

Protein transport into the nucleus

Veena K. Parnaik

Centre for Cellular and Molecular Biology, Hyderabad 500 007, India

The nucleus is partitioned from the cytoplasm in a eukaryotic cell by the double membrane of the nuclear envelope. The envelope is perforated by pores that regulate the nuclear transport of macromolecules. Nuclear proteins, after synthesis in the cytoplasm, must subsequently enter the nucleus. Several studies have established that these proteins contain highly basic nuclear localization signal sequences, which target them through the nuclear pores. Furthermore, recent evidence suggests that nuclear import of proteins critical for embryonic development, cell division and differentiation can be regulated in novel ways.

Eukaryotic cells are characterized by a defined nuclear envelope which partitions the nuclear contents from the surrounding cytoplasm. Karyophilic, or nuclear, proteins are transported into the nucleus via the nuclear pores in a highly selective manner. With the advent of improved techniques for studying nuclear localization of proteins and more information about the mechanisms of other cellular transport systems, there is currently considerable interest in studying nuclear transport processes. Specific nuclear transport of a protein has been shown to require the presence of an amino-acid sequence, the nuclear location signal or NLS, that confers the property of nuclear location. Nuclear entry probably occurs in at least two stages: there is an initial recognition event involving NLS, followed by an energy-dependent translocation of the nuclear protein through the pore. Although transport of proteins into the nucleus is basically a problem in cellular sorting of proteins to their correct destinations, nuclear transport has recently been shown to have the additional features of intricate regulatory controls. These play a crucial role in control of cell-cycle events and in regulation of gene expression during cell differentiation, especially during embryonic development.

In this review, I first present details of the structure of the nuclear envelope. I then discuss identification and characterization of NLS sequences, and current models of their mode of action, as well as other requirements for nuclear transport; present recent studies on proteins with complex requirements for transport and other features of regulated transport; and, finally, examine a possible role for nuclear transport in gene regulation. Several aspects of this topic have been ably reviewed by

others^{1,2}. The emphasis in this article will be on recent findings. Various features of RNA transport have been adequately reviewed earlier³, and will not be discussed here.

Structure of the nuclear envelope

The nuclear envelope (see Figure 1) is a double membrane structure, containing nuclear pores and nuclear lamina. The outer membrane is closely associated with the endoplasmic reticulum, whereas the inner membrane is embedded in a network of high molecular weight insoluble proteins that comprise the lamina, which in turn surrounds the peripheral chromatin^{4,5}. The space between the two membranes is continuous with the lumen of the endoplasmic reticulum. The inner and outer membranes are periodically spanned by nuclear pores, which act as transport channels between the nucleus and the cytoplasm. The major proteins of the envelope are the nuclear lamins (60–70 kDa) and a group of integral membrane proteins of molecular mass 45–55 kDa. Other proteins, including pore complex proteins, have been detected only by sensitive procedures such as monoclonal antibodies^{6,7} or by radiolabelled techniques⁸.

The nuclear pore complex

The nuclear pore complex is a large protein assembly with a mass of about 10^8 daltons and spanning about 1200 Å across the envelope. Roughly 10^3 – 10^6 pores are present per nucleus, rapidly metabolizing cells generally

