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Macromolecular Exchanges Between the Nucleus and Cytoplasm

Carl M. Feldherr*

Department of Anatomy and Cell Biology, University of Florida, College of Medicine, Gainesville, Florida 32610

Abstract The control of transcription and translation is of fundamental importance in cell biology. In this regard, the nuclear envelope is in a unique position to contribute to the regulation of these events, by directing macromolecular exchanges between the nucleus and cytoplasm. Such exchanges occur through the nuclear pore complexes, mainly by signal-mediated processes. Different signals are required for import and export. Specific cytoplasmic or nuclear receptors initially bind the signal-containing substrate, and the complex subsequently interacts with the pores. Additional factors then assist in translocation across the envelope. Current research is focused mainly on further characterization of transport receptors, translocation factors, as well as components of the nuclear pore complex, i.e., the nucleoporins. The ultimate goal is to understand the molecular interactions that occur among the different components of the transport apparatus, the energy sources for transport, and how variations in transport capacity are generated. J. Cell. Biochem. Suppls. 30/31:214–219, 1998. © 1998 Wiley-Liss, Inc.

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The controlled translocation of macromolecules (both proteins and RNA) between the nucleus and cytoplasm is fundamental for the maintenance and regulation of normal cellular activities, including growth and division. These exchanges take place through the nuclear pores, which are discontinuities in the double membrane of the nuclear envelope. The pores are approximately 800 Å in diameter, and are occupied by a supramolecular structure, referred to as the nuclear pore complex (NPC). The major structural element of the NPC is the spoke-ring assembly. This element exhibits eight-fold symmetry, and fills most of the pore area. Cytoplasmic filaments extend from the outer, cytoplasmic surface of the spoke-ring assembly, and a net-like, nuclear basket extends from the inner surface. A cylindrical element, also referred to as the transporter, is located around the central axis of the NPC [for details of pore structure see reviews by Pante and Aebi, 1995; Goldberg and Allen, 1996].

In vertebrate cells, it is estimated that the NPC has a mass of approximately 125 MDa [Reichelt et al., 1990], which, given the octagonal nature of these structures, suggests that the pores are composed of about 50–100 different proteins, the nucleoporins. The first nucleoporin to be identified was a 210 kD, mannose-rich, transmembrane protein [Gerace et al., 1982]. Shortly thereafter, a novel family of nucleoporins containing O-linked N-acetylglucosamine was discovered in vertebrate cells [reviewed by Starr and Hanover, 1992]. The first of these glycoproteins to be cloned was p62. In addition to glycosylation sites, p62 contains a number of XFXFG repeats, as well as hydrophobic heptad repeats, which could form complexes with other nucleoporins. XFXFG repeats, as well as GLFG and FG motifs have been identified in many but not all nucleoporins [reviewed by Rout and Wente, 1994]. In yeast, a favorite organism for studying nuclear transport, the NPC has the same basic elements that are found in vertebrate cells (i.e., a spoke-ring assembly, cytoplasmic filaments, a nuclear basket, and a transporter element), but there are also important differences. The mass of the yeast pore is only 60 MDa, and, as would be expected, there are accompanying differences in the size and morphology of the structural elements [Yang et al., 1998]. Some of the yeast

*Correspondence to: Carl Feldherr, Dept. of Anatomy and Cell Biology, University of Florida, College of Medicine, Gainesville, FL 32610. E-mail: feldherr@anatomy.med.ufl.edu

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nucleoporins are homologs of those found in vertebrates, and also contain the same repeat motifs; however, the yeast nucleoporins do not appear to be glycosylated [reviewed by Doye and Hurt, 1997].

Signal-Mediated Nuclear Import

After the pores were first identified by electron microscopy, it was commonly believed that passive diffusion through these sites could account for macromolecular exchanges between the nucleus and cytoplasm. In the mid 1980s, Dingwall et al. [1982] found that, in addition to diffusion through the pores, signal-mediated processes also function. In this classical study, it was demonstrated that a specific domain located in the "tail" region of each of the pentomeric subunits of nucleoplasmin (a 122 kDa nuclear protein found in *Xenopus* oocytes) is required for nuclear import. By using nucleoplasmin-coated colloidal gold particles as a transport substrate, Feldherr et al. [1984] were able to show that transport occurs through a gated channel located in the center of the NPC. The same channel also serves as the diffusion pathway. Its patent diameter is approximately 90 Å [Paine et al., 1975], but it is able to dilate to over 230 Å during signal-mediated transport [Feldherr et al., 1984]. It was subsequently shown that nuclear import is a two-step process, involving initial binding of the substrate to the surface of the pore complex, followed by a second, energy dependent, translocation step [Newmeyer and Forbes, 1988].

