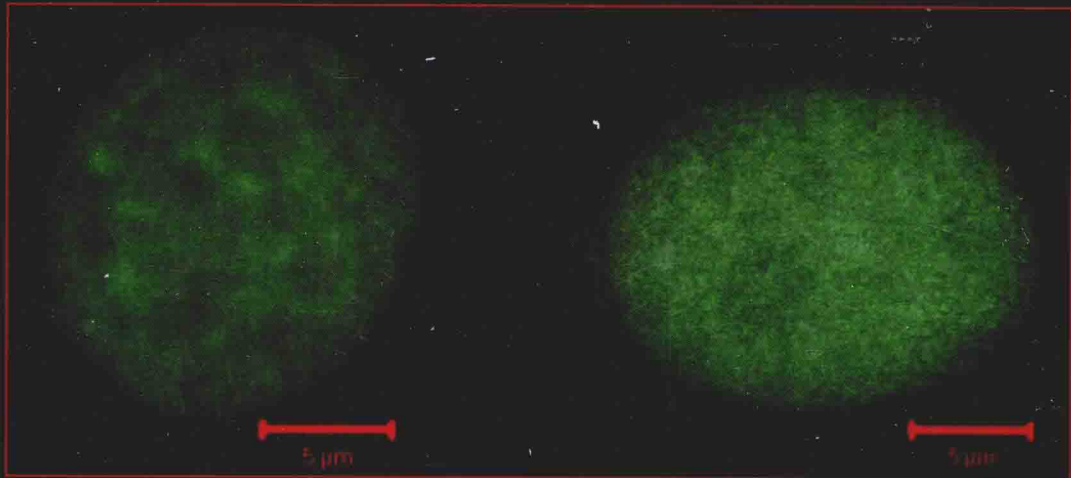

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NUCLEAR MECHANICS AND GENOME REGULATION



Edited by
G.V. Shivashankar



Methods in Cell Biology

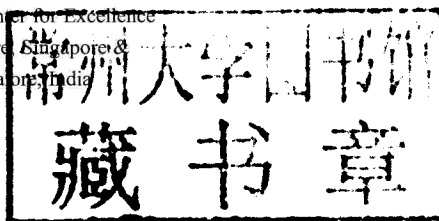
VOLUME 98

Nuclear Mechanics & Genome Regulation

Edited by

G.V. Shivashankar

Department of Biological Sciences and Research Center for Excellence
in MechanoBiology, National University of Singapore, Singapore &
National Center for Biological Sciences, TIFR-Bangalore, India



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CONTRIBUTORS

Numbers in parentheses indicate the pages on which the authors' contributions begin.

Padra Ahmadi, (295) Mechanics and Genetics of Embryonic and Tumoral Development group, UMR168 CNRS, Institut Curie, 11 rue Pierre et Marie Curie, F-75005, Paris, France

Soneela Ankam, (241) Division of Bioengineering, National University of Singapore, Singapore

Dipanjana Bhattacharya, (57) National Centre for Biological Sciences, Tata Institute of Fundamental Research, Bellary Road, Bangalore 560065, India

Lesley Y. Chan, (241) Division of Bioengineering, National University of Singapore, Singapore; NUS Graduate School of Integrative Science and Engineering, National University of Singapore, Singapore; and Bioprocessing Technology Institute, A*Star, Singapore

Matthew W. C. Chan, (179) Matrix Dynamics Group, Faculty of Dentistry, University of Toronto, Fitzgerald Building, Toronto, ON, Canada M5S 3E2

Kris Noel Dahl, (97) Department of Chemical Engineering and Department of Biomedical Engineering, Carnegie Mellon University, Pittsburgh, Pennsylvania 15213

Rumi De, (143) Indian institute of Science Education and Research, Kolkata, Mohanpur 741252, Nadia, West Bengal, India

Dennis E. Discher, (207) Biophysical Engineering Laboratory, School of Engineering and Applied Science, University of Pennsylvania, Philadelphia, Pennsylvania 19104

Emmanuel Farge, (295) Mechanics and Genetics of Embryonic and Tumoral Development group, UMR168 CNRS, Institut Curie, 11 rue Pierre et Marie Curie, F-75005, Paris, France

