

# STRUCTURE AND FUNCTION OF THE NUCLEAR PORE COMPLEX

*Douglass J. Forbes*

Department of Biology, University of California at San Diego, La Jolla, California  
92093

KEY WORDS: nuclear transport, nuclear signal sequence, nuclear pore

---

## CONTENTS

INTRODUCTION .....	495
NUCLEAR TRANSPORT .....	496
<i>An Overview</i> .....	496
<i>Structure of the Nuclear Pore Complex</i> .....	498
<i>Protein Import</i> .....	500
<i>Protein Export</i> .....	508
<i>RNA Export</i> .....	509
<i>RNA Import</i> .....	510
<i>Genetic Approaches to RNA and Protein Transport</i> .....	511
<i>Control of Nuclear Transport</i> .....	512
NUCLEAR PORE PROTEINS .....	512
<i>Proteins of the Vertebrate Nuclear Pore</i> .....	512
<i>Proteins of the Yeast Nuclear Pore</i> .....	515
NUCLEAR PORE ASSEMBLY AND DISASSEMBLY .....	516
<i>Nuclear Pore Assembly and Reconstitution</i> .....	516
<i>Disassembly at Mitosis</i> .....	518
<i>Annulate Lamellae</i> .....	518
FUTURE PROSPECTS .....	519

## INTRODUCTION

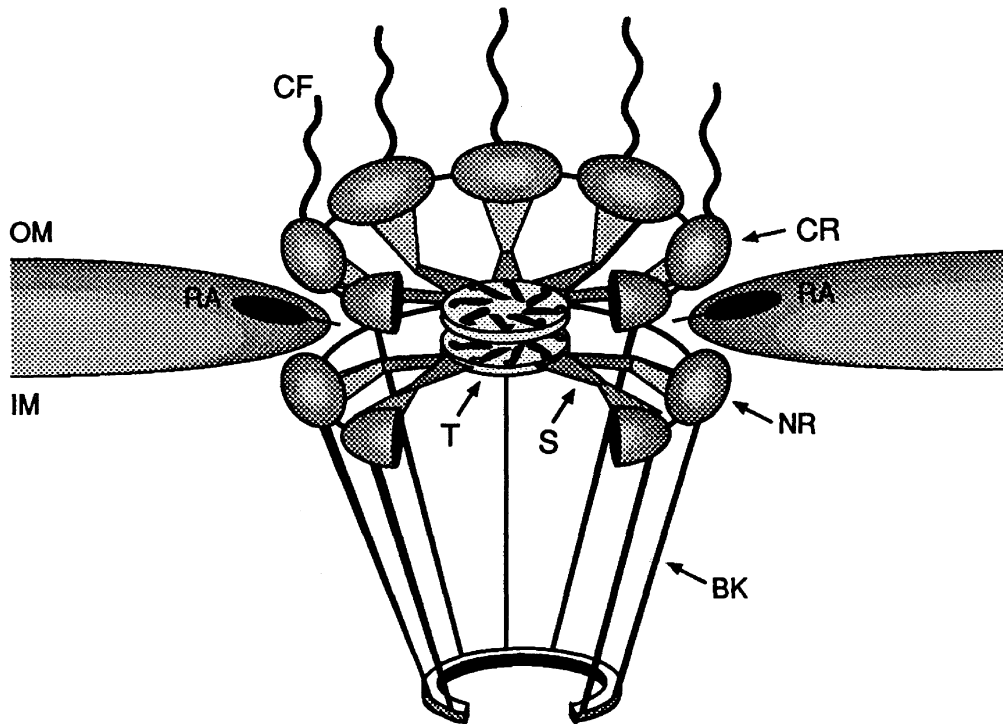
The cell is a dynamic entity; molecular messages of many sorts converge upon the cell to evoke immediate intracellular changes. In the cytoplasm these changes induce a cascade of events most often directed towards the nucleus.

Studies of developmental determination, signal transduction, oncogenesis, and viral infection have increasingly focused on nuclear transport as a point of regulation. The nuclear pore acts as primary arbiter of which signals enter the nucleus and which remain behind. Despite occupying this central position, the nuclear pore is one of the least understood of major cellular structures. However, significant progress has been made in the past five years toward a greater understanding of the pore and nuclear transport. New pore proteins have been identified, the genes for several have been cloned, and a detailed mechanism for transport has been proposed. The nuclear pore has been reconstituted *in vitro*, one or more signal sequence receptors have been identified, and genetic mutants in pore proteins have been isolated. Unexpectedly, a new basket-like structure has been found attached to the inner side of the nuclear pore. In addition, a candidate signal for mRNA export from the nucleus, as well as distinct but related signals that promote the import of small nuclear RNAs have been identified. Numerous novel examples of the regulation of transport in development and viral infection have been observed. This review affords, in a limited space, an overview of the recent progress on nuclear pore structure and function, potential pitfalls of the approaches used, as well as future prospects for progress. The reader is also referred to two excellent comprehensive reviews (Garcia-Bustos et al 1991; Akey 1992) and to more specialized reviews for further detail (Dingwall & Laskey 1991; Goldfarb & Michaud 1991; Hunt 1989; Miller et al 1991a; Mattaj 1990; Maquat 1991; Nigg et al 1991; Silver 1991; Feldherr 1992; Goldfarb 1992; Izaurralde & Mattaj 1992; Nigg 1992; Richter & Standiford 1992; Yamasaki & Lanford 1992a,b).

## NUCLEAR TRANSPORT

### *An Overview*

Traversing the double membranes of the nuclear envelope are numerous channels, the nuclear pores, which link the nucleus to the cytoplasm (Figure 1). In the 1970s the pore was found to contain both a 90 Å channel that allowed for the passive diffusion of small molecules, as well as a larger regulated channel that transported nuclear proteins and RNAs. The existence of an aqueous channel that allows the free diffusion of small proteins, metabolites, and even oligonucleotides (Chin et al 1990) was first determined by the injection of radioactively labeled non-nuclear proteins of various sizes into the cytoplasm of *Xenopus* oocytes (see Garcia-Bustos et al 1991 for a review). Large proteins were excluded from the nucleus, while small proteins (<20–40 kd) quickly equilibrated between nucleus and cytoplasm (Paine & Horowitz 1980). Peters and colleagues (Lang & Peters 1984; Lang et al 1986; Peters 1986) performed similar injections with a series of graded dextrans,



