuclear envelope.⁷¹ On the inner nuclear membrane SUNs, and a host of inner nuclear membrane proteins, bind to lamins and proteins of the nucleoskeleton. The nucleoskeleton is composed primarily of two types of lamin proteins as well as lamin binding proteins, and other structural proteins; lamins and inner nuclear membrane proteins bind to DNA and chromatin.^{23,82,107}

Lamins fall into two categories, A-type lamins (lamin A and lamin C), and B-type lamins (lamin B1 and lamin B2). A-type lamins are coded for by the single gene *LMNA*, while B-type lamins are coded for by the genes *LMNB1* and *LMNB2*. A-type lamins are thought to be the primary components of nuclear rigidity.^{40,59} The lamin proteins can bind to DNA both directly and indirectly through complexes of proteins, including transcriptional repressors and regulators.¹⁰⁶ Thus, there is a degree of interconnection throughout the cell: from the extracellular matrix, through integrins, to focal adhesions, to the cytoskeleton,

through the LINC complex, to the nucleoskeleton and into the chromatin.⁷⁴ Much like chromatin condensation, cytoskeletal connection and other aspects of cell structure, the degree of this connection likely adapts with cell phenotype. During development and differentiation, heterochromatin formation and association with the nuclear lamina are believed to alter the mechanics of cells at later states of differentiation.^{38,80} Genes associated with the lamina are generally repressed.^{23,106} However, the loss of chromatin-lamina association is not in itself a means of gene activation, but instead serves as a necessary part of a multistep mechanism for stochastic gene activation.^{57,80}

CHROMATIN DYNAMICS AND THE TEMPORAL NATURE OF GENOME FUNCTION

Although we described chromatin states above as closed heterochromatin and open euchromatin, chromatin in the nucleus is continuously remodeling between the varying degrees of condensation. Evidence of this is provided by the presence of opposing bivalent (i.e., both activating and repressing) histone modifications at some sites,¹⁰⁰ with one modification dominating the transcriptional behavior.^{20,81} Each step of condensation and repression (or decondensation and activation) occurs through a multitude of protein-DNA and protein-protein binding events associated with different residence times.⁷³ This wide spectrum of characteristic binding time scales results in another layer of control. Additionally, these factors may be cooperative or inhibitory by altering residence times of other regulators¹ to favor certain outcomes. Along these lines, recent work on the binding of chromatin regulators to DNA exposed different patterns of regulator combinations for specific chromatin environments, genes of common functions and regulatory elements.⁸¹

When not bound to stable complexes, chromatin fluctuates and translocates throughout the nucleus *via* anomalous diffusion.^{17,101} In yeast, GFP-tagged regions of chromatin are able to move $\sim 0.5 \mu\text{m}$, equivalent to half the radial length of the nucleus, in 10 s.³⁶ Chromatin movements, as well as the physical mechanisms and the biological factors that regulate increased movements in the presence of exogenous factors, have been studied *via* intranuclear particle tracking microrheology.^{56,98,102} Despite the possibility for movement, individual chromosomes occupy distinct territories within the nucleus and actual movements correspond to regions of $\sim 1/1000$ th of the nuclear volume in humans,¹⁶ although overlap and incursion by loops from other chromosomes is frequent.¹⁰¹ The presence of many dense chromosome territories results in highly restricted motion within the human nucleus. This confers another advantage through molecular crowding, which enhances interactions by increasing effective concentrations and the collision frequency of interactions, while decreasing the probability of less favorable conformations (preferring native structure to more variety).^{26,64,72} The organization of chromosome territories within the nucleus is nonrandom and cell-type specific,^{34,70,79} with more gene dense chromosomes concentrated at the interior as discussed previously. This is believed to aid in gene regulation, with implications for incidence of chromosomal translocations in cancer mutation rates.^{78,105} Additionally, protein concentrations within the nucleus are heterogeneous, allowing for individual genes to be positioned in unique protein environments that play a role in expression patterns.⁷⁰ In this light, the dynamic repositioning of chromatin can serve to

facilitate functional changes by moving to regions for specialized function dictated by their protein composition.