Kalderon et al. [1984] were the first to characterize a nuclear localization signal (NLS) for protein targeting. They reported that a short basic domain, PKKKRKV, was both necessary and sufficient to direct the nuclear import of SV40 large T antigen. Although there is no consensus signal, similar lysine rich sequences have been identified in numerous other nuclear proteins. Subsequent to the identification of the "simple" large T-like NLSs, a more complex, bipartite signal was found in nucleoplasmin [Robbins et al., 1991]. Bipartate signals contain two basic amino acid clusters, separated by a variable spacer region. They are as common as the large T-like NLSs, and, again, there is no consensus sequence. Together, these two types of signals are referred to as classical NLSs.

Early searches for an NLS receptor were performed using cross-linkers, affinity chromatography, and blotting procedures. Although a number of putative NLS binding proteins were identified, functional data demonstrating that these proteins were actually required for transport, was not available. Direct evidence for the existence of nuclear transport factors, more specifically cytoplasmic factors, was first reported by Newmeyer and Forbes [1990], who demonstrated that *Xenopus* egg extracts could support signal-mediated import in isolated nuclei, and by Adam and Gerace [1991] using digitonin permeabilized cells. The latter study demonstrated that cytosolic extracts could support transport in permeabilized culture cells and that a 54–56 kD, NLS-binding protein significantly increased transport capacity. The permeabilized cell system developed by Adam and Gerace [1991] represented a major technical advance in the field. It has proven to be a relatively simple, reproducible, in vitro assay system for transport factors, and is routinely used in many laboratories.

With the availability of an in vitro assay, rapid progress was made in the identification of the cytoplasmic factors required for both docking of the transport substrate to the pores (the first step in nuclear import) and translocation through the pores (the second import step). It is now clear that the initial event in the import of proteins containing either of the classical NLSs is binding to the cytoplasmic factor importin-α/karyopherin-α, which functions as an adapter. The substrate/adapter complex then binds to importin-β/karyopherin-β, which mediates docking to the cytoplasmic filaments of the nuclear pore complex. Translocation through the transporter element requires additional factors, particularly Ran, a 25 kDa GTPase, and a 14 kDa protein referred to as p10/NTF2 [reviewed by Nigg, 1997; Gorlich, 1997; Ohno et al., 1998]. The exact role of Ran is not yet clear; however, it has been suggested that it provides directionality to nucleocytoplasmic exchanges. This appears to be dependent on the fact that Ran is present mainly in its GDP form in the cytoplasm, whereas Ran-GTP is the predominant form in the nucleus. This asymmetric distribution results from the fact that the GTPase-activating protein, Ran GAP1, is cytoplasmic, whereas the Ran nucleotide-exchange factor, RCC1 is localized in the nucleoplasm [Melchior and Gerace, 1998]. The function of p10/NTF2, which is able to bind Ran-GDP and nucleoporins containing FG repeats, also remains un-