*Figure 1* A structural model of the nuclear pore is shown with a portion at the front of the pore cut away to show detail. Seven of the eight globular components of the cytoplasmic ring of the pore (*CR*) are shown connected to spokes (*S*), as are four of the eight globular components of the nucleoplasmic ring (*NR*). The spokes are separated by 90 Å channels and support a central transporter (*T*) of 360–380 Å. A potential structure for the transporter consisting of two irises of eight arms each, as hypothesized by Akey (1990), is shown, with the transporter in the closed form. A central 90 Å channel is also present. Radial arms (*RA*) extend into the luminal space between the outer (*OM*) and inner (*IM*) nuclear membranes and connect to the pore scaffold (= *CR* + *NR* + *S*). The diameter of the pore is 1200 Å excluding the radial arms, 1450 Å with the radial arms. Cytoplasmic filaments (*CF*) are shown extending from the cytoplasmic ring. A basket-like structure of filaments (*BK*) extends from the nucleoplasmic ring of the pore. It should be noted that the spokes in the drawing are in actuality much thicker; in addition, the opposite rings of spokes are normally in close contact. They are drawn in this fashion to promote the visualization of individual structures. The drawing is a modified version of models proposed by Unwin & Milligan (1982), Akey (1990, 1992), and Hinshaw et al (1992).

which clearly defined the diffusion channel as having a diameter of 90–110 Å. Subsequent structural work indicates that there may be eight such 90 Å channels radially arrayed around the central regulated channel (see below), which makes the analogy of the nuclear pore as a molecular sieve a particularly apt one.

In the resting state, the pore acts as a physical barrier to all large proteins. It is now clear that nuclear proteins contain a signal sequence that allows them to be specifically imported through the pore. Even gold particles up to 250 Å in diameter, if coated with signal sequence-bearing nuclear proteins, are transported into the nucleus through the pore, as visualized by electron

microscopy (Feldherr et al 1984; Dworetzky & Feldherr 1988). These experiments indicate that translocation involves an opening of the central channel of the pore from 90 up to 260 Å (Dworetzky et al 1988). Nucleoplasmin-coated gold particles transit exclusively through the central 50–260 Å portion of the 1200 Å pore, thus indicating that this region is the site of active transport. This conclusion is supported by visual observations of large RNP particles exiting from the nucleus (Scheer et al 1988; Mehlin et al 1991). From the fidelity of translocation, it is thought that the pore does not open transiently to allow any or all non-specific large molecules to enter, but opens only wide enough to allow passage of the signal sequence-bearing protein or macromolecule whose signal initiated the opening.

ATP has been shown to be essential for nuclear import (Newmeyer et al 1986 a,b). In its absence, fluorescently labeled nuclear proteins are excluded from the nucleus. Interestingly, under these conditions a significant fraction of the nuclear protein binds to the cytoplasmic rim of the nucleus. Indeed, nucleoplasmin-coated gold particles in the absence of ATP bind to the nuclear pore on or near the spokes and transporter, both in vitro (Newmeyer & Forbes 1988) and in vivo (Richardson et al 1988), which demonstrates that this is likely an arrest at an intermediate step in transport. These experimental manipulations have allowed dissection of nuclear transport into two steps: (a) pore binding, an ATP-independent step, and (b) translocation through the pore, which requires ATP. Pore binding was seen only when a wild-type signal sequence was present, indicating that this step is signal sequence-dependent (Newmeyer & Forbes 1988; Richardson et al 1988). Incubation at low temperature (4°C) blocks transport after the pore-binding step, consistent with translocation being the only energy-requiring step of the two (Richardson et al 1988; Breeuwer & Goldfarb 1990). It was further found in injected oocytes that gold particles arrested at the binding step often line up along filaments extending from the pore into the cytoplasm (~36 Å diameter × 2000–5000 Å long), which suggests that these may be tracks directed to the pore (Richardson et al 1988).

### *Structure of the Nuclear Pore Complex*

The majority of what is now known of the physical structure of the pore has been gained from electron microscopic studies (Gall 1954; see Franke 1974, Akey 1992 for reviews). The nuclear pore is a large and complex structure of 124 million daltons, or approximately 30 times the size of an eukaryotic ribosome (Reichelt et al 1990). It is 1200 Å in diameter and 500 Å in thickness, as it spans the two nuclear envelopes. The nuclear pore complex consists of four separate structural elements: (a) the scaffold, which includes the majority of the pore as seen in traditional EM studies (pre-1989), (b) the central hub or transporter of the pore, which appears to carry out the functions

of active transport of proteins and RNAs, (c) short thick filaments attached to the cytoplasmic side of the pore, and (d) a newly discovered basket attached to the nucleoplasmic side of the pore (Figure 1).

The basic organization of the pore scaffold at a resolution of 90 Å was established by electron microscopy of nuclear envelopes manually dissected from *Xenopus* oocytes (Unwin & Milligan 1982). When observed in cross-section, the 1200 Å scaffold appears as a stack of three closely apposed rings: the cytoplasmic ring, the nucleoplasmic ring, and a central ring of thick spokes. Each ring has eightfold symmetry. The spokes are connected at their inner edge and support a central hub of ~360–380 Å. Interspersed between the spokes are large 90 Å aqueous channels, which presumably allow the observed passive diffusion of small proteins and metabolites between nucleus and cytoplasm (Milligan 1986). Subsequent refinement of this structure has produced a three-dimensional map that divides the scaffold into a minimum of 64 subunits (Hinshaw et al 1992). The mass of the scaffold portion of the pore (112 md; Reichelt et al 1990), if divided by 64, gives an average value of 1750 kd for an average small subunit, further emphasizing the large size of the pore complex (Hinshaw et al 1992). A two-dimensional model derived from image analysis of frozen hydrated nuclear pores largely agrees with these studies, but indicates that the small subunits can be further resolved by electron microscopy into even smaller subunits (Akey 1989). Thus the basic pore scaffold, which represents the majority of the pore mass, can be perceived of as constructed from many smaller non-identical modules. The scaffold of the pore is thought to (a) maintain the fusion of the two nuclear membranes that creates the 900 Å hole in the nuclear envelope, (b) provide the 90 Å diffusional channels, and (c) support the smaller central transporter that regulates actual import and export.