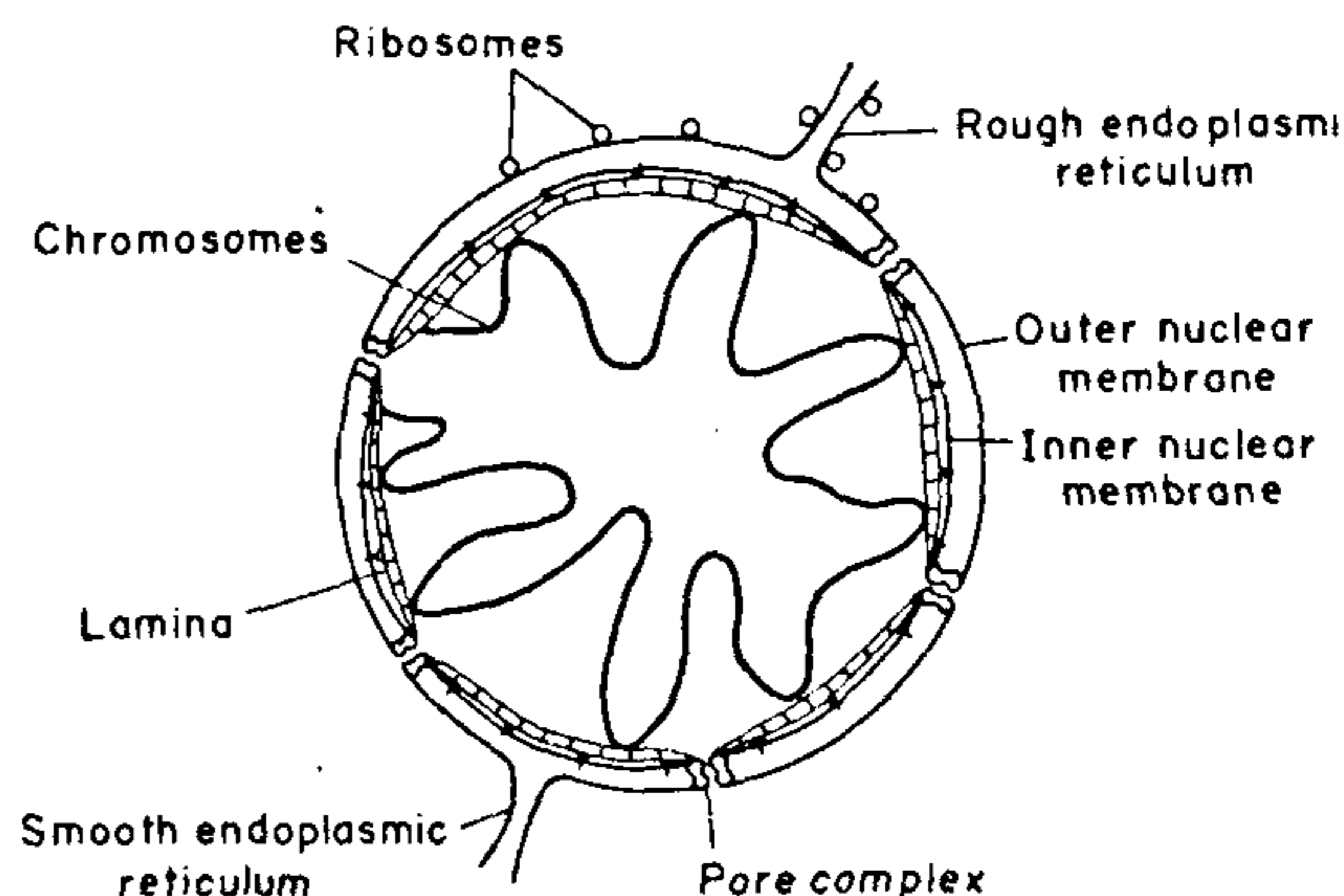


Figure 1. Diagrammatic representation of the nuclear envelope.

containing more pores than inert cells. The morphology of the pore complex is highly conserved across species. Electron-microscopic studies indicate that the pore consists of two outer rings of eight subunits, with one ring each on the cytoplasmic and nucleoplasmic faces of the pore, coplanar with the envelope⁹. Each subunit is connected to an inner ring of spokes that surrounds a central channel. Fibrils approximately 30 Å in diameter are seen to extend to over 200 Å from the surface of the pore. These may have a role in transport¹⁰. The pore channel appears to be actively involved in transport through a central 'transporter' assembly for signal-dependent transport of macromolecules. The resting diameter of the channel is 90 Å, thus allowing an upper size limit for passive diffusion of 20–40 kDa. However, recent studies have shown that even small nuclear proteins such as histones (~20 kDa) show facilitated, energy-dependent transport, unlike small non-nuclear proteins¹¹. On the other hand, the pore can actively transport very large particles such as karyophilic gold particles, ribonucleoproteins (RNPs) and pre-ribosomes. On the basis of image analysis of electron micrographs of frozen preparations of nuclear envelopes and nuclear pore complex–lamina structures, a 'double-iris' model has been proposed recently, which describes how the pore may enlarge to allow the passage of large particles¹². Each pore may contain up to 100 different proteins. However, only a small number of pore proteins have been biochemically well characterized. Table 1 gives a summary of their properties.

