Preface

Nuclear transport: an emerging opportunity for drug targeting

1. Location, Location, Location

Although the recent boom in real estate values around the country appears to have slowed, the old adage, “location, location, location” rings true for not just property, but for cell biology and drug delivery as well. The cell is a highly organized structure with defined locations for each pathway and reaction that occurs. Although it has long been realized that different processes occur at different sites within the cell, many of these processes and sites have been overlooked or thought of in simplistic terms. The most blatant example of this is the nucleus, which for years was thought of as a sack whose function was to house the cell’s genome. Indeed, a current textbook used in many upper division undergraduate and graduate cell biology courses shows the nucleus as a large sphere whose surface is covered with holes (nuclear pores) and is filled with squiggly lines depicting chromosomes. That this is the site of all transcription, DNA replication, and cell cycle control within the cell is not evident. Indeed, the nucleus is a highly dynamic organelle with defined regions and functions. Further, these “holes” that dot the nuclear surface serve as the only conduits for second messengers, proteins, RNAs and DNAs in the non-dividing cell. Thus, without the proper localization of these molecules to the appropriate compartments (nuclear or cytoplasmic), no control over gene expression or cell division can occur. The reviews in this issue will address various aspects of this nucleo-cytoplasmic trafficking and subcellular localization and provide insights into how and why this topic is germane to drug delivery.

2. Role of nuclear trafficking in gene expression and signal transduction

Apart from 37 genes that are expressed within the mitochondria, all transcription within the mammalian cell occurs in the nucleus. Although expression of many of these genes is constitutive, expression of the majority of the genes in the human genome is tightly regulated at the transcriptional level. Combinations of transcription factors, some general, some cell- or developmental-specific, interact with each other and the promoters of these genes to bring about regulated expression. While we typically envision a promoter as a line with numerous circles and squares on top of it representing the transcription complexes, this is only part of the regulation. Like all proteins, transcription factors are synthesized in the cytoplasm. Because they function in the nucleus, they are useless unless they migrate to their site of action. Thus, the nuclear envelope becomes a major player in gene regulation by controlling the entry and exit of transcription factors.

The nuclear envelope also plays a large role in signaling cascades, since most of these pathways culminate in changes in gene expression. Extracellular signals can bind to cell surface receptors, initiating cascades of protein phosphorylation and subsequent nuclear translocation of various kinases, phosphatases, or transcription factors (Fig. 1). By blocking nuclear transport of one or more of these intermediates, the signaling cascade can be blocked. Alternatively, some signals are membrane permeant (such as steroids), and can bind to cytoplasmic receptors that can only migrate to the nucleus upon ligand binding.

As much as translocation into the nucleus is vital
Fig. 1. Transport across the nuclear envelope. Extracellular signals can stimulate the regulated nuclear import or export of transcription factors, tumor suppressors, cell cycle regulators, and other proteins, resulting in changes in gene expression. Endogenous and exogenously added RNAs can associate with different sets of proteins in the cytoplasm and nucleus that can alter their subcellular localization. Exogenously added plasmids can associate with NLS-containing cellular proteins in the cytoplasm to facilitate the nuclear entry of DNA in non-dividing cells. Similarly, viruses carry similar NLS-containing proteins that bind to their genomes to facilitate nuclear targeting of the viral genomes.

to these pathways, so is translocation out of the nucleus. Effective signaling cascades have two properties: signal amplification and shut off mechanisms. Nuclear export is a major form of this control. Numerous transcription factors and cell cycle control proteins are shuttled out of the nucleus when the appropriate signals are given, shutting off the gene expression that they initiated. It could even be argued that it is more important to turn off a signal than to turn it on, so that effective control can be maintained. Thus, although nuclear import and export mechanisms are very downstream events in signal transduction pathways, without them, the pathways are nonfunctional.

3. Nuclear trafficking and drug delivery

Numerous drugs must enter the nucleus to be effective. These include DNA intercalating drugs, drugs that alter chromatin structure, transcription inhibitors, and cell cycle inhibitors, among others. Because most of these are small molecules, they are free to diffuse across the nuclear envelope through the nuclear pore complexes. However, other extracellularly delivered agents can be too large to enter the nucleus by simple diffusion. These include proteins, RNAs and DNAs (Fig. 1). While extrachromosomal DNA may not be a “normal” or physiological molecule in the cell, its nuclear localization is
integral to the life cycles of many viral pathogens and necessary for the success of transfections and gene therapy. Moreover, the movement of DNA from the cytoplasm to the nucleus remains one of the major barriers to efficient gene transfer and expression. Without localization of DNA to the nucleus, no transcription or “gene therapy” can take place. This is true whether viral or non-viral vectors are being used. While great emphasis has been placed on developing reagents to deliver DNAs across the plasma membrane, relatively little attention has been devoted to developing ways to deliver DNA into the nucleus of the non-dividing cell.