Elucidation of the structure of the central transporter of the nuclear pore has been derived from analyzing nuclear pores preserved and photographed in vitreous ice, with subsequent eightfold averaging of the EM images obtained (Akey & Goldfarb 1989; Akey 1990, 1991, 1992). This rapid freezing technique has allowed retention of a structure (the central plug) that was often lost in previous studies, and which at that time was thought to be a macromolecule in transit through the pore. This 360–380 Å central plug, which was termed the transporter, is now seen as a proteinaceous ring, which can be further resolved within individual pores into one of four recognizable and distinct forms. Each form has eightfold symmetry and, by assuming that they represent sequential intermediates in nuclear import, the authors have placed these forms as a model for transport in the following order: a nuclear protein first binds at the periphery of the transporter ring; it then moves to the central channel where it docks and induces the channel to open; lastly, the protein translocates through the open channel into the nucleus. Akey (1990, 1992)

has further elaborated his image analysis of the transporter, which predicts that the structural model best fitting the four observed forms of the central transporter would consist of two irises of eight arms each. The two irises are predicted to be stacked atop one another and to open sequentially, each like the diaphragm of a camera, to let a nuclear protein or RNA through (Figure 1). In response to a signal sequence for import, the first iris would open, let a nuclear protein pass into the pore, the second iris would then open, further passage would ensue, the first iris would close, then the second, and translocation would be complete. Such a mechanism would explain why the pore has such high fidelity of transport and does not allow the inward leakage of inappropriate proteins. The arms of the irises are further suggested to be mechano-ATPases to explain their proposed ability to pivot during the opening of the pore. [A separate hypothesis is that the transporter is a cytoplasmic vault docked in the pore (Rome et al 1991).] The double iris model is both provocative and speculative. Further work will be required to address this interesting model.

Recent electron microscopic evidence indicates that the pore also is in contact with important accessory structures. Individual pores appear to be connected to one another by the nuclear lamina and by an additional set of pore-connecting fibers (Steward & Whytock 1988; Allen & Douglas 1989). On the cytoplasmic face of the pore, thick fibers ( $\sim 33$  Å diameter) that extend into the cytoplasm have been observed by low voltage scanning electron microscopy (Ris 1991). Several strong indications that such filaments exist (Scheer et al 1988) and are a staging area for nuclear proteins to bind to prior to transport (Richardson et al 1988) have previously been observed. On the nucleoplasmic side of the pore, exciting and convincing evidence for a large basket-like structure has also emerged from the SEM study of Ris (1990, 1991). This basket structure appears to consist of eight filaments 1000 Å in length, extending from the nucleoplasmic ring of the pore to a smaller 600 Å ring (Figure 1; Ris 1990, 1991; Jarnik et al 1991; Jarnik & Aebi 1991; see also Scheer et al 1988). This basket disassembles in the absence of  $\text{Ca}^{2+}$  and reforms when  $\text{Ca}^{2+}$  is added (Jarnik et al 1991; Jarnik & Aebi 1991), which may be why the structure was not observed in previous studies in  $\text{Ca}^{2+}$ -free buffers. The function of the basket is the subject of speculation at this point, but its nucleoplasmic location may indicate a role in RNA export.

### *Protein Import*

**IDENTIFICATION OF SIGNAL SEQUENCES FOR PROTEIN IMPORT** Extensive progress has been made in the identification of the signal sequence for nuclear protein import. A nuclear signal sequence may occur anywhere within the sequence of a protein and is not removed during transport; this allows reentry of the protein into the nucleus at the end of each mitosis. Such sequences are,

in general, positively charged, short in length, and contain a helix-destabilizing amino acid such as proline or glycine (Chelsky et al 1989; Richter & Standiford 1992). There is, unfortunately, no consensus nuclear signal sequence. The canonical signal to which all others have been compared is that of the SV40 T antigen, Pro-Lys-Lys-Lys-Arg-Lys-Val, as defined by deletion analysis (Lanford & Butel 1984; Kalderon et al 1984a,b). Within this sequence, mutation of the second lysine to threonine greatly reduces import. This mutant sequence (Pro-Lys-**Thr**-Lys-Arg-Lys-Val) is frequently used as a negative control both in vivo and in vitro. However, because of the disruption of the linear sequence of charge and the overall reduced charge, this peptide may be an inadequate control in biochemical studies where simple binding is assessed, and a reversed wild-type peptide may be a better choice (Adam et al 1989).

Most of the subsequently identified signals have also been found by deletion analysis, and many resemble the SV40 T antigen signal sequence. Many others, however, more closely resemble the first identified signal sequence, that of the *Xenopus* protein nucleoplasmin, initially defined by selective protease digestion (Dingwall et al 1982). The nucleoplasmin gene has been cloned (Burglin et al 1987; Dingwall et al 1987) and its signal sequence found by subsequent deletion analysis to be larger (16 amino acids) and more complex than that of the T antigen, consisting of two interdependent basic domains separated by 10 spacer amino acids (Dingwall et al 1988). Mutations can be tolerated in either basic domain without destroying the targeting ability, but cannot be tolerated in both (Dingwall et al 1988; Robbins et al 1991). It is now thought that the bipartite nucleoplasmin signal sequence will be the type most often found in other proteins (Dingwall & Laskey 1991).

An intriguing variation on the way in which signals target proteins to the nucleus has been found in the rat glucocorticoid receptor. This protein possesses a signal sequence not unlike that of the SV40 T Ag, but also contains a second novel signal domain of 255 aa. This signal domain becomes active only in the presence of the appropriate hormone (Picard & Yamamoto 1987; Picard et al 1990). The T antigen-like signal sequence is silent in the context of the intact receptor, possibly masked by the hormone-dependent signal domain. Similar hormone-dependent signal domains have been found in the human androgen receptor (Simental et al 1991) and in the rabbit progesterone receptor (Guiochon-Mantel et al 1989), but not in the estrogen receptor (Picard et al 1990). Thus there are at least three types of motifs in multicellular organisms that can target proteins to the nucleus; it seems likely that others exist. An indication that this may be so and that there may be species differences comes from consideration of the N-terminal signal sequence for the yeast MAT  $\alpha$ 2 protein, Lys-Ile-Pro-Ile-Lys (Hall et al 1984).

This sequence differs significantly from the above signals, and while functioning in yeast, fails to function in mammalian cells (Lanford et al 1990). For further information on and comparison of signal sequences, several recent reviews provide a more comprehensive discussion of the full panoply of nuclear signal sequences (Garcia-Bustos et al 1991; Dingwall & Laskey 1991; Richter & Standiford 1992; Yamasaki & Lanford, 1992).

