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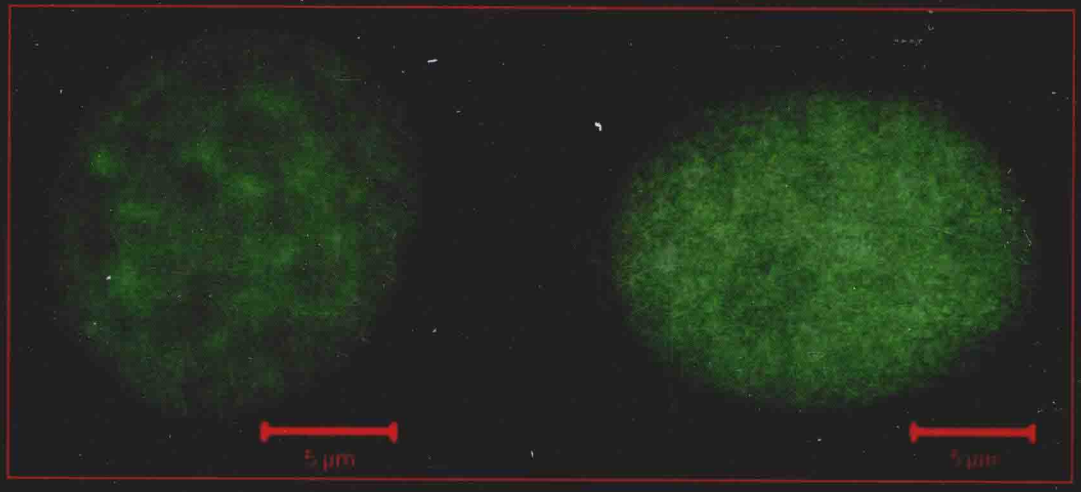
Nuclear Mechanics and Genome Regulation



Edited by

G.V. Shivashankar

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PREFACE

Biological cells are active mechanical systems sensing local microenvironments using specialized cell-surface receptors. Physicochemical signals from the extracellular matrix impinge on cellular geometry resulting in altered functional nuclear landscapes and gene function. These alterations regulate diverse biological processes including stem-cell differentiation, developmental genetic programs, and cellular homeostatic control systems. Although the cytoskeleton is a well-appreciated critical component of cellular morphology, emerging evidence suggests that it may also have important consequences for maintenance of nuclear architecture; its mechanical properties and genome function. Regulation of genetic programs in response to cellular geometric cues requires mechanisms that act at a distance. A number of signaling pathways are activated in response to mechanical signals converging on regulatory factors, which translocate to the nucleus via diffusive processes. Recent evidence also highlights the physical transmission of active stresses via cytoplasmic-nucleus connections to remodel chromatin assembly. The physicochemical signals that arrive at the nucleus have to be further sorted to appropriate regulatory sequences within the 3D architecture of the cell nucleus to effect changes in genome function. Although the location of regulatory sequences on the 1D DNA polymer is known from genome sequencing, its 3D location when folded into chromatin via histone and nonhistone proteins within the nucleus is largely unknown. In addition, a number of essential posttranslational modifications of histone proteins determine both specificity and accessibility to regulatory sequences on the genome.

We are just beginning to appreciate the impact of cellular geometry on nuclear mechanics and genome regulation. In addition, the mechanical integrity of the cell nucleus and nuclear mechanical signaling are found to profoundly influence cellular homeostatic controls: driving cells toward differentiation, proliferation, or apoptosis. Further, diseases such as cancer are conjectured to originate at a single-cell level in its local mechanical environment, within tissue contexts. The chapters in this book describe both methods and *advances* in our understanding of the spatio-temporal organization of genome assembly, its integration to mechanical properties of the cell nucleus and how mechanoregulation of gene function may be defined in interphase cells and during their differentiation and development. The last section discusses the growing number of diseases associated with altered nuclear organization. Clearly, understanding the mechanical aspects of the cell nucleus and how it impinges on genome function in living cells has become a central theme in modern cell and developmental biology and biophysics. With the advent of new methods and approaches, some of which are described in this book, there exists now a promising future in this emerging research frontier.

G.V. Shivashankar

CONTENTS

SECTION A

Cell Nucleus: Organization & MechanoBiology

CHAPTER 1

Fluorescence Fluctuation Microscopy to Reveal 3D Architecture and Function in the Cell Nucleus

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Abstract

The three-dimensional (3D) architecture of the cell nucleus is determined not only by the presence of subnuclear domains, such as the nuclear envelope, chromosome territories, and nuclear bodies, but also by smaller domains which form in response to specific functions, such as RNA transcription, DNA replication, and DNA repair. Since both stable and dynamic structures contribute to nuclear morphology, it is important to study the biophysical principles of the formation of macromolecular assemblies within the nucleus. For this purpose, a variety of fluorescence fluctuation microscopy techniques can be applied. Here, we summarize our current knowledge on the 3D architecture of the mammalian cell nucleus and describe in detail how the assembly of functional nuclear protein complexes can be analyzed in living cells using fluorescence bleaching techniques, fluorescence correlation spectroscopy, raster image correlation spectroscopy, and mathematical modeling. In conclusion, the application of all these techniques in combination is a powerful tool to assess the full spectrum of nuclear protein dynamics and to understand the biophysical principles underlying nuclear structure and function.

