Methods in Molecular Biology

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The Nuclear Envelope

Methods and Protocols

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The nuclear envelope (NE) is a double membrane system enclosing the nucleus and is the central distinguishing feature of all eukaryotes. In addition to protecting the genetic material, the NE regulates the trafficking of proteins, RNAs, and ribosomes between the nucleus and cytoplasm. More recently, the identification of hundreds of NE transmembrane proteins (NETs) has revealed that the NE is also involved in diverse structural and signaling networks, both within the nucleus and through connections between the nucleus and the cytoskeleton. The importance of these networks is highlighted by the involvement of NETs and NE intermediate filaments, the nuclear lamins, in a wide range of inherited diseases. Indications that NE composition is highly tissue-specific further implicate the NE in enabling the level of complexity in gene regulation required to support tissue evolution in higher organisms.

Despite its considerable importance, the NE is among the least understood cellular organelles. This largely reflects the inherent difficulties in studying the NE and its component proteins. For example, lamins, as intermediate filaments, are highly insoluble. NETs embedded in the outer membrane tend to bind cytoskeletal proteins and these properties together make them difficult to work with. NETs in the inner membrane have these same issues and in addition often bind chromatin. Thus, even fragments lacking membrane-spanning regions tend to be insoluble. On top of this, the complex organization of the NE and its dynamic nature, undergoing disassembly and reassembly with each cell cycle, makes standard methodologies such as FRAP, coIP, ChIP, and quantification by Western blot subject to additional constraints that require modifications of procedures. The extraordinary complexity of the nuclear pore complex (NPC)—the largest complex in biology—also leads to specific refinement of standard protocols.

This volume provides a wide range of protocols used in studying the NE, with special attention to the experimental adjustments that may be required to successfully investigate this complex organelle in cells from various organisms. Many of these modifications have been only passed on within the laboratories working for many years in the field. We feel this volume is particularly timely now that many new laboratories have joined this extremely dynamic and rapidly growing field.

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