A number of studies have demonstrated that a true signal sequence is able to induce nuclear import when artificially added to a non-nuclear protein. Nuclear signal sequence peptides can either be covalently crosslinked to a marker protein, or the DNA sequence encoding the signal can be fused to a marker gene and the resulting fusion protein assayed for import. One study employing the latter molecular genetic approach found that the SV40 T antigen signal sequence was capable of targeting pyruvate kinase to the nucleus when inserted into various positions within the protein as long as it was not buried in the interior (Roberts et al 1987). This argues that the accessibility of the signal is important and such a contextual effect has been observed by others (Nelson & Silver 1989). Despite this constraint, the receptor system that recognizes the signal sequence must be remarkably liberal; nuclear signal sequence peptides, when crosslinked to internal lysine groups of non-nuclear proteins, can confer nuclear entry even in this unusual nonlinear conformation (Goldfarb et al 1986; Lanford et al 1986; Yoneda et al 1987a; Chelsky et al 1989). As stated above, even large gold particles (100–260 Å), when coated with proteins bearing chemically crosslinked signals, induce the pore to expand its resting channel diameter and are easily transported through the pore. Interestingly, by using nuclear proteins with increasing numbers of signal sequences to coat the particles, it was found that the more sequences a protein carried the wider the pore channel opened, to a maximum of ~260 Å (Dworetzky et al 1988). The rate of import is also increased as the number of signal sequences increases per protein (Lanford et al 1986; Roberts et al 1987; Dworetzky et al 1988; Finlay et al 1989; Lanford et al 1990). It is perhaps to take advantage of this effect of multivalency that many native proteins bear more than one signal sequence (Richardson et al 1986; Dang & Lee 1988; Morin et al 1989; Underwood & Fried 1990), while others oligomerize and in doing so achieve a similar multivalency (Dingwall et al 1982; Peters 1986).

Modulation of the activity of a given signal sequence can also occur. For example, the rate of nuclear transport of a fusion protein bearing a single SV40 T antigen signal sequence can be dramatically increased if flanking T antigen sequences, which serve as sites of phosphorylation, are included in the fusion protein (Rihs & Peters 1989). Phosphorylation of the flanking sequence may act by increasing the affinity for a signal sequence receptor. A casein kinase II site present in this flanking sequence is essential for the rate increase, as shown by mutation, and may indeed be present near the signal



sequences of many proteins (Rihs et al 1991). Interestingly, the final level of accumulation is the same in the presence or absence of the flanking sequence; only the rate is affected. In other analyses, a *cdc2* kinase phosphorylation site was also found near the T antigen signal sequence. Phosphorylation of this site has no effect on the rate of import, but instead reduces the final extent of nuclear accumulation of the fusion protein (Jans et al 1991). Modification of individual signal sequences or of flanking regions may thus play a greater role in import than was originally thought.

**SIGNAL SEQUENCE RECEPTORS** The existence of nuclear signal sequences predicts that there must also exist one or more receptors for these signals. The findings that nuclear transport can be saturated by free signal sequence (Goldfarb et al 1986), that there exists a mutant SV40 T antigen protein that interferes with the import of wild-type T antigen (Schneider et al 1988), and the demonstration that small nuclear proteins do not diffuse into the nucleus at low temperatures but behave as if they are complexed with a large carrier protein (Breeuwer & Goldfarb 1990) are a few of the results that indicate there is indeed a nuclear signal sequence receptor. Theoretically, the receptor or receptors might be permanent proteins of the pore or, alternatively, cytosolic proteins that carry nuclear proteins to the pore, in much the same way transport to the ER involves the delivery of nascent proteins by a signal recognition particle (SRP). Gerace and colleagues have found two cytosolic candidates for a nuclear signal sequence receptor in rat (60 and 70 kd) by searching for cellular proteins that can be crosslinked to radioactively labeled SV40 T Ag signal sequences (Adam et al 1989). They have more recently isolated from a bovine erythrocyte cytosol a cluster of related proteins (54–56 kd) in large quantities and have shown that these proteins stimulate transport two to threefold when added to an *in vitro* nuclear transport assay (Adam & Gerace 1991). The putative receptor presumably functions by binding the signal sequence of a nuclear protein and carrying that protein to the pore, either by diffusion or, perhaps, by a more directed but as yet undiscovered means, such as along cytoskeletal tracks (Adam & Gerace 1991). A further possibility is that the receptor then goes on to ferry the protein through the pore.

In a separate and interesting approach, Yoneda et al (1988) prepared the negatively charged mirror image peptide of the SV40 T Ag signal sequence, Asp-Asp-Asp-Glu-Asp, which they predicted might be present in a putative receptor. They raised polyclonal antisera to this peptide and found that the antisera labeled the rat nucleus in a punctate pattern at the nuclear rim. When injected into cells, the antisera blocked the subsequent import of nucleoplasmin. They have since isolated a protein of 69 kd from rat nuclear envelopes, using first an antibody affinity column (anti-DDDED), followed by a nucleoplasmin affinity column (Imamoto-Sonobe et al 1990). One caveat of this

study is that the antibody to Asp-Asp-Asp-Glu-Asp can, under certain circumstances, recognize multiple proteins; the authors await the production of an antiserum raised to the p69 protein itself to demonstrate that monospecific anti-p69 antisera can also block nuclear transport (Imamoto-Sonobe et al 1990).

The two approaches above have used functional criteria that suggest the proteins in question are involved in transport. A number of other groups have used either crosslinking or ligand-blotting strategies to identify potential signal sequence receptors. In humans, a single 69 kd protein was identified (Li & Thomas 1989), while in rats, multiple proteins of 55, 70, 100, and 140 kd were detected by crosslinking to various nuclear signal sequences (Yamasaki et al 1989; Pandey & Parnaik 1991). One of the problems that has arisen during all of the studies above and is a caveat for others interested in pursuing signal receptors is that the signal peptides used are small, relatively general peptides. As such, they might be expected to interact with proteins whose intracellular function is to bind to many peptides. Such proteins include the chaperone hsc70 and protein disulfide isomerase (PDI), a luminal ER protein of 55 kd. The latter functions by binding to and rearranging the disulfide bonds of many proteins. Compounding the problem, the synthetic nuclear signal peptides often contain an artificially added cysteine for other crosslinking studies. Yamasaki & Lanford (1991) have nicely shown by purification and protein sequencing that a major rat 55 kd protein, which proves to be the luminal ER protein PDI, binds artifactually to nuclear signal sequences and is purified along with a minor non-PDI 55 kd signal-binding activity. This latter protein may be identical to the receptor of Adam et al (1989, 1991). Yamasaki & Lanford (1991) also find that their 70 kd protein is related to the ER protein, ERp72, and binds only to signal sequences that contain an added cysteine, thus giving a false positive result. Meier & Blobel (1990) have observed 55 and 140 kd putative receptors by using a ligand-blotting strategy with the SV40 T Ag signal sequence as a probe of Western blots of total rat nuclear protein. The 140-kd protein (Nopp140) has subsequently been purified and antisera raised to it; immunofluorescence and cloning demonstrate surprisingly that it is a nucleolar protein and, more excitingly, that it moves on intranuclear tracks from the nucleolus to the nuclear envelope (Meier & Blobel 1992).