I. Introduction

A. Three-Dimensional Organization of the Cell Nucleus

The cell nucleus is responsible for the storage, propagation, maintenance, and expression of the genetic material it contains (Diekmann and Hemmerich, 2005). These duties are executed by biochemical activities, namely DNA compaction/decompaction, DNA replication and segregation, DNA repair, and RNA transcription/processing, respectively. The corresponding machineries are highly structured, yet dynamic macromolecular assemblies (Misteli, 2007) which must work on chromatin with high fidelity in a crowded nuclear environment (Richter *et al.*, 2007). In addition, the mammalian cell nucleus contains a variety of subnuclear domains, nuclear bodies, or subnuclear compartments (Fig. 1). DNA in the form of chromatin is easily visualized as individual chromosomes in mitotic cells. In the interphase cells, chromosomes decondense into so-called chromosome territories (CTs), which occupy distinct volume regions (Fig. 1) (Cremer *et al.*, 2006; Heard and Bickmore, 2007). Staining of interphase chromatin using DNA dyes does not reveal CT structures but allows the discrimination between transcriptionally active euchromatin and transcriptionally silent heterochromatin. Constitutive heterochromatin is mainly composed of pericentromeric DNA, and in this case, the chromosome's centromere/kinetochore complex can be found embedded within this chromatin region (Fig. 1) (Probst and Almouzni, 2008). The nucleus obtains structural support through the nuclear lamina, which is attached to the nuclear double membrane, together forming the nuclear envelope (Fig. 1) The nuclear envelope controls traffic of molecules between the cytoplasm and the nucleoplasm but has also emerged as a critical determinant in genome architecture (Starr, 2009). As a consequence of this important

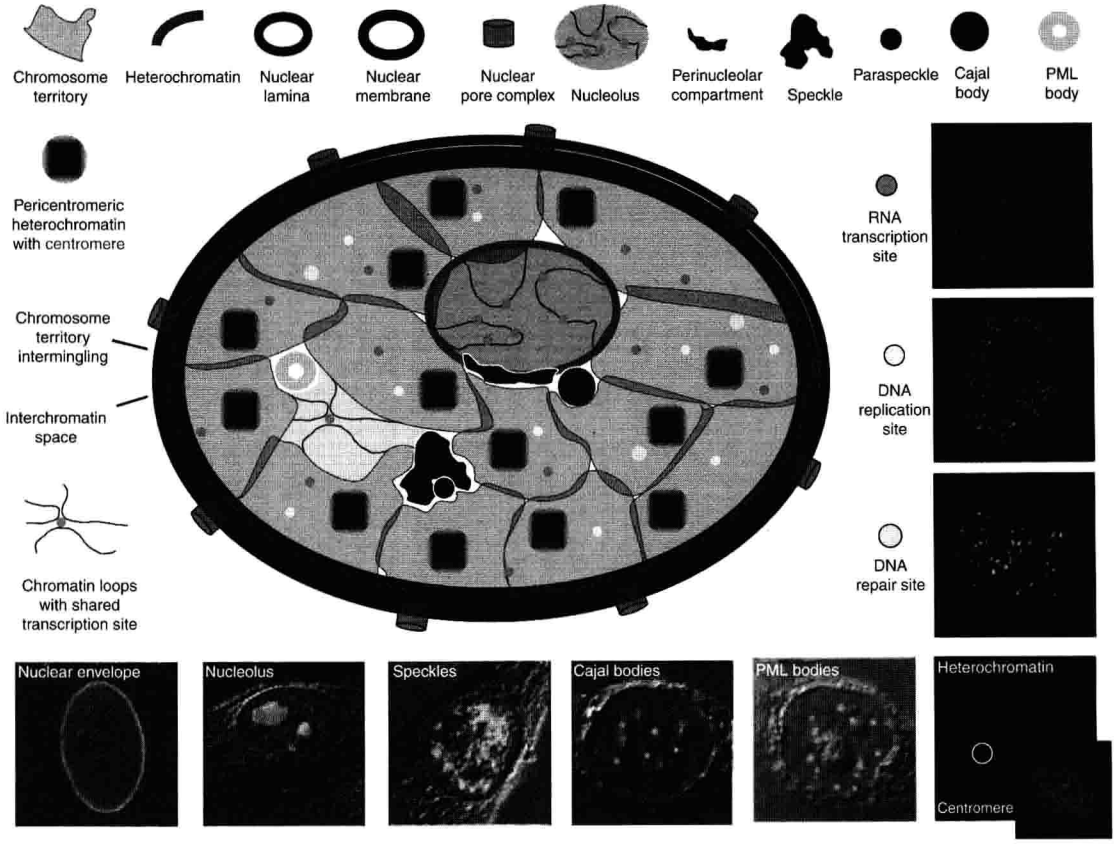

Fig. 1 Nuclear architecture.The mammalian cell nucleus contains chromatin in the form of chromosome territories (CTs). CTs may overlap at their touching borders (intermingling) or create the so-called interchromatin space (white). Constitutive heterochromatin (dark gray in the center cartoon, blue in the bottom right panel) is mainly found as pericentromeric chromatin in patches throughout the nuclear volume, at the nuclear periphery, as well as around nucleoli. Structural hallmarks in the periphery of the nucleus include nuclear pore complexes, the nuclear membrane (dark green), and the meshwork-like nuclear lamina. Chromatin loops with associated transcription factories may extrude out of CTs within the nucleolus as well as throughout the nucleoplasm. Transcription (orange), replication (yellow), and DNA repair processes (light blue) usually occur in small domains with a diameter below 100 nm. A diverse set of nuclear bodies, such as speckles, paraspeckles, perinucleolar compartment, Cajal bodies, or promyelocytic leukemia (PML) bodies can be visualized by confocal immunofluorescence analysis (bottom panels). (See Plate no. 1 in the Color Plate Section.)