The nuclear lamina

The nuclear lamina is a ubiquitous component of the nuclear envelope in widely different species of vertebrates, invertebrates and yeasts. The lamina is composed of two major kinds of polypeptides; the A-type lamins, which become solubilized during mitosis and are represented by lamins A and C in most cells, and the relatively insoluble B-type lamin^{13,14}. Lamin B is strongly associated with the nuclear membrane, and remains bound to membrane vesicles during envelope

disassembly at the time of mitosis and subsequent reformation of the envelope. The lamina filaments are structurally very similar to the cytoplasmic intermediate filaments. The lamins contain an internal segment of ~350 amino acids which forms an alpha-helical domain conserved in intermediate filament-type proteins and is involved in formation of the filament backbone. The biochemical basis of the interaction of the lamina with the inner nuclear membrane is not clearly understood. Binding studies with purified lamin B and lamin-depleted nuclear membranes suggest that lamin B is anchored to the membrane via a 58-kDa receptor protein in avian and yeast cells¹⁵. Using protein cross-linking reagents of the bis(imidoester) class, Fatima and Parnaik¹⁶ have identified membrane-binding proteins of molecular mass 54, 50 and 45 kDa that are closely associated with the lamina of mouse liver nuclear envelopes. Preliminary evidence from binding assays with lamin B suggest that the molecular mass of the lamin-B receptor in the mouse system is 54 kDa, and this may be similar to the 54-kDa membrane-binding protein detected by cross-linking studies (S. Pandey and V. K. Parnaik, unpublished work).

The composition of the lamina can change significantly during development. The stage-specific expression of different lamins during early embryonic development has been studied in detail in amphibians. Prior to the mid-blastula transition in *Xenopus* embryos, a single maternally derived oocyte lamin, LIII, can be detected. After the mid-blastula transition, three somatic-cell lamins (LI, LII and lamin A) appear sequentially in the embryonic cells. LIII expression ceases by the tail-bud stage and selectively reappears only in muscle and neuronal cells, and in the female germ line¹⁷. In mammals, only lamin B is expressed during early development, whereas the A-type lamins are detectable once organogenesis has commenced¹⁸. Expression of a unique lamin has been observed in male germ-line cells¹⁹. The functional significance of lamina rearrangements is poorly understood but may be related to changes in the architecture of chromatin during different stages of development.

Table 1. Properties of known nuclear pore proteins.

Protein(s)	Molecular mass (kDa)	Properties	Reference
gp190	190	Membrane anchorage of pore complex	62
p62	62	Structural component	63*
Eight proteins	45, 54, 58, 63, 100, 145, 180, 210	Contain O-linked GlcNAc	6*
Seven proteins ('nucleoporins')	35, 40, 43, 62, 65, 69, 110	Contain O-linked GlcNAc	7*

*There are likely to be some common proteins described in these different studies.

Identification of NLS sequences

Since nuclear proteins are not translated as transient, precursor polypeptides, the mature form of the protein should contain a signal for nuclear localization within its molecular structure. Experimental evidence for the existence of a signal in a discrete polypeptide domain was first obtained with nucleoplasmin²⁰, a large pentameric protein involved in chromatin assembly and present in *Xenopus* oocyte nuclei in substantial amounts. Proteolysis of the pentamer produces a 'core' pentamer, which lacks the carboxyl 'tail' of each

subunit. When the 'tail' molecule is microinjected into *Xenopus* oocyte cytoplasm, it rapidly enters the nucleus; but the 'core' molecule is unable to do so. However, the 'core' molecule with a single intact subunit (with a 'tail') can enter the nucleus. These results clearly showed the existence of a signal in the 'tail' portion that was essential for nuclear entry. Further studies with nucleoplasmin have also been able to distinguish between the classical view, that nuclear transport of proteins occurs by passive diffusion through pores followed by their selective retention in the nucleus, and current models of selective entry of proteins. When 'core' and 'tail' molecules of nucleoplasmin were microinjected into nuclei, both remained in the nucleus. If the 'tail' regions were required for selective retention in the nucleus, then the 'core' molecules should have diffused back into the cytoplasm, but they did not. Thus the 'tail' region contains a nuclear entry signal, which has subsequently been shown to contain a highly basic sequence²¹.

