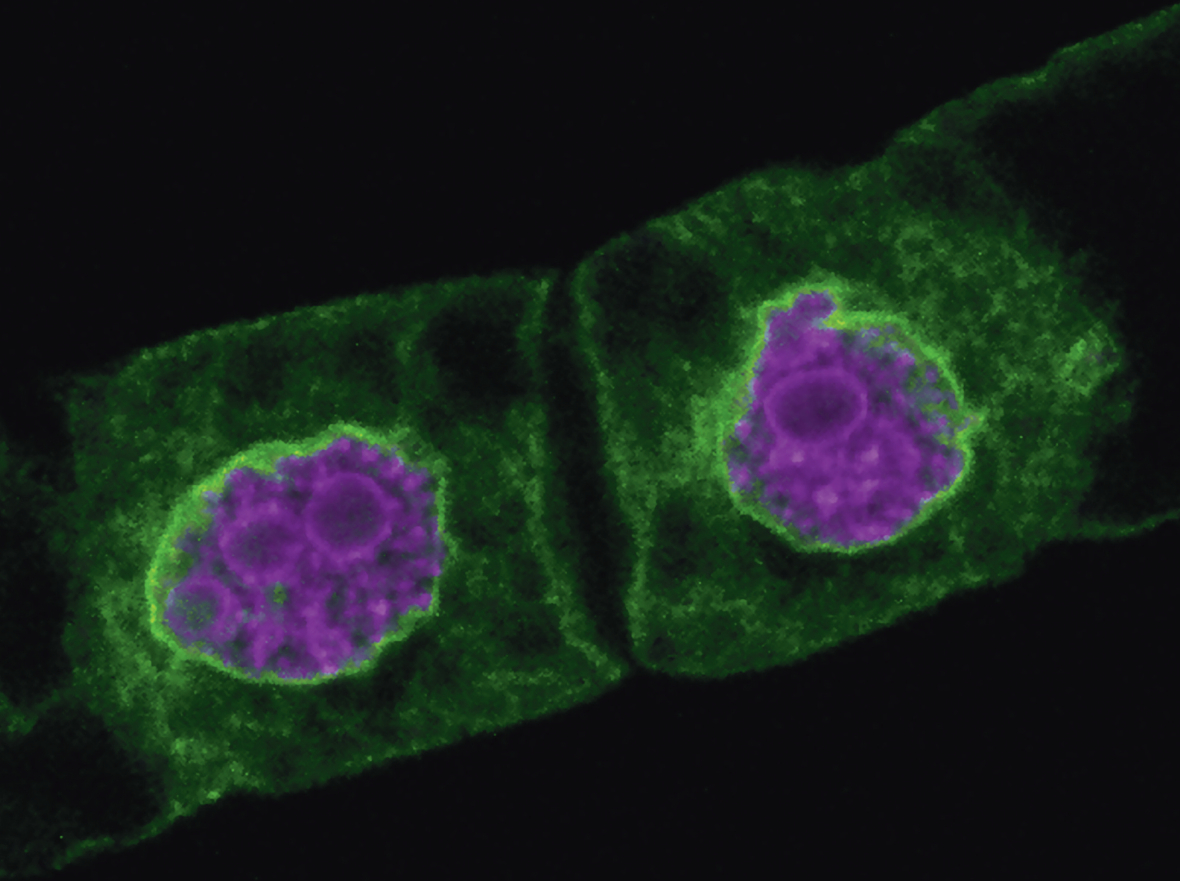


Annual Plant Reviews, Volume 46
Plant Nuclear Structure,
Genome Architecture and Gene Regulation



Edited by David E. Evans, Katja Graumann and John A. Bryant



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Plant Nuclear Structure, Genome Architecture and Gene Regulation

Edited by

David E. Evans

*Department of Biological and Medical Sciences
Oxford Brookes University, Oxford, UK*

Katja Graumann

*Department of Biological and Medical Sciences
Oxford Brookes University, Oxford, UK*

John A. Bryant

Biosciences, University of Exeter, Exeter, UK

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Editorial offices: 9600 Garsington Road, Oxford, OX4 2DQ, UK
The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, UK
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LIST OF CONTRIBUTORS

Susan J. Armstrong

School of Biosciences
University of Birmingham
B15 2TT
UK

Andreas Binder

Faculty of Biology, Genetics, University of Munich (LMU)
Großhaderner Straße 4
82152 Martinsried
Germany