In contrast to DNA, which must enter the nucleus to function, different exogenously added RNAs function in different compartments, and their appropriate localization is necessary for their activity. For example, antisense RNAs can function by one of three mechanisms: inhibition of translation initiation, inhibition of splicing, or activation of RNaseH-mediated target cleavage. Although it is widely assumed that antisense works primarily by inhibiting translation, most added RNAs accumulate within the nucleus within minutes, suggesting that the other two mechanisms may predominate. Similarly, small interfering RNAs (siRNAs) appear to function in mammalian cells in the cytoplasm but are processed from larger precursor RNAs (that are delivered experimentally to the cell) by the enzyme dicer, which is nuclear. Thus, these RNAs must enter the nucleus after delivery to the cell to be processed for activity in the cytoplasm. Again, the importance of nuclear trafficking is obvious.

4. Topics in nuclear trafficking

In the first review, Gasiorowski and Dean provide an overview of nuclear transport mechanisms and highlight recent developments in the regulation of nuclear trafficking. As stated above, regulation of the nuclear localization of proteins is vital to their function. It has been known for a number of years that the phosphorylation state of certain nuclear proteins can alter their localization, but several other post-translational modifications, including acetylation and modification with the ubiquitin homologue SUMO, have been identified recently that appear to modulate nuclear import and export. Together with the identification of substrate-specific nuclear import machinery, these newly characterized mechanisms of regulation demonstrate how important the control of a protein’s cellular localization is. The authors continue with a discussion of the use of pharmacologic agents to modulate nuclear transport of various targets.

Lee and Hannink follow with a comprehensive discussion on the molecular mechanisms used to regulate transcription factor nuclear localization. As stated above, if a transcription factor cannot enter the nucleus, it cannot function, so spatial regulation is vital to gene expression. Special emphasis is placed on several transcription factors that are known to play major roles in gene expression and are regulated by multiple mechanisms to ensure proper nuclear localization.

The next two reviews focus on the delivery of nucleic acids to the nucleus. Whittaker discusses mechanisms used by viruses to target their genomes to the nucleus. This is critical for both naturally occurring viral infections and for gene delivery using viral vectors. By understanding how various viruses target their genetic material to the nucleus, we can design ways to both inhibit these processes (to treat viral infections) and stimulate them (to increase gene transfer). Both DNA and RNA viruses are discussed, and the common strategies used by them are highlighted. Munkonge and colleagues follow with a discussion of how plasmids enter the nucleus, again in the context of gene delivery and gene therapy. They place special emphasis on evaluating recent studies using synthetic NLS peptides coupled to DNA to help facilitate nuclear targeting, and conclude with alternative strategies that may be more effective.

The final review by Campbell and Hope focuses on an aspect of nuclear trafficking that is almost always overlooked, namely transport to the nuclear envelope. Although everyone has a basic understanding of the nature and the extent of the cytoskeleton in the cytoplasm, extension of this knowledge to its impact on cytoplasmic movement is often not appreciated. Proteins, DNAs, and RNAs are very large macromolecules whose diffusion is restricted in the cytoplasm. Recent results demonstrating that many viruses and certain proteins utilize microtubule-based
motor systems to move toward the nuclear envelope are discussed. These results are highly significant and will have a very large impact on how we think about nuclear targeting, since we can no longer ignore what happens between the plasma membrane and the nuclear envelope.

5. Future perspectives

It is our hope that this set of reviews will allow the reader to develop a greater appreciation for the role of the nuclear envelope in regulation of gene expression and gene delivery. Compartmentalization is a highly effective way to consolidate and conserve resources, and provides great opportunity to regulate activity. By characterizing the regulatory mechanisms of nuclear transport of proteins, viruses, and DNA, we will be able to devise better ways to increase and decrease the efficiencies of these processes, depending on our goals.

David A. Dean
(Theme Editor)

Division of Pulmonary and Critical Care Medicine
Feinberg School of Medicine
Northwestern University
303 E. Chicago Ave.
Tarry 14-707
Chicago, IL 60611
USA
E-mail: dean@northwestern.edu