Identification of amino-acid sequences responsible for nuclear localization has been achieved for several nuclear proteins. The earliest definitive results came from work on simian virus 40 (SV40) large T antigen, a tetrameric protein with a subunit molecular mass of 90 kDa. By generating specific point mutations, Kalderon *et al.*²² showed that conversion of Lys-128 to Thr abolished nuclear localization of T antigen. Deletion of Lys-128 and amino acids in its vicinity also had the same effect. These alterations, however, did not change the DNA-binding activity of T antigen. An independently isolated mutant T antigen²³, which was exclusively localized in the cytoplasm, was shown to have Lys-128 replaced by Asn. Fusion proteins containing a seven-residue NLS of T antigen (see Table 2) and a non-nuclear protein (pyruvate kinase)²⁴, and non-nuclear proteins (bovine serum albumin, immunoglobulin G) with a chemically cross-linked NLS²⁵ have been shown to be transported efficiently into the nucleus. Furthermore, native NLS confers nuclear localization on a protein that also bears a mutant sequence in which Lys-128 is replaced by Thr, thus confirming that failure of the mutant sequence to bring about nuclear locali-

zation is not due to nonspecific binding to some cytoplasmic component. Although there is no consensus NLS, several karyophilic proteins, such as SV40 large T antigen, nucleoplasmin²¹, adenovirus Ela protein²⁶ and SV40 polypeptides VP2 and VP3 (ref. 27), contain a signal sequence of a short stretch of highly basic amino acids flanked by proline or glycine. However, other signals present in proteins such as the yeast regulatory proteins MAT- α 2 (ref. 28) and GAL4 (ref. 29) are not homologous to the prototype SV40 large T antigen NLS, whereas NLS of the yeast ribosomal protein L3 shows limited homology³⁰ (see Table 2). It has been suggested that the kinetics of nuclear entry of the SV40 large T antigen may be enhanced by the presence of residues flanking the minimal NLS, which are also known to be targets of phosphorylation of the protein *in vivo* (but do not constitute a second NLS)³¹.

Complex signals have recently been found for some nuclear proteins. For example, the NLS of one of the polymerases of influenza virus³² appears to be a bipartite signal consisting of two positively charged sequences (lacking proline) some distance apart. Both regions have been shown to be essential for nuclear localization. Similarly, the two signals for polyomavirus T antigen together seem to be more efficient in its transport, though either one alone is partially functional³³. Studies with *Xenopus* protein N1 (ref. 34) and the adenovirus DNA-binding protein³⁵ indicate that these proteins also contain discontinuous signals. The most plausible role for multiple signals is that they function cooperatively in the different stages of translocation of the protein through the nuclear pore. An intriguing finding is that multiple copies of a mutant NLS of SV40 T antigen can induce nuclear localization of a conjugated protein³⁶. A word of caution should be added here regarding studies with fusion proteins or peptide-protein conjugates. Several factors appear to influence the role of NLS in such experiments: for example the number of peptides linked per molecule of protein³⁶, the type of cultured cells used in localization studies³⁷, and, in some cases, the protein to which the NLS is conjugated³⁸.

Table 2. Characteristics of some nuclear location signals.

Protein	Sequence*	Reference	Nature of NLS	Location in protein
SV40 large T antigen	P-K-K-K-R-K-V	22	Prototype	Middle
SV40 VP2/VP3	P-N-K-K-K-R-K-L	27	Prototype	Middle
AdV5 E1A	L-S-V-K-R-P-R-C-N	26	Prototype	C-terminal
Polyoma large T antigen	P-K-K-A-R-E-D	33	Bipartite	Middle
	V-S-R-K-R-P-R-P			Middle
Influenza virus PBI	R-K-R-R	32	Bipartite	Middle
	K-R-K-Q-R			Middle
Yeast MAT- α 2	M-N-K-I-P-I-K	28	Unique	N-terminal
Yeast L3	P-R-K-R	30	Prototype?	N-terminal

*Standard one-letter code for amino acids

Mechanism of protein translocation

The first direct evidence for involvement of the nuclear pore complex in protein transport was provided by Feldherr *et al.*³⁹ They showed that colloidal gold particles coated with nucleoplasmin rapidly accumulated in the nucleus upon microinjection into *Xenopus* oocyte cytoplasm, and could be seen to pass through nuclear pores in electron micrographs. However, gold particles coated with 'core' nucleoplasmin remained in the cytoplasm and did not associate with nuclear pore complexes. These experiments also gave the first indication that nuclear proteins interacted with nuclear pore proteins to change the diameter of the pore to facilitate their uptake, since the gold particles (diameter ~ 200 Å) were considerably larger than the pore orifice of 90 Å.

