
Preface

The nucleolus is a prominent structural and functional compartment of the cell nucleus. The main function of the nucleolus is the transcription and processing of ribosomal RNA and the first steps of ribosome assembly; however, it is also involved in numerous other cellular activities, comprising stress response, RNA metabolism, and cell cycle regulation. This volume in the Methods in Molecular Biology series aims at providing an up-to-date compilation of current methodological approaches utilized for the exploration of nucleolar structure and function.

The book is divided into three parts: (I) Imaging of the Nucleolus, (II) Analysis of Ribosomal RNA Transcription and Processing, and (III) Genomics and Proteomics of the Nucleolus.

Part I brings together various techniques to visualize nucleolar structure and function in different model organisms. The methodologies range from immunofluorescence and fluorescence in situ hybridization approaches through correlative light and electron microscopy to live-cell imaging and use *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, *Spodoptera frugiperda*, *Danio rerio*, *Rattus norvegicus*, *Mus musculus*, and *Homo sapiens* cells as model systems.

Part II includes a collection of protocols, which were developed to analyze the basic task of the nucleolus, the synthesis of ribosomal RNA in *S. cerevisiae* and in mammals. Since the production of ribosomal proteins and several steps of the ribosome assembly take place outside of the nucleolus, this part is concerned exclusively with methods to analyze ribosomal RNA transcription and processing. The protocols describe in vitro transcription assays, purification of RNA polymerase I, furthermore quantitative analyses of ribosomal RNA synthesis and processing by in vivo labeling and northern blot techniques.

Part III focuses primarily on high-throughput wet laboratory and computational experimental approaches, which deliver essential data for the system-level understanding and integrative analysis of different nucleolar functions. The chapters cover the following topics: ribosomal DNA sequence assembly in eukaryotic genomes, investigation of ribosomal DNA methylation and fluorescence-activated nucleolus sorting in *Arabidopsis thaliana*, purification of RNA polymerase I-associated chromatin from *S. cerevisiae* for “omics” studies, genomics of ribosomal DNA chromatin, and RNomics and proteomics of the human nucleolus.

The diversity of the protocols mirrors the multifaceted biology of the nucleolus, and the book should provide biochemists, molecular biologists, cell biologists, and bioinformaticians with a reliable practical handbook to facilitate the investigation of this nuclear compartment at the advanced level.

I had the distinct honor to work on the compilation of this book with 43 authors, the Series Editor, Professor John M. Walker, and David Casey at Humana Press, Springer, and would like to express my gratitude for their great contribution.

Regensburg, Germany

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The Nucleolus

Methods and Protocols

Németh, A. (Ed.)

2016, XI, 279 p. 55 illus., 43 illus. in color., Hardcover

ISBN: 978-1-4939-3790-5

A product of Humana Press