CHAPTER 5

Nuclear Import of Plant Proteins

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In this Chapter, we will focus primarily on protein import into the nucleus of plants. As in other eukaryotes the partitioning of genetic information into the nucleus necessitates the import and export of macromolecules such as proteins, nucleic acids and protein/nucleic acid complexes across the nuclear envelope. These transport processes are essential and are subject to stringent regulatory controls. In plants, it is clear that in addition to the maintenance of basic cellular processes, the regulated import of proteins plays a vital role in development. As we will discuss, the nuclear import of proteins in a selective manner is essential in responses of plants to light and results in the dramatic morphological changes that occur as plants switch from growth in the dark to growth in the light.[1] Protein translocation across the nuclear envelope is also a process that is utilized by pathogenic viruses[2] and tumor-inducing bacteria in the genus *Agrobacterium*[3] to transport protein/nucleic acid complexes into the nucleus for replication and even incorporation of pathogen DNA into the host genome.

The import pathway for proteins, themselves key factors regulating nuclear transport processes, is the best understood of the nuclear transport processes in plants. Numerous import signals have been characterized, and we are beginning to identify and understand the major components of the import machinery. As expected many of these factors are conserved between plants, animals and fungi, but there are surprising results indicating subtle differences in preferences for nuclear localization signals (NLSs),[3,4] the mechanics of import receptor function[5], and potential plant-specific import factors.[6] As we move forward plants are contributing new knowledge such as potential mechanisms for the targeting of proteins to the nuclear envelope and nuclear pore complex (NPC).[7] Recent efforts have begun to focus on other nuclear transport processes in plants such as the export of proteins and nucleic acids.[8,9]

Protein Import in Animals and Yeast

Our knowledge of nuclear transport in vertebrates and yeast is more advanced than our understanding in plants. Among the reasons for this historically are: (1) the ease of recovering nuclei from *Xenopus* oocytes that are intact for morphological studies, (2) the development of in vitro systems in *Xenopus* for reconstituting the assembly of nuclei and NPCs, (3) the reconstitution of cytosol-dependent nuclear import in permeabilized mammalian cells, (4) the rapid genetics possible in yeast, (5) the large biomedical research community and potential importance of nuclear processes for medicine. However as we are discovering nuclear translocation is a critical aspect of plant growth and development and thus has broad implications for agriculture in terms of disease and stress resistance and other crop improvements, which are the keys to feeding an expanding worldwide population. To place our knowledge of plant nuclear import in perspective, we will overview processes in animals and yeast throughout the

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

Chapter highlighting important advances and unique aspects in plants. Import from the perspective of non-plant systems is covered in greater detail in other Chapters. There are also excellent recent reviews that are focused on the nuclear/cytoplasmic transport of proteins, nucleic acids and their complexes[4,10-22] as well as the structure and function of the NPCs in vertebrates and yeast.[23-30]

Nuclear Translocation in Plants

Nuclear Pore Complex

The channels through which all transport substrates must pass are the NPCs which are macromolecular complexes embedded in the double-membrane nuclear envelope. The NPCs are estimated to have a mass of 125 MDa in higher eukaryotes and to be composed of 50 to 100 different proteins collectively known as nucleoporins. Morphologically, an NPC is composed of a nucleoplasmic and a cytoplasmic ring. Eight spokes are found within the rings that extend toward a central channel resulting in eight 9 nm channels thought to function in the diffusion of small molecules across the nuclear envelope. A basket-like structure extends from the nucleoplasmic face and fibrils have been observed extending from the cytoplasmic face. Thus far, fewer than 20 nucleoporins have been purified from vertebrates. The NPCs in yeast are less complex (mass about 66 MDa) and are not dissociated and reassembled during mitosis as in higher eukaryotes; nevertheless the development of methods to purify intact complexes[31] has permitted the identification and sequencing of all 30 of its nucleoporins. Even with such information available understanding the assembly and function of the NPC will be a daunting task.[32]

A number of vertebrate NPC proteins have been implicated in nuclear import.[30] They include Nup358 which is located on the cytoplasmic filaments. Nup 358 has multiple Ran binding sites and binds to the protein transporter importin β (See *Components and Mechanisms of Protein Import*) via FXFG (single amino acid code where X represents an amino acid with a small or polar side chain) repeats[33] which are characteristic of many nucleoporins. Other nucleoporins reported to bind to importin β include Nup153,[34] Nup214,[35] Nup116p, Nup100p,[36] and p62.[37] The p62 protein was one of the first nucleoporins to be purified, and like many nucleoporins from vertebrates it is modified by single *O*-linked *N*-acetylglucosamine (O-GlcNAc) residues. While the O-GlcNAc is probably not essential for import, the binding of the lectin wheat germ agglutinin (WGA) inhibits nuclear import in vertebrates and has been used for the identification and purification of nucleoporins from higher eukaryotes (for review see ref. 38).

From electron microscopy studies beginning in the 1970s we know that nuclear pores in plants are morphologically similar to those of other organisms.[39] However there have been few reports in which plant nucleoporins have been purified (for review see ref. 40). Scofield et al[41] localized a protein at the NPC and identified a 100 kDa polypeptide in a nuclear matrix fraction from carrots using an antibody to the yeast nucleoporin NSP100; however successful purification was not reported. Other studies indicate that nuclear envelope fractions from maize and tobacco nuclei contain a subset of proteins in the NPC fraction that can bind NLSs specifically.[42-44] The binding site is at the NPC indicating a role for plant nucleoporins in protein import.[42] This finding is consistent with the unusually tight association of at least one plant importin α NLS receptor with the nuclear envelope in purified nuclei and intact cells.[45,46]

Using WGA as a probe it is clear that GlcNAc-modifications are present at the periphery of the nucleus,[47-49] and electron microscopy has shown that some of these modifications are present at the NPC.[47] In fact biochemical characterization of tobacco nuclear fractions has shown that the glycans are attached to proteins via an *O*-linkage and the moities are longer than five sugar residues in length ending with a terminal GlcNAc residue. This is a novel