While the correlation of radial position with chromosomal translocations and gene expression as well as positional correlation with gene density of chromosomes shows statistical significance across populations of cells of the same type,^{58,79} it is important to recognize that these average positions give no indication of the true position within a single cell, but rather the ensemble average.^{7,44} There is no absolute organization or deterministic guarantors of gene and chromosomal position; instead positions are driven by physical properties of chromatin and a myriad of DNA–protein and protein–protein interactions guiding this organization. Photobleaching experiments during cell division show daughter cells partially maintaining chromosome positioning,⁷⁹ and the lack of complete conservation of chromosome position or complete randomization shows the degree to which probabilistic mechanisms successfully maintain positioning patterns without deterministic control.

Physical models have attempted to recapitulate aspects of chromatin organization and dynamics with some success. The loop models accurately depict the spatial organization of the genome in the nucleus emanating from fluorescence *in situ* hybridization (FISH) experiments. By contrast, the fractal globule model finds concurrence with chromosome conformation capture (3C) experiments that yield information about chromosomal interactions.³ Additionally, conventional polymer dynamics models of de Gennes reptation or the Rouse chain model have been applied strictly to chromatin dynamics.^{12,43,104} However, the mere consistency of data with underlying models obscures what are likely much more complex phenomena.⁹⁸ The lack of congruence across experimental techniques demonstrates these models are likely oversimplifications of the observed phenomena and only tailored to select features. The failure of these models to universally describe chromatin is nearly always associated with the flawed (but necessary) assumption of equilibrium. Thus, we suggest that there is no universal physical model that can describe the nucleus from the DNA to the micron scale nucleus from nanoseconds to hours. To model a nucleus, proper timescales and length-scales must be considered and sampled appropriately together and be integrated into a composite model of gene expression that considers the biology and the physics of this unique organelle.

MECHANOTRANSDUCTION AND MECHANICAL MODULATION OF GENE EXPRESSION

At the scale of a single gene, stimulated transcriptional activation involves the removal of inhibitors, the binding of transcription factors as well as accessibility to polymerase enzymes. These single gene sites have been shown to mechanically unfold and decondense prior to transcription.⁵⁰ Recent studies visualizing polymerase activity also shows that the condensation of the local chromatin environment surrounding the gene can impact transcription speeds by 400% within the same cell.⁴ Thus, the local chromatin mechanical environment both at the site of expression and surrounding the expression region can greatly impact expression time.

In visualizing another length scale, stimulation of endothelial cells with vascular endothelial growth factor (VEGF) results in significant wide-scale decondensation of chromatin,⁹⁸ coincident with the expression of a large scale changes in gene expression.¹⁵ This change in VEGF-stimulated chromatin condensation is associated with altered actin-mediated cell contractility within the cell⁸⁸ as well as increased actin-nuclear coupling.⁹⁹ Direct visualization of nuclear deformation and increased cytoskeleton-driven chromatin agitation in the presence of VEGF⁹⁹ also suggest that cytoskeletal forces impact chromatin structure. Generally, forces within the cell have been shown to cause chromatin remodeling.^{45,49} Other studies have shown that direct force generation on cells causes changes in chromatin organization, in most cases chromatin decondensation, that is coincident with changes in gene expression.^{9,45,49,98} Similarly, nucleolar bodies are reorganized in response to substrate stiffness and force.⁶⁷ The mechanisms by which altered extracellular force or cytoskeletal force impact altered chromatin condensation are still being elucidated. There are likely a combination of mechanical and chemical factors. Mechanically, we suggest there are forces propagated through the LINC complex; decoupling of the LINC complex by a dominant-negative KASH domain inhibits force transmission into the nuclear interior.⁹² There are numerous chemical factors that likely play a role as well, including the Hippo: YAP-TAZ pathways that are involved in mechanotransduction and implicated in gene expression for growth and oncogenesis.^{30,63}

Combining the understanding of (i) an integrated cell mechanical structure with (ii) dynamic control over gene regulation results in the concept that forces generated within the cytoskeleton of the cell can result in remodeling of chromatin and functional nuclear structures (Fig. 1) that result in changes in genome expression.^{45,49} Remodeling can also come from forces imposed from outside of the cell such as compressive forces or fluid shear stress.⁹ These stresses likely act globally and nonspecifically to increase chromatin agitation and, therefore, the probability of expression by enhancing the kinetic events of binding, remodeling, and the mobility of components.^{45,49,98}

