CHAPTER 13

Research Methodologies for the Investigation of Cell Nucleus

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It is impossible to detail all of the techniques used in the nuclear transport field within the allotted space. Since some methods (EM, cell and molecular biology, etc.) are dealt with in other Chapters, here I will focus on the approaches that are less familiar to the cell and molecular biologist (e.g., patch-clamp and atomic force microscopy). I believe that some of the observations I make are significant when interpreting experimental measurements.

Introduction

Any particle traveling directly from cytosol to nucleus, or vice versa, must go through the nuclear pores. The pores connect the inner and outer nuclear membranes (INM and ONM, respectively) of the nuclear envelope (NE). When the pores have no macromolecule traversing inside them, they are filled with the nucleocytoplasmic liquid (probably a mixture of both cytosol and nucleosol). Their denomination as pores, however, does not convey their supramolecular nature of 50 to 130 megadaltons (yeast and vertebrates, respectively). To emphasize their supramolecular architecture, nuclear pores are often referred to as nuclear pore complexes (NPCs). This explanation is necessary to understand the complexities of this structure when compared to much less massive membrane pores such as plasmalemmal ion channels (e.g., Na^+-, K^+-, Ca^{2+}- and Cl^-channels).

One more point deserves clarification for those who have just crossed into either cell biology or physiology. Nuclear transport investigators, mostly cell/molecular biologists, understand that the NPC has a channel inside it (i.e., one that can be filled with liquid). Physiologists, instead, are used to the concept that a channel (i.e., ion channel) is a membrane protein (or several proteins) with a pore inside. And, while physiologists think that (ion) channel gating means the fast statistical opening of the channel gates (a switch-like mechanism), nuclear transport researchers use the term NPC gating in the sense of a relatively sluggish multi-step translocation of macromolecules along the liquid channel of the NPC.

All the many methods used to study nucleocytoplasmic transport are based on physical and chemical principles. Accordingly, they may be classified as biochemical approaches if the purpose is to determine the role of particular molecular species in intracellular/intranuclear cascades. Alternatively, they may be identified as biophysical approaches if the purpose is to identify the structures involved in a particular phenomenon. Methods can also be viewed as purely biophysical if the aim is to determine physical properties of a particular structure or its function. Clearly, the more the approaches, the stronger the case will be made for a particular conclusion. However, quantity not always means quality for it is clear that one must use good judgement in order to reach meaningful data.

Nuclear Import and Export in Plants and Animals, edited by Tzvi Tzfira and Vitaly Citovsky.

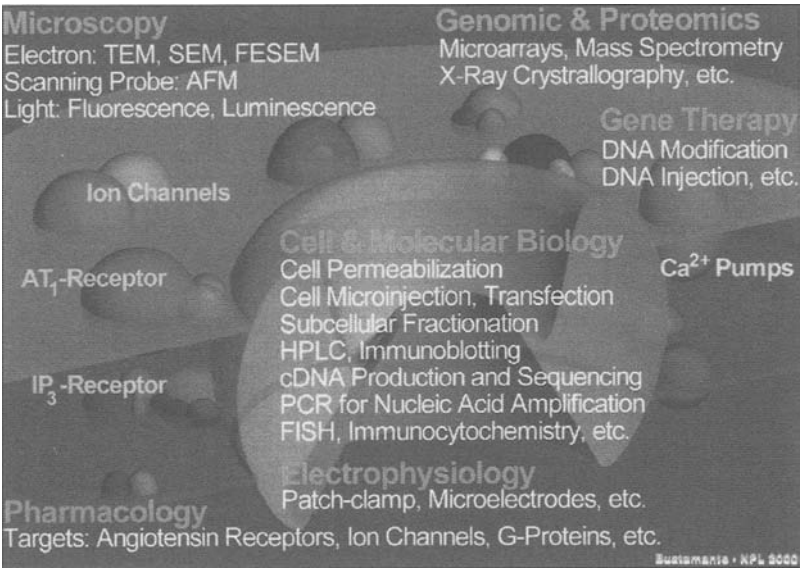

Figure 1. Nuclear transport methods. The foreground groups methods according to various areas. The background represents a single NPC connected to receptors and surrounded by various structural elements, including ion channels, pumps, etc.

In what follows, I shall try to describe most of the methods currently used to study nuclear transport. In Figure 1 I attempt to summarize them. Rather than trying to classify each method within a particular field (e.g., biochemistry), I simply describe the method, for it may appear a pure exercise of semantics to apply such a classification. Reviews on the subject have appeared[1] and I encourage the reader not only to look up reviews but also to data mine the literature with Internet search engines. Most of the references related to this Chapter can be obtained from freely accessible databases. The major database used in the preparation of this Chapter is PubMed.[2] Since this book will be accessible from the Internet, most of the references are given with their Internet link for easy access. Some of the links lead to the free access of the full text article. Due to space limitations, I have also left out some classical and less critical references.

Methods

Electron Microscopy (EM)

As shown elsewhere in this book, EM has been successful in the study of the structure and function of NEs and NPCs.[3-6] Both transmission and scanning EM (TEM and SEM, respectively) have been useful in identifying the physical structures responsible for specific functions of NPCs as well as in analyzing various transport mechanisms. Both, however, must be applied with caution. For example, in either TEM or SEM, the presence of macromolecular transport requirements, prior and/or during fixation of the sample, appears to be an important determinant of whether or not a central plug is observed.[4] Thus, whether the plug is intrinsic to the NPC has been subject of debate.[4]

TEM images are formed by the absorption of electrons during their passage through the sample. The absorptive properties of matter are referred to as its electron-density. The more electron-dense the material, the more the absorption and the darker it will appear in the image. As with other imaging techniques, pre-treatment and enhancement of the image must be made