Joanna Boruc

Department of Molecular Genetics
The Ohio State University
Columbus
OH 43210
USA

John A. Bryant

Biosciences
University of Exeter
Exeter
EX4 4QD, UK

David E. Evans

Department of Biological and Medical Sciences
Faculty of Health and Life Sciences
Oxford Brookes University
Headington Campus
Oxford
OX3 0BP
UK

F. Chris H. Franklin

School of Biosciences
University of Birmingham
B15 2TT
UK

Martin W. Goldberg

Department of Biological and Biomedical Sciences
University of Durham
Durham
DH1 3LE
UK

Katja Graumann

Department of Biological and Medical Sciences
Faculty of Health and Life Sciences
Oxford Brookes University
Headington Campus
Oxford
OX3 0BP
UK

Iris Meier

Department of Molecular Genetics
The Ohio State University
Columbus
OH 43210
USA

Eugenio Sanchez-Moran

School of Biosciences
University of Birmingham
B15 2TT
UK

Kim Osman

School of Biosciences
University of Birmingham
B15 2TT
UK

Martin Parniske

Faculty of Biology, Genetics
University of Munich (LMU)
Großhaderner Straße 4
82152 Martinsried
Germany

Monica Pradillo

Departamento de Genética
Facultad de Biología
Universidad Complutense
Madrid
28040 Spain

Nicola Y. Roberts

School of Biosciences
University of Birmingham
B15 2TT
UK

Juan L. Santos

Departamento de Genética
Facultad de Biología
Universidad Complutense
Madrid
28040 Spain

Christophe Tatout

GReD Laboratory
UMR CNRS 6293
INSERM U1103
University Blaise Pascal
Aubière
France

Mélanie Thomas

GReD Laboratory
UMR CNRS 6293
INSERM U1103
University Blaise Pascal
Aubière
France

Emmanuel Vanrobays

GReD Laboratory
UMR CNRS 6293
INSERM U1103
University Blaise Pascal
Aubière
France

Javier Varas

Departamento de Genética
Facultad de Biología
Universidad Complutense
Madrid
28040 Spain

Xiao Zhou

Department of Molecular Genetics
The Ohio State University
Columbus
OH 43210
USA

PREFACE

This volume was conceived to bring together reviews describing recent advances in knowledge and understanding of plant nuclear structures and functions, including that of the nuclear envelope. The book is particularly timely in that recent progress has been rapid in key areas including description and characterization of proteins of the nuclear envelope and nuclear pore complex, novel insights into nucleoskeletal structures, as well as developments related to chromatin organization, function and gene expression. Together these advances provide a framework for comparative understanding of nuclear envelope structure and function in a range of organisms and for understanding its evolution.

Current knowledge of the dynamic structure of plant DNA and chromatin is discussed by Sanchez-Moran in Chapter 5. Despite intensive study of histones and other chromosome-associated proteins, interactions to achieve the complex structures required both in interphase and during cell division remain poorly understood. The structures require several levels of organization, the first being the nucleosomal fibre comprising DNA wrapped around a core of histones. This is a dynamic structure and mechanisms for its remodelling are described. The nucleosomal fibre is then wound into a structure termed the chromatin fibre, which is arranged in loops associated with a multi-protein chromosome scaffold, the third level of structure. This interphase structure undergoes rapid dynamic change in mitosis with further condensation for replication and division. The importance of the structural organization of chromatin for processes such as transcription, replication, repair, recombination, condensation and segregation is also discussed. As in metazoans, plant chromatin is organized into regions of hetero- and euchromatin with heterochromatin adjacent to the NE.

Recent advances in understanding heterochromatin structure are presented in Chapter 6 by Vanrobays *et al.* Heterochromatin, originally thought to be condensed, gene-poor and 'silent', is now known often to be preferentially localized to the nuclear envelope and nucleolus and its significance is becoming clear as an epigenetic state required for many functions of the genome, including gene regulation, segregation of chromosomes and maintaining stability of the genome. Despite limited knowledge of it, in most species heterochromatin is the main form of chromatin and key questions remain to be answered. How is spatial organization of heterochromatin maintained through the cell cycle as DNA is replicated, chromatin condensed, and the nuclear envelope disrupted and reformed? Interactions between nuclear

envelope, nucleoskeleton and chromatin are likely to be very significant and are discussed together with other theories for heterochromatin positioning.