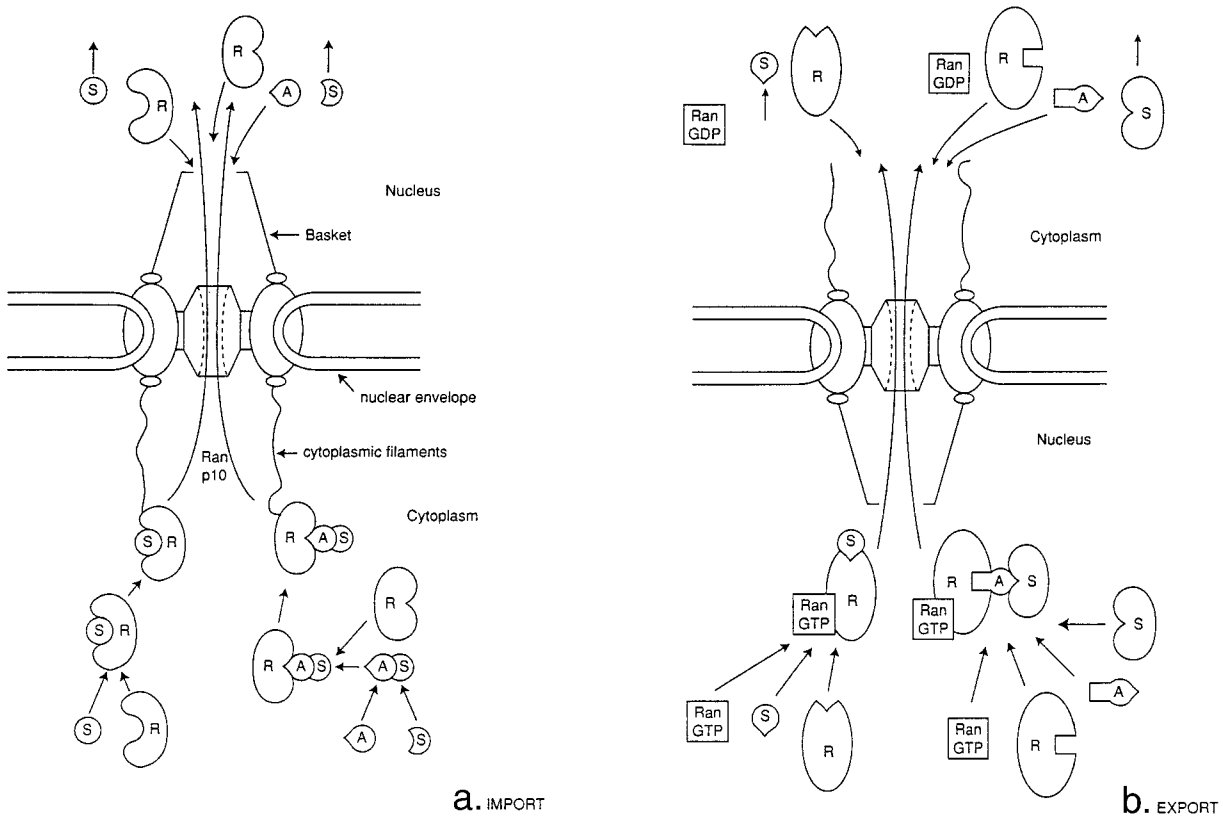

Fig. 1. **a:** The signal-mediated nuclear import of NLS containing substrates (S), involves either direct binding to a cytoplasmic receptor (R; a member of the importin-β/karyopherin-β superfamily), or, as is the case for the classic NLSs, initial binding to an adapter protein (A), and subsequent association with the receptor. The receptor/substrate complex then binds to the cytoplasmic filaments, and its translocation through the central transporter element of the pore complex is facilitated by additional factors, including Ran and p10/NTF2. Once in the nucleoplasm, the complex dissociates and the receptors and adapters return to the cytoplasm. **b:** The export of substrates (S; proteins and RNA) from the nucleus also involves initial binding to a receptor (R), which may or may not require an adapter protein (A). Ran-GTP is essential for formation of transport complexes within the nucleus. Following translocation through the pore complex, the receptor and adapter recycle to the nucleoplasm.

known; however, it has been suggested, based on in vitro binding studies [Nehrbass and Blobel, 1996], that it facilitates a stepwise adsorption and desorption of the substrate-receptor complex during translocation. After translocation is completed, the complex dissociates in the presence of the high concentration of Ran-GTP in the nucleoplasm. Importin-α and -β/karyopherin-α and -β return to the cytoplasm, and the NLS-substrate remains in the nucleus. The import process is summarized in Figure 1a.