The search for signal sequence receptors has proceeded apace in yeast laboratories. A yeast protein of 70 kd was found to bind the labeled signal sequences of the SV40 T Ag, nucleoplasmin, the yeast Gal 4 protein, and histone H2B protein by ligand blotting, and seemed to localize specifically to the nucleus (Silver et al 1989), although later studies indicate that this localization may not be the whole story (Stochaj & Silver 1992). Antisera to the 70-kd yeast protein interact with 70-kd proteins from HeLa, *Drosophila*,

and *Zea mays*. A similar strategy, followed by Lee & Melese (1989), identified a 67-kd nuclear protein. This latter protein, now named NSR1, has been cloned and is, like the 140-kd rat protein mentioned above, a nucleolar protein. It contains two RNA recognition motifs, as well as very basic and acidic domains. Deletion of NSR1 from yeast is not lethal, but results in abnormally slow growth. Phosphorylation of NSR1 is required for its signal sequence-binding activity (T. Melese, personal communication), as it is for binding of the p70 of Silver et al (Stochaj & Silver 1992), but NSR1 is not the p70 studied by Silver et al. Although demonstration that these proteins are the functional yeast signal sequence receptors awaits further evidence, they are promising candidates. It will be interesting to discover in both yeast and mammalian cases whether there is more than one authentic signal sequence receptor and whether the receptor(s) carry nuclear proteins to the pore or actually ferry them through the pore. This latter mechanism might be suggested if the shuttling nucleolar proteins, which bind signal sequences on blots, are found to be authentic receptors *in vivo* (Borer et al 1989; Meier & Blobel 1990; Lee et al 1991). Clearly, by analogy with other signal-receptor systems, the nuclear signal sequence receptors could consist of both soluble and structure-bound elements.

**IN VITRO ASSAYS FOR NUCLEAR PROTEIN IMPORT** Initial studies of nuclear transport relied exclusively on microinjection into *Xenopus* oocytes and cultured mammalian cells. Each of these systems excels in the ability to analyze what constitutes a signal sequence. However, manipulation of the external milieu or pore structure in these *in vivo* systems is difficult, albeit not impossible (Goldfarb et al 1986; Richardson et al 1988; Breeuwer & Goldfarb 1990; Yoneda et al 1987a; Dabauvalle et al 1988a; Wolff et al 1988). A quick and direct *in vitro* assay that faithfully mimics authentic nuclear transport was developed to address these problems. The assay makes use of nuclei, a fluorescently labeled transport substrate, and an extract that keeps the nuclei in an intact state (Newmeyer et al 1986a,b; Dreyer 1987). One of the major problems that *in vitro* nuclear transport assays face is the difficulty in isolating intact nuclei and, sometimes, in subsequently keeping the nuclei intact. In consequence, the first transport assay made use of an extract of *Xenopus* eggs, which contains all the cytosolic and vesicular components necessary to assemble nuclei. This extract thus maintains added rat liver nuclei in an intact state or, if necessary, heals them. In the assay, nuclei are added to the extract in the presence of an ATP-generating system, allowed to equilibrate for 30 min, the transport substrate is added, and transport is assayed 15 min to 2 hr later by visualization by fluorescence microscopy. Up to 17–40-fold accumulation of the substrate occurs. Transport does not occur at 4°C, but was found to require a wild-type signal sequence, ATP, an intact nuclear envelope, and cytosol

(Newmeyer et al 1986a,b). Substrate-coated gold particles are quickly imported through the pores, which confirmed authentic import (Newmeyer & Forbes 1988). For questions related to transport, rat liver nuclei, which are easily isolated and stored, are the nuclei of choice; for other questions, nuclei can be assembled de novo from chromatin or DNA before transport is assayed. As transport substrates, rhodamine isothiocyanate (RITC)-labeled nucleoplasmin or an RITC-labeled synthetic transport substrate, consisting of multiple SV40 T-antigen signal sequences (ss) crosslinked to human serum albumin (RITC-ss-HSA; >67 kd), are typically used.

Possession of an in vitro assay proved valuable in identifying an inhibitor of nuclear transport, the lectin wheat germ agglutinin (WGA) (Finlay et al 1987). WGA was found to bind to the area contained within the cytoplasmic ring of the pore, presumably at the transporter or to structures leading to it (Finlay et al 1987; Akey & Goldfarb 1989). In vitro and in vivo, WGA blocks the transport of nuclear proteins, but not the diffusion of small dextrans (Finlay et al 1987; Yoneda et al 1987b; Dabauvalle et al 1988b; Wolff et al 1988). Inhibition is reversible by N-acetylglucosamine addition. Using signal sequence-coated gold particles, WGA was shown not to effect signal sequence recognition or binding of nuclear proteins to the pore, but to arrest import after the pore-binding step (Newmeyer & Forbes 1988). This arrest mirrors that seen after ATP depletion (Newmeyer & Forbes 1988; Richardson et al 1988). WGA appears to act by binding to one or more N-acetylglucosaminylated pore proteins and blocking a crucial step in translocation through the pore. A recent study using WGA-gold shows binding to sites between the central plug and cytoplasmic ring and, interestingly, to the distal ring of the nuclear basket (U. Aebi, personal communication).

The transport of nucleoplasmin, which could also be visualized when a fluorescent substrate was used, could be competed away with excess unlabeled ss-HSA, which argues that these proteins use the same receptor (Finlay et al 1989). A similar conclusion was reached in vivo (Breeuwer & Goldfarb 1990). A detailed protocol for the *Xenopus* nuclear transport assay and its quantitation has been published (Newmeyer & Wilson 1991).