Plant genomes vary greatly in size but in all cases the genome is contained within a very small compartment and it is clear that complex three-dimensional organization is needed in order for the many processes required for function. This three-dimensional structure requires interactions between chromatin, the envelope, the nuclear pores and the rest of the cell. In Chapter 4, Goldberg discusses from an ultrastructural and biochemical perspective the presence of an equivalent of the highly ordered lamina and nucleoskeleton described in metazoans. Such a structure appears to be required for nuclear function, but until recently its protein composition has eluded plant scientists. Plant cells have no proteins homologous to the lamins or other intermediate filament protein. Recent electron microscope studies in Goldberg's laboratory of the inner face of the plant nuclear envelope reveal a filamentous structure interconnecting the NPCs. This appears to be organized similarly to the lamina of *Xenopus* oocytes. Protein candidates for a plant nucleoskeleton have recently been suggested from a number of approaches; these long coiled-coil nuclear-localized proteins show some similarities to nucleoskeletal proteins of the metazoans and Goldberg presents the growing, but as yet incomplete, evidence for their role. The likely (direct or indirect) interactions of these proteins with the proteins of the nuclear envelope via a 'Linker of Nucleoskeleton and Cytoskeleton' complex is also considered in Chapter 2 by Graumann and Evans. Therein, the authors describe that, in common with metazoans, plants have one key family of proteins that in other kingdoms constitutes the inner nuclear envelope component of this bridging complex, namely the Sad1/Unc84 (SUN)-domain protein family. Absence of a variety of other inner nuclear envelope components involved in nuclear envelope-chromatin interactions in other kingdoms suggests that the SUN- domain proteins play a particularly significant and broader role in plants. In many respects however, the higher plant SUN-domain proteins show remarkable conservation in structure to those of other organisms. They are smaller than their metazoan counterparts, being closest in size to the yeast homologue Sad1. In addition, the authors discuss first evidence of proteins interacting with SUN-domain proteins in plants that show similarity in structure and mechanism to the Klarsicht/Anc-1/Syne Homology (KASH)-domain proteins of other kingdoms, which complete the nucleo-cytoskeletal bridging complexes. Chapter 2 also focuses on other protein components of the plant nuclear envelope as well as its lipid composition and highlights many of the cellular and nuclear processes in which the plant nuclear envelope plays key roles.

Structure and position of chromosomes must be achieved both for successful mitosis and meiosis. Evidence for SUN- domain protein involvement in the breakdown and reformation of the nuclear envelope in plant mitosis is presented in Chapter 2 together with suggestions of conserved mechanisms between kingdoms. Meiosis, while more complex, has received considerable attention and the role of telomeres is presented by Roberts *et al.* in Chapter 7,

who reveal emerging evidence for their role in early events in the movement and synapsis of homologous chromosomes. Studies in *Arabidopsis* suggest that paired telomeres loosely cluster at the nuclear periphery in meiotic prophase 1; it is suggested that this facilitates chromosome alignment and synapsis. The proteins involved in the attachment of telomeres to the nuclear envelope remain elusive; however, in common with the yeast and metazoans, a role for SUN-domain proteins is suggested. Exploration of the structural protein interactions in meiosis is being vigorously pursued.

Recent characterization of proteins of the plant nuclear pore complex (NPC) has revealed that the structure more closely resembles those of vertebrates than yeast or fungi. In Chapter 3, Zhou *et al.* describe the significant progress made recently in identifying 30 constituent proteins of the plant NPC as well as characterizing plant NPC structure. While the overall architecture of NPCs is conserved in eukaryotes, the plant NPC are set apart by several unique features and absence of a number of vertebrate nucleoporins. Significantly, the anchorage of RanGAP, involved in the generation of the RanGTP/GDP gradient required for nuclear import and export (a mechanism conserved between kingdoms) has been shown to differ significantly between plants and other organisms. In mammals, for instance, RanGAP is anchored to the pore complex by sumoylation. In plants, this function is taken over by interaction with proteins associated with the nuclear pore complex termed the WPP (tryptophan proline proline) interacting proteins (WIPs) and WPP interacting tail-anchored proteins (WITs). Apart from structural differences, the authors also discuss plant-specific functions and non-trafficking processes that plant nucleoporins are involved in, including mitotic functions, plant development, hormone and abiotic stress responses and plant-microbe interactions. The latter topic is the primary focus of Chapter 8 by Binder and Parniske. Using *Lotus japonicus* as a model system, loss of function mutants of several nucleoporins result in impaired mycorrhizal association as well as root-nodule symbiosis linked to failure of nuclear calcium signalling. In *Arabidopsis thaliana*, nucleoporins have been shown to be required for the two major forms of response to fungal pathogens, namely pathogen-associated molecular pattern (PAMP)-triggered and disease resistance (R) gene-mediated defence signalling. This is presented in the context of expanding knowledge of the nuclear pore complex and other proteins of the nuclear envelope and suggests important targets for attention in relation to the introduction of nitrogen fixation into cereals and in the development of crops showing enhanced resistance to fungi. The authors also focus on the challenges of correlating specific functions with individual nucleoporins due to the complexity of interactions and functions of NPC components and functional redundancies.