Additional import pathways, other than the one utilized by the classical NLSs, have been reported for the nuclear import of heterogeneous nuclear ribonucleoproteins (hnRNPs) and ribosomal proteins [reviewed by Pemberton et al., 1998]. The most thoroughly studied hnRNP is A1 [Pollard et al., 1996], a protein that recycles between the nucleus and cytoplasm. The import signal for A1 is contained in a 38 amino acid motif, referred to as the M9 domain. This domain lacks the basic amino acid regions found in classical NLSs, and, in addition, M9 functions as an export signal. The cytoplasmic receptor for the M9 signal is a 90 kD protein, designated transportin, which has 28% identity with importin-β/karyopherin-β. A1 interacts directly with the receptor; an adapter, equivalent to importin-α/karyopherin-α is not required. The ribosomal protein L25 contains an NLS that resembles a classical bipartite signal; functionally, however, it is not transported by the classical pathway. Instead, the nuclear import of L25 requires an importin-β/karyopherin-β homolog, termed Kap123p [Rout et al., 1997]. This receptor, as is the case for transportin, binds directly to its substrate. In vitro binding studies suggest that Kap123p might also serve as a receptor for numerous other ribosomal proteins.

Nuclear Export

The export of macromolecules from the nucleus occurs through the same central transporter element employed for protein import. In addition to utilizing a common exchange site, there are other similarities between the two processes; however, there are also significant differences [reviewed by Dahlberg and Lund, 1998; Nakielny and Dreyfuss, 1997; Weis, 1998]. Signal-mediated export, like import, requires a transport complex consisting of a receptor, an adapter which may or may not be required, and a substrate, which can be either a protein or RNP particle. Ran-GTP is necessary for the formation of the export complex, but GTP hydrolysis is not required for transport. Nuclear export is summarized in Figure 1b.

Nuclear export signals (NESs) were originally identified in the HIV-1 Rev protein, PKI (the inhibitor of protein kinase A), and the hnRNPs A1 and K [reviewed by Moore, 1996]. The NESs that have been characterized do not resemble classical NLSs. The Rev and PKI sequences (LQLPPLERLTL and LALKLAGLDI, respectively) are leucine-, rather than lysine-rich domains. The A1 and K signals are considerably larger than the classical signals (38 and 24 amino acids, respectively); they are not basic, and also function in import (see above). The receptor for leucine-rich NESs is CRM1 or exportin 1. This factor functions in the export of late HIV-1 mRNAs (the adapter is Rev), 5S rRNA (the adapter is transcription factor IIIA), U snRNAs, and mRNAs. In the latter two instances, the adapter proteins have not been identified. The export of tRNA requires a different receptor, exportin t, which binds directly with tRNA, and, thus, does not require an adapter. A third receptor, CAS, is responsible for recycling importin-α/karyopherin-α back to the cytoplasm following import. It is also likely that there are other, unidentified receptors that have a redundant function; for example, if the hnRNPs A1 and K act as transport adapters, as has been suggested [Pinol-Roma, 1997], receptors that bind A1 and K NESs should facilitate mRNA export, along with exportin 1.

It is of interest that all of the known import and export receptors belong to the importin-β/karyopherin-β superfamily [reviewed by Ullman et al., 1997; Wozniak et al., 1998]. A common feature of these proteins, appears to be a helix-loop-helix domain in their N-termini. Since all of the receptors utilize the same exchange site through the pores (the central transporter element), such a domain could presumably interact with specific nucleoporins (perhaps one or more of the repeat motifs) that are essential components of the translocation machinery. It should also be pointed out that there are additional members of the superfamily, of unknown function, that represent potential receptors.

The Function of the Pores in Regulating Cellular Activity

There is evidence that nuclear transport capacity can vary, depending on cellular activity [reviewed by Feldherr and Akin, 1994]. This dynamic aspect of nuclear transport greatly enhances the potential regulatory role of the nuclear envelope. Much of the data relating to variations in transport have been obtained from in vivo experiments in which nuclear import was assayed using nucleoplasmin-coated colloidal gold. The size of the particles ranged from 20 to 300 Å in diameter. In proliferating fibroblasts, the largest particles that enter the nucleus are approximately 230 Å in diameter; however, as the cells become quiescent, the functional size of the pores can decrease by as much as 100 Å. It is important to note that a change in pore size of this magnitude could significantly reduce the efflux of RNP particles, especially ribosomal subunits. Although this is consistent with observed decreases in cytoplasmic rRNA in quiescent cells, a direct correlation between rRNA content and transport has not been established. The cause(s) of the permeability change that accompany quiescence is not known; however, a recent study on developing *Xenopus* oocytes suggests a possible mechanism [Feldherr et al., 1998]. The functional size of the pores in stage 1 oocytes, which synthesize primarily 5S- and tRNAs, is significantly less than in stage 2 oocytes, which produce larger rRNAs. The exclusion limits for particles entering the nucleoplasm are estimated to be approximately 200 Å and 230 Å, respectively. Analysis of transport factors further demonstrated that the relative concentrations of the importins/karyopherins, and Ran were the same at both stages; however, p10 was more abundant in the stage 1 cells. Increasing the concentration of p10 in stage 2 oocytes, by microinjection, resulted in a decrease in transport capacity to the stage 1 level. Whether altered p10 concentra-