The involvement of an active transport system for the nuclear entry of proteins was first predicted by *in vitro* assays for nuclear uptake of purified proteins. These assays displayed a requirement for a signal sequence, ATP dependence, temperature dependence, and presence of an intact nuclear envelope^{40,41}. Some of these assay systems were also shown to be sensitive to wheat germ agglutinin (WGA), which could inhibit transport by binding to the *N*-acetylglucosamine moieties of certain pore proteins⁴¹. Recent studies with mouse nuclei⁴² as well as another report on yeast nuclei⁴³ have shown that proteins translated *in vitro* from SP6 plasmid-generated synthetic mRNAs can also be specifically localized to nuclei *in vitro*. These transport assays have the additional advantage of providing methods for studying the effects of various mutations in the NLS on transport without the associated problems of the *in vivo* instability of mutant proteins. *In vitro* assays have been widely used for transport-inhibition studies with antibodies to different pore-complex proteins to demonstrate the role of specific proteins in transport (discussed in detail a little later in this section).

From initial experiments designed to understand the role of the NLS it was apparent that the nuclear uptake of proteins could be separated into at least two distinct steps: a rapid, signal-dependent binding of the protein at the nuclear pore, followed by a slower, ATP-dependent and WGA-sensitive translocation of the protein across the pore^{44,45}. Since signal-dependent nuclear entry of proteins had earlier been shown to exhibit saturation kinetics, suggestive of a receptor-mediated process, recent studies in several laboratories have been aimed at identification of NLS receptors. The occurrence of NLS receptors was initially demonstrated by an indirect method, using antibodies raised to putative receptor sequences (anti-Asp Asp Asp Glu Asp) for the NLS for SV40 large T antigen in immunofluorescence studies with rat nuclei⁴⁶. These

results suggested the presence of NLS receptors of molecular mass 59 and 69 kDa in nuclear pores, and 65, 54 and 50 kDa in salt-detergent extracts of nuclear envelopes (but none in cytosol). More direct approaches, using chemical cross-linking studies, have identified a high-affinity NLS receptor of 60 kDa, which is present in nuclei of rat cells⁴⁷ and is also detected in the cytoplasm. In studies on identification of NLS receptors in mouse cells using photolabelled synthetic NLS peptides⁴⁸, we (Pandey and Parnaik⁴⁸) have demonstrated the presence of a high-affinity 60-kDa receptor in nuclei and envelopes, and three receptors (67, 53 and 47 kDa) of slightly lower affinity in envelopes, but none in cytosol. We have localized the 60- and 67-kDa receptors to the nuclear pores and the 53- and 47-kDa receptors to the periphery of the envelope or pores. Other photolabelling studies have detected the presence of NLS receptors of 76, 67, 59 and 58 kDa in nuclear envelopes of rat cells⁴⁹. The high-affinity 60-kDa NLS receptor is consistently observed; however, detection of other receptors appears to depend on the methodology used. NLS receptors have also been detected in yeast cells⁵⁰. As cytosolic receptors have been identified by certain methods but not by others, there is some question regarding their presence and possible role in nuclear transport. This is discussed in more detail in the next section.

The availability of monoclonal antibodies to nuclear pore proteins has led to identification of a group of pore proteins (named 'nucleoporins') with O-linked *N*-acetylglucosamine residues that appear to play an essential role in nucleocytoplasmic transport⁷. One member of this class of proteins, NUP1, has been shown to be essential for cell viability in yeast⁵¹. Akey and Goldfarb⁵² used WGA and a pore-specific monoclonal antibody (against a 62-kDa protein) to localize the nucleoporins to a discrete structure in the centre of the pore, termed the transporter. Using cryoelectron microscopy, they observed that gold particles with adsorbed nucleoplasmin bound to the transporter and also to the pore periphery. They propose that nucleoplasmin first binds at the periphery of the pore and then to the transporter, before finally being translocated through the pore. At present it is not known whether any of the nucleoporins detected by monoclonal antibodies correspond to any of the NLS receptors that have been identified.

