CHAPTER 9

Nuclear Protein Import:

Distinct Intracellular Receptors for Different Types of Import Substrates

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Entry into the eukaryotic cell nucleus occurs through multiple pathways involving specific targeting signals, and intracellular receptor molecules of the importin/karyopherin superfamily which recognise and dock the nuclear import substrates carrying these signals at the nuclear pore. Subsequent to translocation through the pore via a series of importin-mediated docking steps at multiple sites within it, release into the nucleus is effected by the monomeric guanine nucleotide binding protein Ran. Different importins possess distinct target sequence-binding specificities, meaning that different importins mediate the nuclear import of different classes of proteins. This extends to different classes of transcription factors which are recognised by distinct importins, and whose transport to the nucleus is modulated by specific regulatory mechanisms. The first step of nuclear import is of central importance, with the affinity of the importin:targeting signal interaction being a critical parameter in determining transport efficiency. In the whole cell context, target signal recognition can be modulated through differential expression of the importins themselves, as well as through competition between different importins for the same nuclear import substrate, and between different nuclear import substrates for the same importin. In addition, there are specific mechanisms to modulate targeting sequence-importin interaction directly through phosphorylation. The fact that there are distinct nuclear import pathways for different types of nuclear import substrates enables the cell to regulate these pathways specifically, ensuring efficient nuclear import of particular proteins as and when required.

Introduction

The last few years have seen important advances in our understanding of the cellular factors that mediate signal-dependent nuclear transport. Multiple pathways have been identified, where different types of proteins are transported either into or out of the nucleus through the action of specific molecules called importins/karyopherins that recognize distinct targeting signals.[1-4] The monomeric guanine nucleotide-binding protein Ran plays a central role in release into the nucleus subsequent to translocation through the nuclear envelope-localized nuclear pore complex (NPC), with the specific Ran-transporter NTF2 (nuclear transport factor 2), and Ran-binding and -modifying proteins playing important auxiliary roles. Since the total cellular concentration of targeting signal receptors and Ran in particular is limiting,[2,5,6] the initial step of target signal recognition is critical in terms of

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getting proteins efficiently to their correct subcellular destination. Regulating this process by enhancing or preventing target signal recognition in response to growth and differentiation signals is a key factor detemining the nuclear entry or otherwise of particular proteins.[2,3,7] This Chapter discusses current knowledge of nuclear protein import in terms of the idea that the existence of multiple differentially regulated nuclear import pathways enables the nuclear import of particular classes of proteins to be carried out efficiently according to the dynamically changing needs of the cell, such as during development, or in response to hormonal or cytokine stimulation. Modulation of importin-target signal recognition is discussed in this context as the main mechanism of regulating nuclear import in the physiological context of the plethora of competing different importin-targeting sequence interactions in the cell.

The Transport Process

The first step of nuclear protein import involves the recognition of targeting signals by members of the importin family and translocation of the proteins carrying them to the cytoplasmic side of the NPC (Fig. 1). For certain classes of protein (see below), the importin α/β1 heterodimer is involved, whereas most other pathways require only importin β1 or an importin β homolog; in all cases, the importin β homolog docks the importin/transport substrate complex to the NPC, and mediates interaction with Ran. The latter, dependent on the action of several key Ran-interacting and regulating proteins including NTF2, Ran binding protein 1 (RanBP1), Ran GTPase activating protein 1 (RanGAP1) and the nucleotide exchange factor RCC1,[4-6,8] mediates release into the nucleus subsequent to translocation of the import substrate through the NPC. Nucleoporins (nups), the FG (single letter amino acid code)-repeat-containing proteins present in multiple copies throughout the NPC, serve as binding sites for different transport factors including importin β homologs, Ran and NTF2, thus representing "docking bays" for transport factors and assemblies as they pass through the NPC via a succession of transient binding interactions.[9-12] Dissociation of the transport complex at the conclusion of translocation through the NPC is effected by Ran in the GTP bound form binding to importin β to trigger release of the nuclear import substrate and importin α (in the case of importin α/β1-mediated nuclear import) into the nucleoplasm. Nuclear RanGTP is maintained at sufficiently high concentration by the combined action of NTF2, which transports RanGDP from the cytoplasm to the nucleus through a series of FXFG-docking events analogous to those of importin β,[11,12] and RCC1, which converts RanGDP into RanGTP.[6,13] Cytoplasmic RanGDP is maintained through RanGAP1, which is predominantly cytoplasmic, as opposed to RCC1, which is nuclear, thus ensuring the asymmetric balance of the guanine nucleotide bound by Ran in the two subcellular compartments.[13] Although non-hydrolysable GTP analogs inhibit nuclear transport, no direct role in either import or export has been demonstrated for GTP hydrolysis,[13,14] whilst the requirement for ATP in the transport process remains controversial.[6,14,15] All transport components are recycled back to the cytoplasm subsequent to nuclear import; importin α has its own specialised nuclear export receptor (the importin β-related molecule CAS) which requires RanGTP for binding to importin α.[16,26]

α Importins

Conventional nuclear localization sequences (NLSs), the short modular peptide sequences sufficient and necessary for nuclear localization of the proteins carrying them, fall into several broad classes. Two of these are highly basic in nature:- those resembling the NLS of the SV40 large tumor antigen (T-ag: PKKKRKV^{132})[17] which comprises a short stretch of basic amino acids, and bipartite NLSs which consist of two stretches of basic amino acids separated by a spacer of 10-12 amino acids.[18] Other types include NLSs resembling those of the yeast homeodomain containing protein Matα2[19] where charged/polar residues are interspersed with