Maria-Elena Fernandez-Sanchez, (295) Mechanics and Genetics of Embryonic and Tumoral Development group, UMR168 CNRS, Institut Curie, 11 rue Pierre et Marie Curie, F-75005, Paris, France

Ying-Hui Fu, (337) Department of Neurology, University of California, San Francisco, San Francisco, California 94158 2324

Sanjeev Galande, (35) National Centre for Cell Science, Ganeshkhind, Pune 411007, India and Indian Institute of Science Education and Research, Pashan, Pune 411021, India

Soumya Gupta, (57) National Centre for Biological Sciences, Tata Institute of Fundamental Research, Bellary Road, Bangalore 560065, India and Department of Biological Sciences and Research Center for Excellence in MechanoBiology, National University of Singapore, Singapore 117543, Singapore

Takamasa Harada, (207) Biophysical Engineering Laboratory, School of Engineering and Applied Science, University of Pennsylvania, Philadelphia, Pennsylvania 19104

- Peter Hemmerich**, (3) Leibniz Institute of Age Research, Fritz Lipman Institute, Beutenbergstr. 11, 07745 Jena, Germany
- Boris Hinz**, (179) Matrix Dynamics Group, Faculty of Dentistry, University of Toronto, Fitzgerald Building, Toronto, ON, Canada M5S 3E2
- Irena Ivanovska**, (207) Biophysical Engineering Laboratory, School of Engineering and Applied Science, University of Pennsylvania, Philadelphia, Pennsylvania 19104
- K. Venkatesan Iyer**, (221) National Centre for Biological Sciences, Tata Institute of Fundamental Research, Bellary Road, Bangalore 560065, India and Department of Biological Sciences and Research Center for Excellence in MechanoBiology, National University of Singapore, Singapore 117543
- Ranveer S. Jayani**, (35) National Centre for Cell Science, Ganeshkhind, Pune 411007, India
- Benjamin Kim Kiat Teo**, (241) Division of Bioengineering, National University of Singapore, Singapore and Mechanobiology Institute Singapore, National University of Singapore, Singapore
- Karolin Klement**, (3) Leibniz Institute of Age Research, Fritz Lipman Institute, Beutenbergstr. 11, 07745 Jena, Germany
- Abhishek Kumar**, (221) National Centre for Biological Sciences, Tata Institute of Fundamental Research, Bellary Road, Bangalore 560065, India and Department of Biological Sciences and Research Center for Excellence in MechanoBiology, National University of Singapore, Singapore 117543
- Frank P.L. Lai**, (323) Institute of Medical Biology, Immunos, 8A Biomedical Grove, Biopolis, Singapore 138648
- Jan Lammerding**, (121) Department of Medicine, Cardiovascular Division, Brigham and Women's Hospital/Harvard Medical School, Boston, Massachusetts 02115
- Thorsten Lenser**, (3) Institute of Computer Science, Friedrich-Schiller-University, Ernst Abbe Platz 2, 07743 Jena, Germany and Carl Zeiss MicroImaging GmbH, Carl-Zeiss-Promenade 10, 07745 Jena, Germany
- Qingsen Li**, (79) Mechanobiology Institute, National University of Singapore, Singapore 117411, Singapore
- Maria Lucia Lombardi**, (121) Department of Medicine, Cardiovascular Division, Brigham and Women's Hospital/Harvard Medical School, Boston, Massachusetts 02115
- Shovamayee Maharana**, (57) National Centre for Biological Sciences, Tata Institute of Fundamental Research, Bellary Road, Bangalore 560065, India and Department of Biological Sciences and Research Center for Excellence in MechanoBiology, National University of Singapore, Singapore 117543, Singapore
- Aprotim Mazumder**, (221) National Centre for Biological Sciences, Tata Institute of Fundamental Research, Bellary Road, Bangalore 560065, India
- Christopher A. McCulloch**, (179) Matrix Dynamics Group, Faculty of Dentistry, University of Toronto, Fitzgerald Building, Toronto, ON, Canada M5S 3E2
- Radfidah A. Mutalif**, (323) Institute of Medical Biology, Immunos, 8A Biomedical Grove, Biopolis, Singapore 138648
- Quasar Saleem Padiath**, (337) Department of Neurology, University of California, San Francisco, San Francisco, California 94158 2324 and Department of Human