A second useful and authentic in vitro assay makes use of digitonin-permeabilized mammalian cells (Adams et al 1990). The nuclear membranes of these cells remain intact as verified by exclusion of an anti-DNA antibody. Nuclei in the permeabilized cell assay accumulate fluorescent transport substrate 30-fold in 30 min. Transport, as in the *Xenopus* system, requires ATP, cytosol, an intact nuclear envelope, and is temperature-dependent and inhibited by WGA. The assay was subsequently shown to work with permeabilized *Drosophila* cells (Stochaj & Silver 1992).

A number of other in vitro assays have been published and are more extensively reviewed in Newmeyer (1990). Most do not require cytosol for

the nuclear association observed, which is troublesome in light of the careful demonstrations that cytosol is required in both the *Xenopus* and permeabilized cell assays. It may be that in these other assays the investigators are looking at pore binding instead of import, or that they are looking at an ATP-dependent binding to DNA, which biochemically mimics nuclear transport. Potential systems for assaying just the pore-binding step of transport have been described by Benditt et al (1989) and Stochaj et al (1991). Certain other of the transport assays may, however, accurately measure transport because they include cytosol (Shirakawa & Mizel 1989) or transport substrates translated in reticulocyte lysate (Kalinich & Douglas 1989; Parnaik & Kennady 1990), which could fulfill the cytosol requirement (Adam et al 1990). It should be noted that because binding to various nuclear structures is easily mistaken for nuclear import, it is very important when developing alternate assays to demonstrate by electron microscopy, if possible, the actual translocation of signal sequence-coated gold particles through the nuclear pore.

**CYTOSOLIC FACTORS REQUIRED FOR IMPORT** An advantage of in vitro assays is the ability to assess the requirement for cytosolic factors for nuclear import. Cytosol, an absolute requirement in the *Xenopus* assay, contains two factors required for transport, NIF1 and NIF2, that are inactivated by the sulfhydryl alkylating agent, N-ethylmaleimide (NEM; Newmeyer & Forbes 1990). NIF1 can be separated from NIF2 by ammonium sulfate precipitation and was shown to be required for the pore-binding step of nuclear protein import and not for nuclear envelope integrity. NIF1 appears to copurify with signal sequence-binding activity in *Xenopus* extracts (D. Newmeyer, personal communication). The permeabilized cell assay of Adam et al (1990) later showed that cytosol was required for import and that the cytosol could be inactivated by treatment with NEM. Cytosol from HeLa cells, *Xenopus* oocytes, and rabbit reticulocyte lysates all worked equally well. Because the investigators were unable to visualize a pore-binding step in the assay, it was not possible to determine whether the factors were required for the binding or translocation step. Recently, however, Adam & Gerace (1991) demonstrated that one NEM-sensitive cytosolic factor is a nuclear signal sequence receptor. At least two other factors, one NEM-sensitive and one NEM-insensitive, were also found to be required in the permeabilized cell assay. Gerace and colleagues (Sterne-Marr et al 1992) further found that an affinity column of pore glycoproteins partially depletes the cytosol of its ability to support transport, which indicates that one or more factors must bind to glycoproteins of the nuclear pore. The factor depleted is not the signal sequence receptor. Perhaps these additional cytosolic factors may be adaptors to bind the receptor to the pore, or in a more speculative model where filaments are involved, might be filament subunits or motors.

Extending the analysis of cytosolic factors required for nuclear import,

Moore & Blobel (1992) combined the permeabilized cell assay of Adam et al (1990) with fractionation of *Xenopus* oocyte cytosol to identify factors required for the binding and translocation steps of import. A fraction **A** promoted binding of ss-HSA to the nuclear rim in an ATP-independent, NEM-sensitive manner and may be related to the cytosolic factor NIF-1 of Newmeyer & Forbes (1990). This fraction can be envisioned as a nuclear signal sequence receptor or some other part of the targeting apparatus. A fraction **B** was found to promote import of rim-bound ss-HSA into the nucleus in an ATP-dependent, WGA-sensitive step (Moore & Blobel 1992). The unique activity of Fraction **B** is intriguing and introduces a new potential player into considerations of the mechanism of action of the nuclear pore, perhaps as a release factor. Determination of the identity of Fraction **B** will be of key interest.

An equally intriguing set of data implicates hsp70 and its cytosolic cognate hsc70 as required for nuclear import. Shi & Thomas (1992) using a similar permeabilized cell assay found that HeLa cell cytosol, when immunodepleted of hsp70 and hsc70, could not support nuclear import. Full import was restored by the addition to the depleted cytosol of bacterially expressed hsp70 or hsc70. The authors concluded that, in addition to previously identified cytosolic factors, nuclear transport requires hsp/hsc70 proteins. The role of hsp/hsc70 in nuclear import is particularly perplexing, since unlike protein import into other organelles, it is thought that nuclear import does not require unfolding of the imported protein. It may prove that hsp/hsc70 is required to better expose nuclear signal sequences or, alternatively, for an as yet unimagined role in import (for further review, see Goldfarb 1992; Yamasaki & Lanford 1992b).

### *Protein Export*

Once inside the nucleus, signal sequence-bearing proteins follow different fates: some, such as histones, bind tightly to internal nuclear structures; others, such as nucleoplasmin, remain soluble in the interior (Zimmer et al 1988; see Peters 1986, for a review). Since these latter proteins, although soluble, remain in the nucleus at a very high concentration relative to the cytoplasm, they clearly lack a signal to exit the pore (Dingwall et al 1982; Peters et al 1986; Dworetzky & Feldherr 1988). Indeed, when gold particles coated with nucleoplasmin or synthetic signal sequences are injected into the nucleus, they are unable to exit (Feldherr et al 1984; Dworetzky & Feldherr 1988). It has been concluded that the pore acts unidirectionally in the import of these nuclear proteins. Certain proteins, however, shuttle between nucleus and cytoplasm, some constitutively and others in response to regulatory signals. These include various nucleolar proteins, hsc70, and the cAMP-dependent protein kinase (Borer et al 1989; Mandell & Feldherr 1990; Mein-

koth et al 1990). It is predicted that these proteins contain an additional signal, i.e. one for protein export. Such a signal is being eagerly sought at this time. Ribosomal subunits, which are assembled in the nucleus and are exported to the cytoplasm, may exit through use of such an export signal present on one or more of their proteins. Ribosomal export has been shown to be ATP-dependent and competitively inhibited by excess injected ribosomes (Bataille et al 1990; see also Gupta & Ware 1989). Interestingly, the lectin WGA, when injected into oocyte nuclei, blocks the export of ribosomes and snRNAs, which argues that the export system of the pore is also sensitive to this import inhibitor (Bataille et al 1990; Neuman de Vegvar & Dahlberg 1990).