It is evident that exploration of plant nuclear structure, genome architecture and gene regulation has widespread implications for crop improvement and food security. Movement of the nucleus occurring as stress and developmental responses are presented in Chapter 2 by Graumann and Evans and include movement in intense light, due to touch and viral and fungal

infection. Such movements are likely to be significant in plant tolerance to stress and infection and to involve nucleo-cytoskeletal bridging complexes at the nuclear envelope. The positional effects of chromatin structure and the structure of the nucleus on gene expression, discussed in Chapter 6 by Vanrobays *et al.*, suggest an area with considerable potential for exploration as tools to study gene targeting to subnuclear localizations become available. It has yet to be established whether localization to the nuclear periphery, pore complex or other regions of the nucleus induces a repressive or activation effect in respect to gene expression. Such effects, if reproducible, have considerable potential for development. Perhaps the most comprehensively studied role for the plant nuclear structures with widespread significance concerns the role of the nuclear pore complex in fungal pathogenesis and symbiosis.

There is a very clear need to expand knowledge of protein interaction networks at the nuclear envelope involving cytoskeleton, nucleoskeleton and chromatin components. Study of the nuclear envelope proteome has been held back by a combination of limited interest by researchers and the technical difficulties of isolating and analysing it. Recent advances – the identification of SUN domain proteins and first evidence for a linker of nucleoskeleton and cytoskeleton complex, the characterization of more than 30 nucleoporins and increasing functional evidence and the tentative characterization of a plant lamina – all provide a framework for rapid advances coupled with increased understanding of chromatin structure and function. Given the outstanding importance of the nucleus and of epigenetic factors, we anticipate that the study of plant nuclear structure, genome architecture and gene regulation will play a very significant role in the near future.

As knowledge and understanding of the structure and properties of the nucleus and nuclear envelope expand, we come tantalizingly closer to understanding the origins of the structures of the eukaryotic cell. John Bryant (Chapter 1) uses the information presented together with knowledge of replication of nuclear DNA and the import of the replication proteins to present and develop current theories of the origins of the nucleus and its envelope. The early presence of the nucleus and nucleoskeleton, predating the arrival of chloroplasts and mitochondria in the proto-eukaryotic cell and the probable formation of the nuclear envelope from invaginations of the plasma membrane are discussed in the light of the development of key features of the higher plant nucleus. Just as we hope that presenting advances in understanding the structure and function of the plant nucleus will stimulate research in this field, it is equally our hope these advances will result in better appreciation of their origins not only in plants but across the orders of living things.

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Chapter 1

INTRODUCTION: MYSTERIES, MOLECULES AND MECHANISMS

John A. Bryant

Biosciences, University of Exeter, Exeter, UK

Abstract: This brief chapter mentions the main structural and functional features of plant nuclei and in doing so, provides a very general introduction to other chapters in the book. It also covers aspects that are not featured elsewhere, especially the replication of nuclear DNA and the import of the replication proteins. Throughout the chapter there is an underlying theme of evolution, relating both to the similarities to and differences from the Archaea and to the possible evolutionary origins of the nucleus.