Genetics, University of Pittsburgh, Graduate School of Public Health, Pittsburgh, PA - 15261

J. David Pajerowski, (207) Biophysical Engineering Laboratory, School of Engineering and Applied Science, University of Pennsylvania, Philadelphia, Pennsylvania 19104

Siew Cheng Phua, (323) Institute of Medical Biology, Immunos, 8A Biomedical Grove, Biopolis, Singapore 138648

Nisha M. Ramdas, (221) National Centre for Biological Sciences, Tata Institute of Fundamental Research, Bellary Road, Bangalore 560065, India

Praveena L. Ramanujam, (35) National Centre for Cell Science, Ganeshkhind, Pune 411007, India

T. Roopa, (221) National Centre for Biological Sciences, Tata Institute of Fundamental Research, Bellary Road, Bangalore 560065, India

Samuel A. Safran, (143) Department of Materials and Interfaces, Weizmann Institute of Science, Rehovot, 76100, Israel

Fanny Serman, (295) Mechanics and Genetics of Embryonic and Tumoral Development group, UMR168 CNRS, Institut Curie, 11 rue Pierre et Marie Curie, F-75005, Paris, France

Bidisha Sinha, (57) National Centre for Biological Sciences, Tata Institute of Fundamental Research, Bellary Road, Bangalore 560065, India

Deepak Kumar Sinha, (57) National Centre for Biological Sciences, Tata Institute of Fundamental Research, Bellary Road, Bangalore 560065, India

G. V. Shivashankar, (57, 221) National Centre for Biological Sciences, Tata Institute of Fundamental Research, Bellary Road, Bangalore 560065, India and Department of Biological Sciences and Research Center for Excellence in MechanoBiology, National University of Singapore, Singapore 117543, Singapore

Colin L. Stewart, (323) Institute of Medical Biology, Immunos, 8A Biomedical Grove, Biopolis, Singapore 138648

Joe Swift, (207) Biophysical Engineering Laboratory, School of Engineering and Applied Science, University of Pennsylvania, Philadelphia, Pennsylvania 19104

Swee Jin Tan, (79) NUS Graduate School for Integrative Sciences and Engineering, National University of Singapore, Singapore 117456 and Division of Bioengineering and Department of Mechanical Engineering, National University of Singapore, Singapore 117576, Singapore

Shefali Talwar, (57) National Centre for Biological Sciences, Tata Institute of Fundamental Research, Bellary Road, Bangalore 560065, India and Department of Biological Sciences and Research Center for Excellence in MechanoBiology, National University of Singapore, Singapore 117543, Singapore

Chwee Teck Lim, (79) NUS Graduate School for Integrative Sciences and Engineering; Division of Bioengineering and Department of Mechanical Engineering; and MechanoBiology Institute, National University of Singapore, Singapore 117411, Singapore

Katherine L. Wilson, (97) Department of Cell Biology, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Klaus Weisshart, (3) Carl Zeiss MicroImaging GmbH, Carl-Zeiss-Promenade 10, 07745 Jena, Germany

Tobias Ulbricht, (3) Leibniz Institute of Age Research, Fritz Lipman Institute, Beutenbergstr. 11, 07745 Jena, Germany

Evelyn K.F. Yim, (241) Division of Bioengineering, National University of Singapore, Singapore; Mechanobiology Institute Singapore, National University of Singapore, Singapore; Department of Surgery, National University of Singapore, Singapore

Zhixia Zhong, (97) Department of Chemical Engineering, Carnegie Mellon University, Pittsburgh, Pennsylvania 15213

Assaf Zemel, (143) Institute of Dental Sciences, Faculty of Dental Medicine, and Fritz Haber Center for Molecular Dynamics, Hebrew University-Hadassah Medical Center, Jerusalem, 91120, Israel