### *RNA Export*

One of the most vital roles played by the nuclear pore is the export of RNA. The nucleoplasmic side of the pore clearly must recognize and export multiple RNA species, including tRNAs, snRNAs, and spliced mRNAs, while leaving unspliced RNAs behind. This traffic is for the most part unidirectional. Gold particles coated with tRNA or polyA RNA when injected into a *Xenopus* oocyte nucleus quickly exit the nucleus, whereas when injected into the cytoplasm such particles are unable to enter the nucleus (Dworetzky & Feldherr 1988). The export of mRNAs has many of the properties of protein import, being a saturable process, sensitive to low temperature, ATP depletion, and WGA inhibition (Dargemont & Kuhn 1992). In the case of mRNAs and snRNAs, it has been shown that the 5' monomethyl cap, acquired at the time of transcription by RNA polymerase II, is required for their export (Hamm & Mattaj 1990). Several cap-binding proteins that have been identified are candidates for proteins important for mRNA export. The cap-binding protein may either be the RNA export receptor or may contain a protein export signal that causes the cap-binding protein-mRNA complex to be exported (see Izaurralde & Mattaj 1992 for references). It has not been ruled out, however, that other proteins are involved (Pinol-Roma & Dreyfuss 1991, 1992). For example, proteins that control correct poly A addition and/or 3' end formation, as well as release from the splicing apparatus, are most likely essential for RNA export.

Other classes of RNAs are also exported from the nucleus. Zasloff et al have found that tRNA export appears to be a carrier-mediated process that is sensitive to mutation within the tRNA sequence (Zasloff 1983; Tobian et al 1985). Guddat et al (1990), analyzing the export of 5S RNA, find that both the L5 ribosomal protein and TFIIIA can complex with 5S RNA and influence export. Many cases of RNA export are specialized ones involving the export of viral RNAs. For example, newly replicated influenza viral RNAs exit the nucleus via the bound viral matrix protein M1 (Martin & Helenius 1991a,b).

The M1 protein upon entering an infected cell and dissociating from the viral RNA is imported into the nucleus via a protein import signal; however, once inside the nucleus, M1 binds to the replicated viral RNAs, an M1 export signal dominates, and the RNAs are exported.

Many other interesting issues with relevance to the nuclear export of RNAs are coming to light in the literature, including the demonstration of tracks for movement of RNA through the nucleus (Lawrence et al 1989; Xing & Lawrence 1991; see also Meier & Blobel 1992). Other examples of RNA regulation, such as the action of the HIV Rev protein, have also been suggested to involve the control of nuclear transport. Instead this protein may control the release of viral RNA from the nuclear-splicing apparatus (Chang & Sharp 1989, 1990), as is seen for bulk RNAs in *in vitro* RNA export assays (Agutter 1991; Izaurralde & Mattaj 1992). The reader is referred to several recent reviews of RNA transport for further discussion (Mattaj 1990; Agutter 1991; Goldfarb & Michaud 1991; Maquat 1991; Izaurralde & Mattaj 1992).

### *RNA Import*

Few cellular RNAs are imported through the pore. The best known examples are the small nuclear RNAs (snRNAs). The snRNAs U1 and U2, which are synthesized with monomethyl caps, are exported to the cytoplasm where the caps are then converted into a trimethyl form and the snRNAs are assembled with proteins into snRNPs. U1 and U2 snRNPs then make use of a bipartite signal for their reimport into the nucleus: the 5' *tri*-methyl cap and the signal sequence(s) for protein import present on the coassembled Sm snRNP proteins (Fischer & Luhrmann 1990; Hamm et al 1990; Fischer et al 1991). Neither signal is functional and/or is exposed without the other. Excess free trimethyl cap competitively blocks the import of the trimethyl capped U1, U2, as well as U4, and U5 snRNAs, but not the import of U3 and U6 RNAs, which implies that the cap is not an important part of the signal for U3 and U6 (Michaud & Goldfarb 1991, 1992). (Excess monomethyl cap has no effect.) Interestingly, saturating concentrations of SV40 signal sequence inhibit the import of U6, but not that of U1, U2, U4, U5, or U3. These and the previous results suggest that there are three distinct pathways (i.e. receptors) for the import of snRNAs, as defined by competitive inhibition (Michaud & Goldfarb 1992; reviewed in Goldfarb & Michaud 1991; Izaurralde & Mattaj 1992). Both WGA and anti-pore antibody (mAb414) block the import of all six snRNAs (Michaud & Goldfarb 1992), thus indicating that all three pathways are sensitive to general nuclear transport inhibitors.

Specialized cases of RNA and DNA import are found with infectious agents, only a few examples of which can be given here. For example, SV40 virions appear to be imported through the pore, a process blocked by WGA and anti-pore antibody (Clever et al 1991). Influenza nucleocapsids are



imported apparently via a signal on the viral NP protein. Import is blocked by WGA and anti-pore antibodies, but not by agents that disrupt microtubules, intermediate filaments, or actin microfilaments (Martin & Helenius 1991a). The single-stranded T DNA ( $2 \times 10^4$  nt) of the *Agrobacterium* Ti plasmid enters a plant cell's nucleus complexed with one copy of *Agrobacterium* VirD2 protein at its 5' end and  $\sim 600$  copies of VirE2 proteins coating its length, both of which proteins contain nuclear signal sequences (Howard et al 1992; Citovsky et al 1992). This long complex is  $\sim 60$  times the thickness of the nuclear pore. One model proposes that VirD2 initiates nuclear import and then the linear array of VirE2 signal sequences sequentially keeps the pore open for complete transport of the complex, a mechanism that could also be invoked for general mRNA export.

