CHAPTER 8

Nuclear Export:

Shuttling across the Nuclear Pore

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One of the distinguishing features of eukaryotic cells is the compartmentalization of genetic information within a membrane-enclosed nucleus. The double membrane of the nuclear envelope separates the nucleus and the cytoplasm, and all macromolecular exchange across the nuclear envelope takes place through large protein channels termed the nuclear pore complexes (NPCs). The molecules that are exchanged between these two compartments range in size from ions and other small molecules to large complexes such as the 50S ribosome and other large ribonucleoprotein complexes. In contrast to ions and small proteins that diffuse across the NPC, macromolecular movement is an active process. Active nucleocytoplasmic transport allows for the proper compartmentalization of nuclear proteins involved in transcription, replication of DNA, and remodeling of chromatin. Transport also is necessary for mRNAs, tRNAs, and rRNAs that are transcribed in the nucleus but ultimately function in the cytoplasm. This growing awareness of the role of nuclear transport in regulating gene expression has paralleled a remarkable increase in our knowledge of the nuclear transport process itself. Far from acting as static "localization signals" the sequences specifying nuclear location act in combination with other signals to alter the steady-state distribution of nuclear proteins. Thus, the concept of nuclear proteins shuttling between nucleus and cytoplasm has emerged as a dominant principle in understanding nuclear import and export. It is impossible to look at nuclear export in isolation without considering nuclear import rates. This has proven to be a barrier in understanding nuclear export as a process with distinct features and requirements. The number of import and export carriers identified has grown to include factors specific for classes of nuclear components and more general factors. In addition, it is clear that movement through the nuclear pore complex is dictated by properties of both the pore and the carrier molecules themselves. These properties are reflected in binding interactions that may facilitate movement across the nuclear pore and perhaps provide for directionality of transport. In this brief chapter, we will give a brief overview of the nuclear pore complex, methods for examining nuclear export and shuttling, the nature of the transport machinery and nuclear export carriers. We will then attempt to combine these into a coherent model for understanding nuclear import and export. We do not claim that this is a comprehensive overview. A number of excellent reviews of this type have recently appeared (see for example refs. 1, 2). What we hope to do is point out some novel aspects of nuclear export and emphasize some recent findings which suggest that factors other than traditional transport carriers may be involved and regulate the process of nuclear shuttling.

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

The Nuclear Pore Complex (NPC)

One key to understanding the process of nuclear import and export of proteins is the fact that the nuclear envelope is a semi-permeable membrane that excludes molecules greater than about 40 kDa if they lack specific targeting sequences. The nuclear membrane is, in fact, a double membrane that may be regarded as an extension of the endoplasmic reticulum. The nuclear envelope is interrupted at regular intervals by the nuclear pore complexes (see 3). These conserved features of eukaryotic cells can accommodate megadalton-sized particles and are themselves greater than 100 megadaltons in mass.

The number of NPCs per cell appears to depend on the need for nuclear transport and varies with cell size, growth and other cellular activity. There are approximately 200 NPCs in a yeast cell[4] and nearly 5000 nuclear pores in a rapidly growing human cell. A mature *Xenopus laevis* oocyte can have as many as 5 X 10^8 nuclear pores (Cordes et al 1995). An eightfold rotational symmetry and a nearly perfect two fold lateral symmetry in the plane of the nuclear membrane characterize all NPCs, regardless of origin. NPCs also exhibit cytoplasmic and nuclear extensions in the form of cytoplasmic filaments and intranuclear baskets. NPCs of higher eukaryotes have a mass of greater than 125 MDa[5] and appear to be composed of some 40 different proteins that are often collectively called nucleoporins.[3,6-8] Proteomics efforts have recently confirmed this number.[9] A large number of mammalian nucleoporins have also been molecularly identified since the major nucleoporin Nup62 was cloned in 1987.[10] These proteins share a number of characteristics. Some of the proteins and their characteristics arising from these various efforts are summarized in Figure 1. The nucleoporins often contain domains consisting of stretches of short peptide repeats containing the GFXFG and GLFG motifs. Mammalian nucleoporins also can contain repeats of the TTPST motif which appear to be sites of O-linked GlcNAc addition.[3,11] Yeast NPCs are smaller and have a mass of roughly 66 Mda.[4] Using a novel proteomics approach, approximately 30 yeast nucleoporins have been identified.[12] Like their mammalian orthologs, the majority of nucleoporins contain characteristic domains consisting of numerous short peptide repeats ending in the dipeptide FG liked the motif in mammalian nucleoporins. These repeats are now thought to play a pivotal part in the mechanism of vectorial movement across the nuclear pore complex (see below).

Methods for Analyzing Nuclear Export

Nuclear export was in essence, discovered more than 40 years ago in studies involving nuclear transplantation in amoebae.[13] As shown in Figure 2, other sensitive assays have been developed to examine nuclear export since those pioneering studies. Such approaches as antibody microinjection, heterokaryon cell fusion[14,15] r microinjection into Xenopus oocyte nuclei[16,17] have been employed. The difficulty in examining nuclear export in isolation has been the complication that nuclear import is often occurring simultaneously. Overcoming this issue has been particularly problematic.[17] The microinjection, heterokaryon fusion, and transplantation studies avoided this issue by examining redistribution of marker proteins. In more recent studies, the nuclear export of the hnRNP A1 protein or the Human Immunodeficiency Virus (HIV)-1 Rev proteins have been useful in sorting out nuclear export pathways. By sorting out the regions important to export, mutational analysis then led to the identification of the signals that mediate nuclear export. These were termed nuclear export signals (NES).[15,18] The best understood of these export signals is the leucine-rich NES present in HIV Rev and scores of other cellular or viral proteins involved in signal transduction, transcription, cell cycle (Table I). However, the description of shuttling proteins not containing leucine-rich NES points out the existence of other nuclear export signals. Our efforts have been centered upon trying to understand the export of the HIV NES using a novel GFP-based reporter construct in which nuclear import and export can be independently regulated.[19] Other in vitro systems have also