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

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SECTION A

Cell Nucleus: Organization & MechanoBiology

CHAPTER 1

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

**Thorsten Lenser^{*,†}, Klaus Weisshart[†], Tobias Ulbricht[‡],
Karolin Klement[‡], and Peter Hemmerich[‡]**

^{*}Institute of Computer Science, Friedrich-Schiller-University, Ernst Abbe Platz 2, 07743 Jena, Germany

[†]Carl Zeiss MicroImaging GmbH, Carl-Zeiss-Promenade 10, 07745 Jena, Germany

[‡]Leibniz Institute of Age Research, Fritz Lipman Institute, Beutenbergstr. 11, 07745 Jena, Germany

Abstract

- I. Introduction
 - A. Three-Dimensional Organization of the Cell Nucleus
 - B. Assembly Mechanisms of Nuclear Structures
 - C. Fluorescence Fluctuation Microscopy Techniques
 - II. Rationale
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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 (Dickmann 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

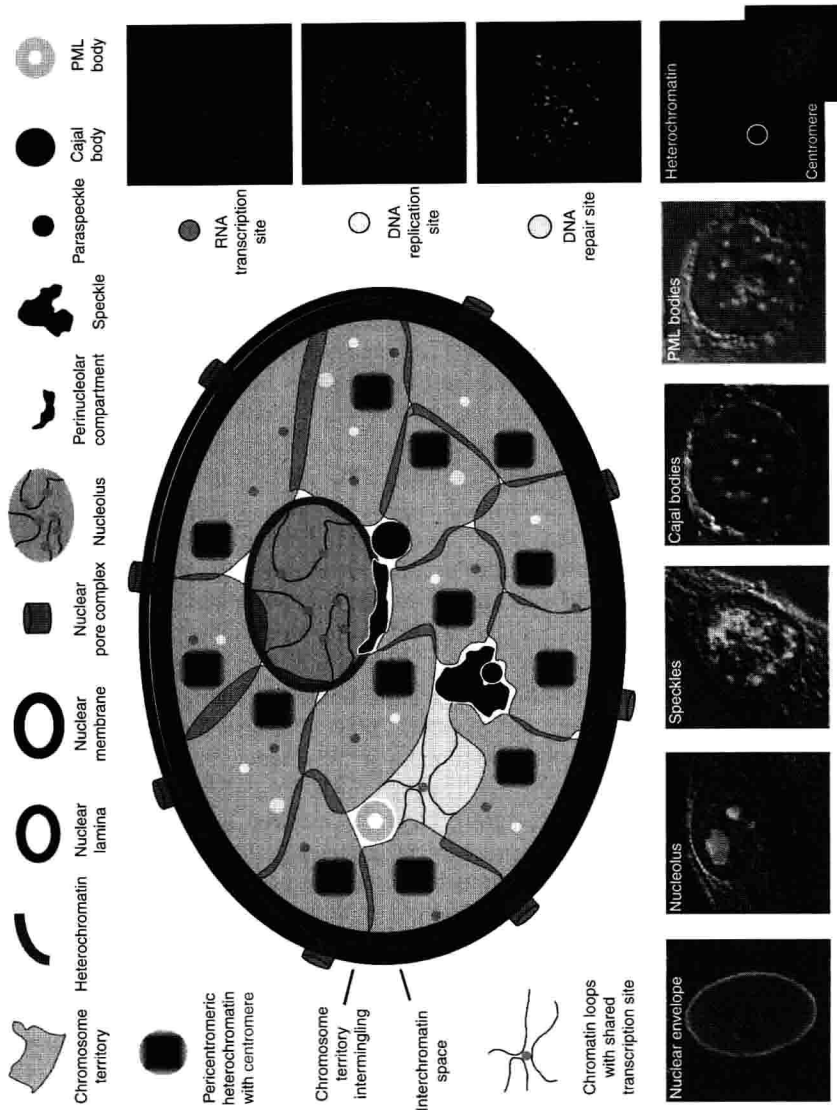


Fig. 1 Nuclear architecture. The mammalian cell nucleus contains chromatin in the form of chromosome territories (CTs). CTs may overlap at their touching borders (intermingling) or create the so-called interchromatin space (white). Constitutive heterochromatin (dark gray in the center cartoon, blue in 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, perinuclear 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.)