Regulation of nuclear localization of proteins

For certain proteins, possession of an NLS may be necessary but not sufficient to ensure nuclear entry. For example, the NLS may be masked by interaction with other proteins, so that the complex does not enter the nucleus. Subsequent ligand binding or other protein

modification such as phosphorylation may dissociate the complex and expose the NLS, thereby promoting nuclear uptake. Even if the NLS is exposed and active, other signals on the nuclear protein may negate its effect by anchoring the protein in the cytoplasm till it is required in the nucleus. Thus there exist several possibilities for regulating nuclear transport of proteins, by both extracellular and intracellular events. These are represented schematically in Figure 2. When the nuclear protein concerned is a transcription regulatory factor, one can envisage how gene expression could be regulated in various ways at the level of nuclear entry of the transcription factor in response to a particular signal. This has been illustrated in an elegant manner with the transcription factor NF- κ B, which is involved in the activation of immunoglobulin kappa chain gene transcription in B lymphocytes⁵³. This factor is detectable in nuclear extracts of activated lymphocytes but is present in the cytoplasm of unstimulated cells as a complex with a cytoplasmic protein I- κ B (65 kDa). The system is activated by phosphorylation, which depends on protein kinase C. The most likely target is I- κ B, and the modification results in the dissociation of the complex and energy-dependent transport of NF- κ B into the nucleus, where it then binds to a kappa-chain enhancer sequence. Another example is that of the steroid-receptor class of transcription factors⁵⁴. Although there is still some controversy as to where they are normally located in the cell, a consensus seems to be emerging that the unliganded receptor is complexed with heat shock protein HS90 in the cytoplasm. When a steroid molecule binds to its receptor, this complex dissociates and the receptor