Keywords: Archaea; DNA replication; evolution; nuclear envelope; nuclear localization signal; origin; protein import

1.1 Darwin and Margulis revisited

In a famous letter sent in July 1879 to Joseph Hooker, the Director of Kew Gardens, Charles Darwin described the origin of the flowering plants as ‘an abominable mystery’. Over 130 years later, the mystery seems to be solved, if not in detail, at least in general terms. It is now thought that flowering plants diverged from a lineage of seed ferns (now a totally extinct group) in the late Jurassic or early Cretaceous period (Doyle, 2006, 2008). Based on extensive phylogenetic analysis, the living plant that most resembles the earliest angiosperms (i.e. which is at the base of the angiosperm phylogenetic tree) is *Amborella trichopoda*, a semi-climbing shrub only found in the rain forests of New Caledonia. So, while a solution to that mystery has been found, a further, and perhaps more fundamental mystery remains. It is a

mystery that involves not just flowering plants but all eukaryotes and at the beginning of the 21st century it is still not completely solved. That mystery is the origin of the nucleus, the organelle that is the subject of this book. As is evident in subsequent chapters, we have extensive knowledge of its structure and activities. It is a truly beautiful organelle – one that induces in many of us a sense of wonder. However, we are not at all sure where it came from although, as will become clear later in the chapter, a few hypotheses are beginning to emerge as front runners.

On the quest to solve the puzzle, one factor to consider is the origin of eukaryotes. It is now accepted that the two other major membrane-bound organelles, mitochondria and chloroplasts, have evolved from bacterial symbionts that invaded or were engulfed by what we could call proto-eukaryotes (as originally proposed by Margulis, 1971a, b, 1981). This idea has been extensively confirmed by genomic and proteomic studies, which also suggest strongly that those proto-eukaryotic host cells were derived from the Archaea and, in terms of energy metabolism, were using a form of glycolysis¹. Further, it is clear that following the endosymbiotic events, transfer of genes from both the non-photosynthetic (i.e. mitochondrial) and the photosynthetic (chloroplastic) endosymbionts to the host's genome occurred on a large scale. Indeed, that the process is still going on (Huang *et al.*, 2004, Rousseau-Gueutin *et al.*, 2011, Wang *et al.*, 2012). But where, and in what state were the genomes of those proto-eukaryotic host organisms?

It was thought for several years that relevant information could be obtained by study of amitochondrial eukaryotes, eukaryotes presumed to date back to before the first endosymbiotic event. However, it is now known that these are secondarily amitochondrial, as revealed by the presence of endosymbiont-derived genes in the nucleus and the vestiges of a mitochondrion (e.g. van der Giezen and Tozar, 2005; Minge *et al.*, 2009). So, these cells cannot tell us what the proto-eukaryote looked like. Nevertheless, it is clear that in more recent instances of gene transfer (as mentioned above), the organelle gene has been integrated into a typical eukaryotic nuclear genome located in a typical eukaryotic nucleus. These structures are no hindrance to gene transfer. Further, the use of bioinformatics coupled with comparative cell physiology and biochemistry in attempts to 'root' the eukaryotic phylogenetic tree all lead to the conclusion that most of the approximately 60 differences between eukaryotes and prokaryotes were developed or developing before the first symbiotic event, the acquisition of mitochondria (de Duve, 2007; Margulis *et al.*, 2007; Cavalier-Smith, 2009).

The eukaryotic features possessed by the proto-eukaryotes are thought to have included the possession of a nucleus, nucleoskeleton and cytoskeleton (Margulis *et al.*, 2007; de Duve, 2007; Cavalier-Smith, 2009). Looking at the

¹But note that in modern Archaea there are several variants of the 'conventional' glycolysis pathway (Sato and Atomi, 2011).

first two of these, these data do not provide any clear clues about where the nucleus came from and there are also questions about the nature of the nucleoskeleton in the earliest eukaryotes. Focussing specifically on this problem, we note that after the first symbiotic event (acquisition of mitochondria), the eukaryotic lineage split into two major branches (Cavalier-Smith, 2002), the unikonts (with one flagellum) that gave rise to, amongst other things, fungi and Metazoa, and the bikonts (with two flagella), one lineage of which became plants by the acquisition of chloroplasts (as mentioned above; see also Keeling, 2010).

Turning now to look at extant lineages, as is shown in Chapters 2 and 4, part of the nucleoskeleton in animals is the prominent lamina, consisting mainly of proteins known as lamins. However, plants lack lamins but do possess a lamina-like structure that has been called the 'plamina' (Fiserova *et al.*, 2009), consisting of plant-specific proteins that are functional analogues of lamins. Finally, in fungi, at least as represented by yeasts, the nucleoskeleton does not have any form of lamina. So, based on the origins of these groups, it is suggested that the proto-eukaryotic nucleoskeleton lacked a lamina and that this has developed subsequent to the uni-kont/bikont split. This gives us a little more information on the early nucleus, but the question of its origin remains.

