CHAPTER 10

The Molecular Mechanisms of mRNA Export

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A general paradigm for nuclear transport was established primarily through studies of protein import and export. Until recently, this paradigm was generally presumed also to apply to the process of RNA export from the nucleus. In particular, it was assumed that general mRNA export was mediated by one or more transport receptors of the importin-β family and that the RanGTP/GDP gradient was required to impart directionality to the process. The highly abundant class of nuclear RNA-binding proteins—the hnRNP proteins—were regarded as primary candidates for mRNA export adapter proteins that could link mRNAs to importin-β family export factors. Within the past few years, however, an explosion of data has largely disproven prior assumptions about the mechanisms of mRNA export, permanently changing the face of the field. The dust is still settling, but what we now see, albeit incompletely, is the outline of a probable major route of mRNA export that is independent of the importin-β family and the Ran GTPase system.

Introduction

The distinguishing feature of eukaryotic cells is the segregation of RNA biogenesis and DNA replication in the nucleus, separate from the cytoplasmic machinery for protein synthesis. Communication between the nucleus and the cytoplasm occurs through aqueous channels in the nuclear envelope called nuclear pore complexes (NPCs).[22,92] Small molecules can pass through NPCs by diffusion, but there is a permeability barrier for larger molecules—those with a relative molecular mass of >40 kDa—which permits transport only of selected cargo with the help of transport receptors. The NPC is a gigantic proteinous complex ranging in size from approximately 50 MDa in the yeast *Saccharomyces cerevisiae* to 125 MDa in higher eukaryotes, and possessing an eight-fold symmetric structure. All nucleocytoplasmic transport occurs via the central aqueous channel found in NPCs. As the maximum diameter of this channel may be only ~25 nm, achieving nuclear transport of large complexes such as ribosomes and viral genomes presents a potentially formidable challenge and must involve a considerable change in the three-dimensional conformation of the transport cargo or of the pore itself.

Active transport through the NPC is a signal-mediated process involving recognition of cargo molecules by a large class of soluble transport factors.[30,66] Transport is bidirectional, energy dependent, and highly regulated. Research into the molecular mechanisms of nucleocytoplasmic transport has been initiated by studies of nuclear protein import. Protein export from the nucleus has been shown to utilize similar mechanisms as protein import, and now researchers are focusing on the transport of another class of macromolecules—RNAs. RNA export, especially export of mRNA, has been less understood because the process is more complex than that of protein export, involving the coordination of several post-transcriptional

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processing events with the formation of RNA-protein complexes (RNPs) that are the actual export cargoes. In the past few years, however, researchers have accumulated a vast amount of information that reveals the existence of a distinct transport system dedicated to mRNA export.[17,79,80] In this chapter, we review recent studies of the molecular mechanisms of nucleocytoplasmic transport, from 'classical' protein transport to the modern view of the mRNA export system.

Ran Dependent Nucleocytoplasmic Transport

Considerable progress has recently been made in understanding the mechanisms underlying the sequence-specific transport of proteins between the nucleus and the cytoplasm and the critical role played by the NPC in this process.[30,66] Nucleocytoplasmic protein transport is promoted by signal-receptor recognition process. Generally, cargo proteins contain peptide motifs that function as nuclear localization signals (NLSs) and/or nuclear export signals (NESs). These include the so called 'classical NLSs', such as SV40 large T antigen NLS, which consists of a short cluster of basic amino acids, the nonclassical M9 signal, which is a 38- amino acid domain of hnRNP A1 used both for nuclear import and export, and HIV Rev-type NESs consisting of an approximately 10-amino acid stretch rich in leucine residues that are found in many nuclear export cargos. Evolutionally conserved transport signal receptors specific for each transport signal have been identified. These members form a protein family known as the 'importin-β family' or as 'karyopherins' that includes more than 20 members in metazoans and 14 members in yeast belong (from now on, these will be referred to as β family receptors). The family members share a partial similarity in sequence and structure as well as a biochemical property of interaction with Ran, a Ras-related small GTP binding protein. Like other GTP-binding proteins, Ran has both GTP- and GDP-bound forms, and the switch between these two forms plays a crucial role in regulating transport by promoting the association and dissociation of transport receptors and their cargoes, as well regulating their interactions with the NPC. Ran requires two regulatory factors, the GTPase activating RanGAP and the guanine nucleotide exchange factor RanGEF, to switch between its two nucleotide bound states. At steady state, these regulators localize to the cytoplasm and the nucleus, respectively; this asymmetric distribution generates a RanGTP/GDP gradient across the nuclear envelope, which is essential for most nuclear transport pathways. Cooperation with the Ran GTPase system allows transport receptors to bind and subsequently release their substrates on opposite sides of the nuclear envelope, which in turn ensures directed nucleocytoplasmic transport.

The most well-characterized β family receptors are importin-β itself and CRM1/exportin-1.[30,66] Importin-β was the first nuclear transport receptor to be identified. It forms a heterodimeric complex with the adapter protein impotin-α, which mediates recognition of classical NLSs in nuclear import cargoes. The importin-α/-β-cargo complex is formed in the cytoplasm, then travels through the NPC to the nuclear interior, where the cargo is released from the receptor upon binding of RanGTP to importin-β. In contrast, RanGTP binding to the export receptor CRM1/exportin-1 is required for its association with proteins that contain leucine-rich NESs. After the ternary complex of CRM1-RanGTP-cargo is translocated to the cytoplasm, the cytoplasmic Ran activators RanGAP and Ran-binding protein 1/2 (RanBP1/2) stimulate GTP hydrolysis, resulting in the conversion of RanGTP to RanGDP. The switch to RanGDP results in the dissociation of the export complex and release of the export cargo. The export receptor then returns to the nucleus for another round of export. Thus, import and export are essentially reverse processes, with their directionality maintained by the presence of RanGTP in the nucleus and RanGDP in the cytoplasm. The interactions between soluble transport factors described in these examples suggested that it was not unlikely that a given receptor could function in the import of some substrates and in the export of others. In fact,