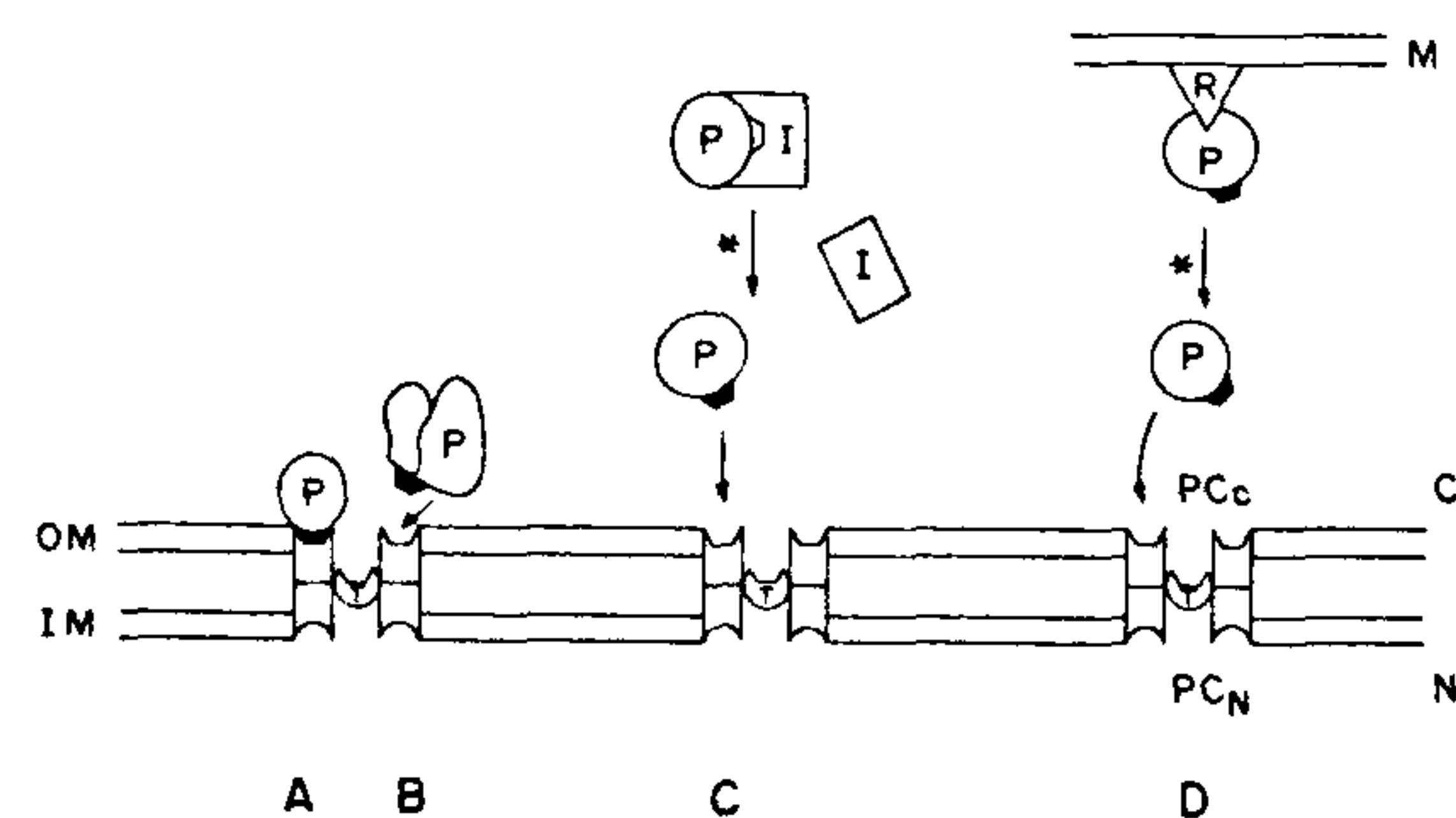


Figure 2. Some pathways for regulation of protein entry into the nucleus. A. Protein (P) bound to the pore complex via its NLS (■); B, protein with a bipartite NLS binding to the pore; C, protein bound to a cytoplasmic inhibitor protein (I) which masks the NLS and prevents transport till an extracellular/intracellular stimulus (★) dissociates the complex and un masks the NLS; D, protein bound to a cytoplasmic anchor (R), probably a membrane protein, which prevents transport although the NLS is active, until an extracellular/intracellular stimulus releases the protein, which can now enter the nucleus. PC_N; Pore complex, nucleoplasmic face; PC_C, pore complex, cytoplasmic face; T, central transporter assembly; OM, outer nuclear membrane; IM, inner nuclear membrane; N, nucleus; C, cytoplasm; M, membrane.

enters the nucleus and is able to bind to DNA. A novel kind of regulation is seen with the potent mitogen platelet-derived growth factor (PDGF)⁵⁵. The A and B chains comprising this protein both contain an NLS at their C-termini. However, shorter versions of the A chain exist, in which the region containing the NLS is spliced out; these do not accumulate in the nucleus. These different kinds of A chains are found in different cell types, where they may display different mitogenic activities.

A recent study with the *fos* oncogenes has important implications for control mechanisms involved in tumorigenesis⁵⁶. The product of the *c-fos* proto-oncogene, which is a transcriptional factor that acts as a master switch for controlling cell division, is normally found in an inactive state in the cytoplasm of cultured cells, in association with another protein (not characterized). The c-Fos protein translocates to the nucleus upon stimulation by serum growth factors or addition of cAMP to the cells. On the other hand, v-Fos proteins, which are tumorigenic mutants of c-Fos, are always found in the nucleus. This bypass of control at the level of nuclear entry may be contributing to the tumorigenic potential of the v-Fos proteins by their ability to stimulate transcription of genes required for cell division constitutively. In the control of normal cell-cycle events, there are well-known examples of proteins that undergo a change in their location during the cell cycle. For example, cyclin and p34^{cdc2} enter the nucleus as yeast cells enter mitosis, but are otherwise found in the cytoplasm⁵⁷. The mechanism of this relocation is totally unknown.

There is another category of proteins, called 'shuttling' proteins since they appear to migrate constantly back and forth between the nucleus and cytoplasm. Two major nucleolar proteins (92 kDa and 38 kDa) are believed to play a role in nucleocytoplasmic transport of ribosomal components and have been shown to shuttle between the nucleus and cytoplasm, though they are normally resident in the nucleus⁵⁸. A possible mechanism for this shuttling is that the two proteins exit from the nucleus as part of a preribosomal particle, then dissociate from this complex in the cytoplasm and re-enter the nucleus in a signal-dependent and energy-dependent manner.

Convincing evidence has been presented by Breeuwer and Goldfarb¹¹ for energy-dependent transport of small nuclear proteins such as histones. Their major finding is that nuclear entry of histone H1 (~20 kDa) after microinjection into the cytoplasm of mammalian cells can be arrested by energy depletion or chilling of cells. This is not observed with small, non-nuclear proteins, which diffuse into the nucleus in an energy-independent manner. The arrest of H1 import into nuclei of chilled cells can be overcome by excess H1 but not excess of a non-nuclear protein, suggesting that a cytoplasmic

protein may be titrated out with excess H1. The authors postulate that such cytoplasmic H1-binding proteins may regulate nuclear transport of H1. Other reports also suggest the presence of cytoplasmic proteins that can bind to NLS sequences⁴⁶. The role of such cytoplasmic proteins in influencing transport of proteins that are normally resident in the nucleus (such as histones or SV40 large T antigen) is not yet clear.

