Contents

Nuclear Organisation and Subnuclear Bodies
Melpomeni Platani and Angus I. Lamond

1 Introduction 1
2 Chromatin and Nuclear Compartments 3
3 The Nucleolus 4
4 Perinucleolar Compartment 7
5 Cajal Bodies, gems 8
6 Speckles – Nuclear Bodies Enriched in Splicing Factors 9
7 Promyelocytic Leukemia Bodies 12
8 Other Forms of Nuclear Bodies 13
9 Conclusion, Perspectives 14
References 15

Searching for Active Ribosomal Genes
Ivan Raška

1 Introduction 23
2 Historical Perspective 28
3 The Ups and Downs of Affinity Cytochemistry: the Case of the Nucleolus 29
4 Mapping of Active Ribosomal Genes 33
4.1 Electron Microscopy and Cytochemistry Approaches 35
4.2 Immunocytochemical Mapping of Nucleolar Chromatin Structures 37
4.3 In Situ Hybridization Mapping of rDNA 37
4.4 In Situ Hybridization Mapping of rRNA 38
4.5 Immunocytochemical and In Situ Hybridization Mapping of Nucleolar Proteins and Ribonucleoproteins 40
4.6 Mapping of the Nucleolar Transcription Signal 41
5 Conclusions 46
References 48

Toxic RNA in the Nucleus: Unstable Microsatellite Expression in Neuromuscular Disease
Keith R. Nykamp and Maurice S. Swanson

1 Overview 57

2 Unstable Microsatellites in Neurological and Neuromuscular Disease 58
2.1 Microsatellites: Definition and Potential Functions 58
2.2 Coding Region Expansions and Potential Effects on Transcriptional Regulation 58
2.3 Myotonic Dystrophy and RNA-Mediated Pathogenesis 61
3 RNA-Mediated Disease 63
3.1 Nuclear RNA Foci in Myotonic Dystrophy 63
3.2 $(CUG)_n$ RNA Structures and Gain-of-Function Models 65
3.3 Guilt by Association: Proteins that Bind CUG and CCUG Repeat Expansions 66
3.4 Cellular Functions Affected by $(CUG)_n$ Expansion 69
3.5 All Together Now: Loss of Myogenic Differentiation in Congenital DM1 Requires $(CUG)_n$ Expansion and the *DMPK* 3′-UTR 71
4 Unstable Microsatellites, Toxic RNA and Implications for Nuclear Structure and Function 72
References 73

Assembly and Traffic of Small Nuclear RNPs
Edouard Bertrand and Rémy Bordonné

1 Introduction 79
2 Biogenesis of Small Nuclear RNPs 79
2.1 U1, U2, U4 and U5 snRNPs 79
2.2 U6 snRNP 84
2.3 U7 snRNP 85
3 Biogenesis of Small Nucleolar RNPs (snoRNPs) 85
3.1 Principal Features and Functions of snoRNA Families 85
3.2 snoRNA Synthesis and Genomic Organization 87
3.3 Trafficking of snoRNA and snoRNP Proteins 88
4 Noncanonical Types of Box C/D and Box H/ACA RNAs 89
5 Similarities Between snRNA and snoRNA Biogenesis 90
6 snRNAs and snoRNAs: a Common Origin? 91
References 92

Intranuclear Pre-mRNA Trafficking in an Insect Model System
Eva Kiesler and Neus Visa

1 The Balbiani Ring Genes of *Chironomus tentans*: a Model System for Studying Gene Expression in Situ 99
1.1 The Active Balbiani Ring Genes 100
1.1.1 Processing of BR pre-mRNA 101
1.2 Proteins Associated with the BR (pre-)mRNA 102
1.2.1 DExD/H Box Proteins in the BR RNP Particle 104
2 The Movement of the (pre)mRNP Particles from the Gene to the NPC 105

2.1 Three-Dimensional-Analysis of Nucleoplasmic BR RNP Particles by Electron Tomography 106
2.2 Protein Components of CFs and FGCs 107
2.2.1 Hrp65 and Other DBHS Proteins 108
2.2.2 The hrp65 Isoforms: Specialized Roles in Gene Expression? . . . 109
2.3 Intranuclear Movement of (pre-)mRNPs 109
2.4 Nuclear Retention of mRNA 111
2.5 CFs and FGCs in Mammalian Cells? 112
3 Conclusions 113
References 114

Photobleaching Microscopy Reveals the Dynamics of mRNA-Binding Proteins Inside Live Cell Nuclei
José Braga, José Rino and Maria Carmo-Fonseca

1 Introduction 119
2 General Concepts of Quantitative FRAP 120
3 FRAP Reveals the Dynamics of GFP-PABP2 and GFP-TAP in the Living Cell Nucleus 124
4 Estimating the Fraction of GFP Fusion Proteins Bound to mRNP Complexes by FLIP 127
5 Simulation of mRNP Mobility Within the Nucleus Using a Random Walk Model 129
6 Concluding Remarks 132
References 133

Imaging of Single mRNAs in the Cytoplasm of Living Cells
Dahlene Fusco, Edouard Bertrand and Robert H. Singer

1 Rationale for Live Cell Imaging of mRNA 135
2 Existing Technologies for the Visualization of RNA in Living Cells 137
2.1 Microinjection of Fluorescent RNA 137
2.2 Fluorescent Oligonucleotide Probes (FIVH-Fluorescent In Vivo Hybridization) 138
2.3 Cell Permeant Dyes 139
2.4 RNA Tagged with Sequence-Specific GFP Fusion Proteins 139
3 Analysis of Single RNA Dynamics in Living Cells 140
4 Time-Lapse Microscopy/Particle Tracking 141
5 In Vivo mRNA Analyses in the Cytoplasm 142
6 Conclusion 147
References 147

Subject Index 151



http://www.springer.com/978-3-540-74265-4

RNA Trafficking and Nuclear Structure Dynamics
Jeanteur, P. (Ed.)
2004, IX, 153 p., Softcover
ISBN: 978-3-540-74265-4