Preface

Bidirectional transport between the cytoplasm and the nucleoplasm is a finely tuned process, whose regulation is critical for the proper function of numerous biological pathways. Exchanges between these two compartments take place through nuclear pore complexes (NPCs), huge macromolecular assemblies embedded within the nuclear envelope (NE). Since the unearthing of this research field, more than 50 years ago, the combination of a large variety of approaches, performed in a broad range of model organisms, has permitted the deciphering of the main features of NPCs, their assembly and dynamics, as well as the precise rules governing and regulating nucleocytoplasmic exchanges. Although this field of research is extremely dynamic and has both contributed to and benefited from a wealth of innovating methods over recent years, no dedicated volume had been devoted to this topic since Elsevier published the *Methods* volume "Nuclear cytoplasmic transport and nuclear pore complexes" in 2006 (Volume 39(4): 275–380, ed. B.M.A. Fontoura). As this topic impinges on multiple fields, scattered protocols could however be found in volumes devoted to nuclear architecture, gene expression, small GTPases, or specific model systems. It was thus timely to set up a dedicated volume, and I am grateful to Phong Tran, senior editor of this series, for stimulating me to assemble this book, and to the many leaders in the field who have devoted some of their precious time to contribute to specific chapters.

Following the introduction, that presents an overview of the history and of the main tools and rules governing nuclear transport, the 21 chapters in this volume provide protocols developed in the most widespread model systems in the field, namely mammalian cells in culture (Chapters 2, 6, 10–12, 15, 16, 18, 19, and 21), yeast *Saccharomyces cerevisiae* (Chapters 3, 5, 7, 14, and 19–21), *Xenopus laevis* oocytes and eggs extracts (Chapters 2, 4, 8, 9, and 18), and *Caenorhabditis elegans* (Chapter 13). The first chapters of this volume mainly focus on methods that have enabled impressive progress in our understanding of NPC structure and dynamics. These include the visualization of the NPC architecture and the localization of its constituents (the nucleoporins, Nups) that have largely benefited from transmission and scanning electron microscopy approaches (Chapters 2–4 and 13) and more recently from super-resolution imaging (Chapter 10), and recent protocols designed to study large Nups or NPC subcomplexes by negative staining (Chapter 5), to allow the precise determination of Nup stoichiometry (based on NE purifications and targeted proteomics, Chapter 6), or to follow the *in vivo* assembly of newly synthesized proteins within complexes (Chapter 7). While such approaches have contributed to refine our view of NPC organization, they no doubt could be usefully applied to improve our understanding of any other complex macromolecular assemblies. NPCs are dynamic structures whose disassembly and reassembly upon open mitosis (Chapters 10 and 13) or *de novo* assembly during interphase (Chapter 11) can be monitored in a quantitative manner using live cell imaging. In addition, *in vitro* assays based on nuclei assembled using cell-free extracts of Xenopus eggs (Chapters 2, 8, and 9) or on

semi-permeabilized mammalian cells (Chapters 12 and 15) have been adapted and refined to decipher the mechanisms that underlie these dynamic changes. To assess the critical function of nuclear pores in bidirectional transport of macromolecules, specific reporters and methods have been designed in each model organism to measure nuclear permeability (Chapters 10, 12, and 13), protein import or export (Chapters 8, 9, 13, and 14), or the fate of the various classes of RNAs that are assembled into ribonucleoparticles (Chapters 18–20). With respect to nuclear protein transport, this volume includes recent adaptations to the well-established *in vitro* assay that relies on digitonin-permeabilized cells (Chapter 15), quantitative mass-spectrometry approaches to identify specific import or export substrates (Chapter 16), and an example of nanodevices that mimic functional NPCs (Chapter 17). Dedicated tools to follow and characterize RNA transport mechanisms are provided in Chapter 18 (that details microinjections in Xenopus oocytes and FISH studies in vertebrates), Chapter 19 (dedicated to tRNA dynamics), and Chapter 20 (ribosome assembly and transport). While not covered by specific chapters, all these tools can be adapted to study nucleocytoplasmic trafficking of a wealth of other macromolecular assemblies, notably viral particles. Finally, the nuclear transport machinery further plays a critical role in multiple cellular processes, including cell cycle regulations (see for instance Chapters 14 and 15), maintenance of genetic integrity, and gene expression. For the latter topic, protocols developed to analyze gene positioning and to evaluate the association of Nups with transcribed genes are detailed in Chapter 21 (and referred to in Chapter 13).

Twenty one chapters cannot entirely cover the multiplicity of model systems and approaches so far used in this ever-expanding field, and I apologize to those whose protocols could not be included in this volume. However, all authors have clearly made considerable efforts to provide, in addition to step-by-step protocols that should enable scientists to reproduce their preferred methods, critical references to related or alternative techniques that have been developed to tackle similar questions. Other currently arising model organisms in the field (such as flies, plants, fungi, ciliates, or protozoans, to cite but a few) are unfortunately not included in this volume, and will clearly deserve specific chapters in the future.

I hope that all readers, junior scientists joining the nuclear transport field or already acquainted with it as well as outsiders of other fields, will appreciate, as much as I did, going through these carefully detailed methods chapters. I am particularly grateful to all authors for their commitment to this collective project. I would also like to thank the editors at Elsevier, Sarah Lay and Zoe Kruze, for their efficient help during this process, and all my collaborators at the Institut Jacques Monod for their constant support and understanding over these last busy months.

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