Role in embryonic development

During the development of an embryo, the processes of tissue differentiation require tissue-specific expression of certain genes. This is governed by the activity of various transcription factors, which may increase or decrease gene expression. The DNA-binding activity of transcription factors, in turn, depends on their effective nuclear concentration. This concentration can be controlled either by new synthesis or by nuclear localization of preexisting factors from the cytoplasm. The latter phenomenon has been elegantly illustrated in establishment of the dorsoventral axis in the *Drosophila* embryo⁵⁹⁻⁶¹.

The pattern elements defined by the dorsoventral system are the mesoderm, the ventral ectoderm which gives rise to the central nervous system, and the dorsolateral ectoderm from which the trachea is derived. This pattern is established by the concerted action of twelve genes: eleven of these comprise the dorsal group and a mutation in any of them leads to development of all cells according to a dorsal fate. Loss of function of the twelfth gene, *cactus*, causes ventralization. The putative morphogen, the dorsal protein (from the dorsal group), is evenly distributed in the egg. However, during cleavage stages the dorsal protein is found to be distributed between cytoplasm and nuclei in a gradient of nuclear localization. It is found in the nuclei of ventral cells and the cytoplasm of dorsal cells, with a continuous gradient in between. But what determines this gradient? The most reasonable possibility is that positional information in the dorsoventral axis is measured by the Toll protein (an integral plasma-membrane protein coded by one of the dorsal-group genes) from signals left by follicle cells in the inner membrane of the eggshell, and then this is passed onto the dorsal protein via two other proteins coded by dorsal-group genes *pelle* and *tube*, to induce its nuclear entry. Loss-of-function mutations in any of the dorsal group of genes results in an inactive dorsal protein localized solely in the cytoplasm. *Cactus*, on the other hand, functionally behaves as if it inhibits the nuclear entry of the dorsal protein. The dorsal protein, which is homologous to the product of the vertebrate cellular proto-oncogene *c-rel*, contains a likely NLS but the protein is normally localized in the cytoplasm of

cultured cells. Deletion of just eight residues from its C terminus results in its nuclear localization. Moreover overexpression of the wild-type protein in cultured cell results in its spillover into the nucleus, as though it were saturating a cytoplasmic binding protein. The Toll protein may act in concert with other dorsal-group proteins to dissociate the dorsal protein from its cytoplasmic anchor (possibly the cactus protein) and induce its nuclear entry.

Conclusions and prospects

Two major conclusions have emerged from research on nuclear transport carried out in several laboratories over the last ten years. Firstly, the basic mechanisms involved in nuclear transport of proteins are broadly similar to other cellular transport processes, in that there is a signal sequence-dependent and energy-dependent transport of proteins across a membrane barrier. Secondly, this basic mechanism can be modulated in several ways to regulate the entry of proteins, especially those involved in replication and transcription of DNA.

However, certain important questions still remain to be answered. What is the biochemical nature of the nuclear pore? How does the pore orifice expand to allow the translocation of large proteins? Are there different receptor 'domains' in the pore for recognition of different classes of signal sequences? Answers to these and related questions on pore structure would require new methods of structural analysis owing to the technical difficulties in obtaining isolated pores or purifying pore proteins by standard procedures. Further studies on regulated transport of different proteins should give new insights into the different ways in which nuclear transport and gene activity can be modulated.

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Factors in host-parasite interactions and immunological unresponsiveness in leishmaniasis

Ishaya Haruna Nock and Jayashri Devi Sharma

School of Environmental Sciences, Jawaharlal Nehru University, New Delhi 110 067, India

Antigenic molecules on the surface membrane of the leishmanias serve as the key to the success of the parasites in residing and initiating a disease process within the hostile macrophage/lymphocyte immune environment of a mammalian host. Primarily, major surface antigens such as the 63-kilodalton glycoprotein (gp63), lipophosphoglycan (LPG) and acid phosphatase (AP) appear to feature prominently in the process. These molecules may influence release of secondary factors such as prostaglandin E (PGE) and other endogenous

peptide molecules, which together initiate and sustain immunological unresponsiveness and allow establishment of infection.

Most parasites reside in safety in their various hosts by circumventing the host defence apparatus that is potentially capable of destroying them. This is achieved through various dynamic processes, described by Bloom¹ as 'games parasites play'.