DISEASE AND DYSFUNCTION

Given the integral role of proteins in organizing the genome, dysfunction associated with any number of proteins and the subsequent alterations in nuclear organization can cause aberrant expression. This is particularly true of the peripheral organization of the genome along the nucleoskeleton. Since lamins bind repressed regions of chromatin, laminopathies associated with mutations in lamin proteins can alter gene function.^{21,57} The result is a perturbation of chromatin–lamina associations,⁵⁷ changes in expression,⁵⁷ and differential nuclear mechanical properties.²¹ This suggests a role for aberrant gene regulation in laminopathies.

Hutchinson-Gilford progeria syndrome (HGPS) is a disease that results in premature aging-type symptoms and arises from a mutation in the *LMNA* gene, causing a truncated lamin A protein with failure to cleave the farnesylated portion of prelamin A. This mutant lamin A is known as 50 lamin A or progerin.^{25,33,54} Progerin is more strongly associated with the nuclear membrane (due to this farnesylation and altered protein structure) changing the physical properties of the nucleus. The lamina in HGPS is thicker³⁹ with reduced protein

turnover.²⁴ Chromatin in HGPS is also altered, and interior chromatin is decondensed as a result of altered lamin structure.^{8,93}

Another disease which affects lamins, and therefore potentially chromatin dynamics, is autosomal dominant leukodystrophy (ADLD). ADLD symptoms are similar to that of multiple sclerosis with demyelination of nervous tissue and is caused by a duplicate copy of the *LMNB1* gene.⁷⁶ An overexpression of lamin B1 is believed to alter lipid synthesis as shown in a transgenic mouse model,⁸⁷ and lamin B1 is believed to alter nuclear heterochromatin distribution.^{14,92} We suggest that changes in chromatin condensation may lead to changes in gene expression and ultimately the ADLD disease phenotype.

The most notable correlation between disease and nuclear structure and chromatin organization is the broad spectrum of cancers. Most cancer cells have dramatically altered nuclear structure⁶¹ including altered nuclear shape, heterochromatin formation and organization as well as nucleolar assembly and function.¹¹⁰ Thus, nuclear stains still serve as the basis for many biopsies today. Altered DNA and chromatin organization associated with cancer metastasis can include defects in histones,⁹⁷ heterochromatin-inducing proteins,²⁷ several DNA-binding proteins involved in higher-order chromatin organization⁴⁶ and transcription factors.³¹ However, it is likely that the series of random mutations that lead to aberrant genome organization provide selective advantages to cancer cells mechanically by allowing for cells to be more deformable into tight interstitial spaces.^{35,55,83,85}

CONCLUSIONS

While heterochromatin and euchromatin provide useful terms to discuss chromatin structure in a single snapshot of a nucleus, these are oversimplifications of the complex dynamic nature of the genome. We have discussed the ability of a cell to assemble and disassemble various nuclear bodies based on cellular function, and the flux of proteins through these bodies. In addition to the heterogeneous distributions of chromatin condensation and protein concentration levels, we highlight the importance of the LINC complex in transmitting forces into the genome. The physical connections through the LINC complex creates a physically connected intracellular network, demonstrating the importance of observing the nucleus, and chromatin, as a mechanically integrated network with the cell. These physical connections allow physical stimulation of the cell, as well as motor protein derived forces, to alter chromatin dynamics. All of these factors make the chromatin inside the nucleus an active and integrated structure inside the cell that senses and responds mechanically to stimuli from inputs within many regimes within the cell.

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Nuclear structural changes that impact gene expression. Forces generated in the cell from actin-myosin or from outside of the cell are transmitted by cytoskeleton-LINC-nucleoskeleton connections to the chromatin. Force induced movements within the nucleus cause or enhance the following structural changes and regions of anomalous flux. This may lead to (i) altered dynamics of transcription factories and improved accessibility of proteins in these regions; (ii) decondensation of chromatin directly by mechano-modulation and indirectly through biochemical pathways; and (iii) altered stability and assembly of subnuclear bodies including nucleoli.