At this point further specific discussion of the origin of nucleus is deferred to the end of the chapter, although it will appear more indirectly from time to time in the next three sections. Attention is now turned to the genome itself. Particular focus will be placed on the general structure of the genome, on its replication and on the implications for the latter process of enclosing the genome in an organelle.

1.2 Nuclei – general features

In plant cells that are not extensively vacuolated, the nucleus is the largest and usually the most obvious organelle. Even in mature cells with large vacuoles, the nucleus is usually clearly visible within the cytoplasm. It is the organelle that contains the bulk of the cell's DNA, the nuclear genome. Indeed, chromatin (Chapters 5 and 6), consisting mainly of a complex of DNA and proteins, is usually the most obvious component of the nucleus. The chromatin is attached *via* scaffold- or matrix-associated regions (SARs/MARs) to the nuclear matrix/scaffold/nucleoskeleton (Chapters 4 to 6). Within chromatin, the highly repeated genes encoding the major ribosomal RNAs (rRNAs) are looped out in structures called nucleoli. The fibrillar centres of the nucleoli are the sites of transcription of these genes and the transcripts are processed in the outer regions of the nucleoli.

The nucleus is bounded by the nuclear envelope or NE (Chapters 2 and 3), which consists, in effect, of three membranous components (shown diagrammatically in the cartoon in Figure 1.1). Firstly, the outer envelope is connected

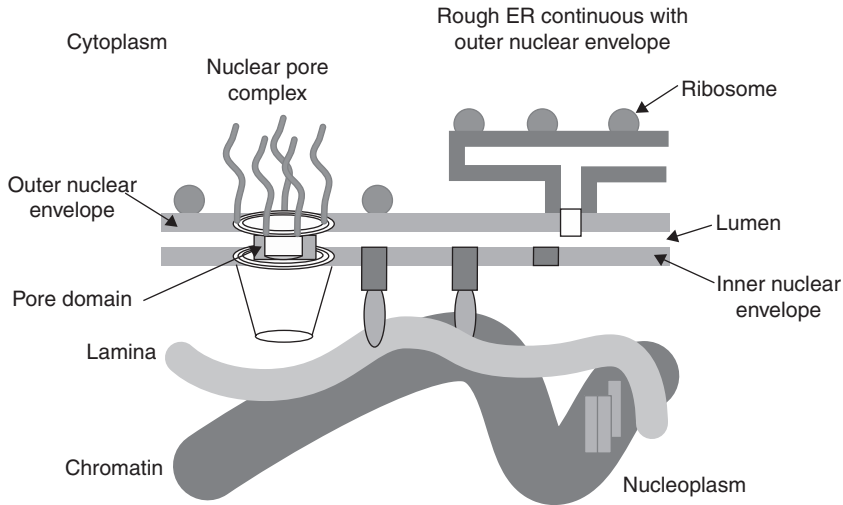


Figure 1.1 Diagrammatic cartoon of the nuclear envelope and nuclear pore complex. (From Evans *et al.*, 2004.) Reproduced by permission of the Society for Experimental Biology.

to the ER and the lipids and proteins of the outer NE are similar to those of the rough ER. Further, as with the rough ER, ribosomes are often present on the outer NE. So, the outer NE may be a site of protein synthesis and is certainly a part of the cell's endomembrane system. Secondly, there is the inner NE separated from the outer NE by the lumen, which is about 30 nm across. The inner surface of the inner NE is closely associated with the nuclear lamina, a structure consisting of filamentous proteins and which forms the main component of the nuclear matrix or nucleoskeleton. Thirdly there is the pore membrane, which links the inner and outer NEs and forms part of the nuclear pore complex or NPC (Chapters 2, 4 and 8).

The containment of chromatin within its own membrane-bound organelle has major implications for the life of the cell. Amongst other things, it permits precise and complex regulation of gene activity and DNA replication 'protected' from more general aspects of cellular metabolism. However, it also imposes constraints. The nucleus does not contain protein-synthesizing machinery, even though proteins may be made on the surface of the outer NE. All the enzymes, together with structural and regulatory proteins necessary for the activities and components of the nucleus, over 1000 proteins in all (Nuclear Protein Database: <http://npd.hgu.mrc.ac.uk/>), must be able to get in from the outside. At the same time, several thousand more proteins, those that are not involved in the life of the nucleus, are kept out. There are also proteins that shuttle between the nucleus and the cytosol. Finally, all the different RNAs that function in the cytosol must leave the nucleus (in the form of nucleoprotein complexes). The NPCs have a major role in the