tions can also account for the transport changes in quiescent cells, or whether other factors are involved has yet to be determined.

If a decrease in pore size accompanies a decrease in overall cellular activity, conditions that increase cell growth and division might be expected to enhance transport. This seems to be the case, at least for SV40 transformed fibroblasts. Transformation results in an increase in pore size of about 40 Å. This change is initiated by large T antigen, and involves an amplification of protein kinase C activity [Feldherr and Akin, 1995].

Future Directions

The initial requirements for determining the molecular basis of signal-mediated nuclear import and export is an understanding of 1) the composition and organization of the pores and 2) the receptors and associated factors required for docking the transport substrate to the pores and subsequent translocation. Current studies on nucleoporins are progressing along several lines. First, cataloging the nucleoporins. Using biochemical, and especially genetic procedures in yeast, the majority of nucleoporins have been identified, and it is anticipated that the remainder will be characterized in the near future [Fabre and Hurt, 1997]. Second, biochemical and genetic approaches are also being employed to establish the nature of nucleoporin interactions; i.e., subcomplex formation. To date, three structural complexes have been identified, the largest of which contains six different nucleoporins. Such data is essential for establishing the functional organization of the pore complex. A third, and related problem, involves the localization of specific nucleoporins within the pore complex. These studies, which rely primarily on immuno-EM analysis, have been instrumental in identifying docking proteins, and proteins that are likely to be necessary for RNA efflux. Fourth, the functions of the nucleoporins, which include nuclear import and export, RNA processing, and the organization of the pores within the envelope, are being investigated primarily in mutant yeast; however, knockout strategies and overexpression of nucleoporins in cultured cells are also proving useful.

Although considerable progress has been made in our understanding of signals, receptors, and accessory factors required for signal-mediated exchange across the nuclear envelope, a number of fundamental questions remain to be answered. The fact that there are numerous homologs to known transport receptors (members of the importin-β/karyopherin-β superfamily) suggests that other pathways for import and/or export exist. If this is the case, it will be important to establish the specific cargoes that utilize these pathways, and to identify adapter proteins, if they are involved. This also raises the possibility that there are additional transport signals (NLSs and/or NESs) that are specific for novel receptors. Although there is strong evidence that Ran provides directionality to the transport process, it is not clear whether it has a direct role in translocation through the pores, or whether it is passively carried across the envelope. Does hydrolysis of GTP by Ran represent the energy source for translocation (there is evidence that this is not the case for export)? If not, what is the energy source? The function of p10/NTF2 in translocation also remains to be determined. The ultimate question is how the substrate/receptor complex, along with accessory factors, interacts with individual nucleoporins to facilitate translocation. Although several in vitro binding studies have dealt with this problem, a final resolution will require considerably more data regarding both the nature of the transport complex and the molecular organization of the pores.

As mentioned above, there is a relationship between cellular activity and the functional state of the nuclear pores. With the exception of one study, which suggested that p10/NTF2 might be involved, the molecular basis of the changes in pore activity has not been investigated. The observed changes in transport capacity also indicate that the gating mechanism, located in the transporter element of the pore complex could have a significant regulatory role, but there is no information concerning either its composition or mode of action. The existence of different receptor mediated pathways, both for import and export could serve as a means of independently regulating the exchange of specific classes of macromolecules. In vivo experiments to analyze the relative activity of different pathways with changes in cellular activity are feasible, and should be performed.

The fundamental importance of nucleocytoplasmic exchanges in cell biology, the number of basic questions that remain to be answered, and the availability of molecular, genetic, and

cellular approaches, make this an especially exciting field.

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