### *Genetic Approaches to RNA and Protein Transport*

One of the most promising methods of studying RNA export may be a genetic one, using the yeast *Saccharomyces cerevisiae*. Several groups are now isolating temperature-sensitive mutants that accumulate polyA<sup>+</sup> RNA at high temperature. One such mutant has multiple defects, including defects in RNA processing, which make it less likely to be a specific defect in the export machinery of the pore (Hopper et al 1990). A second mutant hunt has identified a promising mutant *mtr-1* (mRNA transport), which has no obvious defects in RNA processing and causes accumulation of polyA<sup>+</sup> RNA as judged in a fluorescence microscopic screen (Kadowaki et al 1992). These authors have found that protein synthesis, mRNA splicing, and a normal titer of the polyA-binding protein are not critical for the export of yeast polyA<sup>+</sup> RNAs. A third group, using a similar strategy, has identified a number of *rat* mutants (RNA trafficking) that also accumulate polyA<sup>+</sup> RNA. These map in six complementation groups. In addition to affecting transport, these mutants variously also affect karyokinesis, rRNA processing, or pore localization (Amberg et al 1991). Two such mutants, *rat2-1* and *rat3-1*, show clustering of the yeast nuclear pores to one side of the nucleus at all temperatures, but RNA accumulation only at the high temperature (Copeland et al 1991), which implies that pore clustering per se cannot be the primary explanation of the export defect. Mutations of this type show great promise for the molecular analysis of RNA export.

Genetic approaches have also been used to study protein import by looking for mutations that prevent accumulation of nuclear proteins, but questions as to whether the mutations, some of which are in ER membrane proteins, cause a lack of integrity of the nuclear envelope rather than a defect in nuclear transport machinery have made this approach a complex one (Sadler et al 1989; Blumberg & Silver 1991). The genetic studies that use mutagenesis of the specific yeast pore proteins, NSP1 and NUP1 (see below), are likely to give more immediate insight into nuclear transport.

### *Control of Nuclear Transport*

In the last few years, import or export through the nuclear pore has been discovered to be a major point of cellular control. Only a few examples will be cited here for lack of space. The proto-oncogene product *c-abl* becomes transforming when prevented from reaching its natural location, the nucleus (Van Etten et al 1989). The decision of the *Drosophila dorsal* protein to become nuclear or stay cytoplasmic in the embryo determines the dorsal-ventral axis of the embryo (see Govind & Steward 1991 for a review). In a third example, when cAMP levels are induced to rise within a cell, the regulatory subunit of cAMP-dependent protein kinase releases the kinase subunit from a cytoplasmic tether; the kinase subunit is then immediately imported into the nucleus (see Meinkoth et al 1990 and references therein). Certain proteins, such as the human cyclins A and B1 (Pines & Hunter 1991), and the yeast SWI5 transcription factor (Nasmyth et al 1990; Moll et al 1991) undergo cell cycle-dependent nuclear transport. Other proteins are transported only at certain stages of development (Dreyer et al 1982; Dreyer 1987; Slavicek et al 1989; Miller et al 1991b). In each of these cases, a given protein is cytoplasmic until induced to become nuclear; this induction can involve release from a cytoplasmic tether, exposure of a signal sequence by protein dissociation, or covalent modification of a signal sequence to activate it. In very few cases does regulation involve modification of the nuclear pore itself. One exception is the intriguing finding that in proliferating cells, the pores are able to transport much larger signal sequence-coated gold particles ( $\sim 230 \text{ \AA}$ ) than the pores of confluent cells ( $\sim 110 \text{ \AA}$ ; Feldherr & Akin 1990, 1991). This might be a mechanism to turn off the export of large RNP particles in confluent cells. A full discussion of the increasing number of examples of control is unfortunately beyond the limited confines of this review, but the reader is referred to several recent reviews on this subject (Hunt 1989; Gilmore 1990; Dingwall 1991; Miller et al 1991; Richter & Standiford 1992; Yamasaki & Lanford 1992). This will continue to be one of the most active areas of interest in nuclear transport.

## NUCLEAR PORE PROTEINS

### *Proteins of the Vertebrate Nuclear Pore*

In the past five years, both biochemical and genetic searches have been initiated for new nuclear pore proteins, yet only a handful of a possible 75–100 have been conclusively identified. Because of the great size of the pore and the continued difficulty in isolating it away from the nuclear lamina and matrix, it has been virtually impossible to determine its protein composition by conventional purification of pores. Extraction of the nucleus with DNase, RNase, and detergent has resulted in nuclear pores tethered within

an insoluble nuclear lamina-matrix aggregate. A size of  $120 \times 10^6$  kd predicts that the pore would contain  $\sim 1200$  proteins if all were 100 kd. Since the pore has eightfold rotational symmetry and twofold mirror symmetry, one can presumably divide by 16 to give a possible estimate of 75 different 100-kd proteins, the majority of which are unidentified. Very recent success at purification of the nuclear pores from the yeast *S. cerevisiae*, however, should bring about a rapid alleviation of the problem (Allen & Douglas 1989; Rout & Blobel 1991). Although the yeast pore proteins discovered through this purification may not have exact analogues in higher eukaryotic systems, it is likely that the pore purification protocol can be adapted to these systems.

As for those proteins already found, at present a single integral membrane pore protein, gp210, has been identified, isolated by its abundance in total nuclear envelope preparations from both rat and *Drosophila* (Gerace et al 1982; Filson et al 1985). Gp210, an integral membrane protein ( $\sim 25$  copies per pore), is thought to play a structural role in the pore, anchoring it to the membrane. The gene encoding gp210 has been cloned and sequenced: it encodes a 204-kd protein containing a 24-residue signal sequence and two transmembrane domains (Wozniak et al 1989). Greber et al (1990) have subsequently used anti-peptide antibodies to demonstrate that gp210 uses only one of the transmembrane domains and that the majority of the protein lies on the luminal side of the nuclear membranes. A 58 amino acid tail at the C-terminus extends into the cytoplasm and is presumably involved in anchoring the pore scaffold. Wozniak & Blobel (1991) have found that the gp210 transmembrane domain or its cytoplasmic tail can target a membrane protein to the nuclear pore. Recent electron micrographs (Jarnik et al 1991) visualize multiple large knob-like structures that protrude into the envelope lumen in a radial pattern around each pore (see also radial arms, Akey 1989), most likely composed of one or more copies of gp210. Interestingly, when antibodies against the luminal domain of gp210 are present within this lumen (Greber & Gerace 1992), pore structure and number are not disturbed, but nuclear transport and diffusion are significantly decreased (fourfold).

Until the mid 1980s, the stage was singularly devoid of other pore proteins, much less of any proteins involved in pore function. Due to several strategies, the dearth of pore proteins has now been somewhat remedied. Davis & Blobel (1986, 1987) and Snow et al (1987) raised monoclonal antibodies to nuclear pore-lamina-matrix complexes from rat and found a number of antisera that gave a punctate nuclear rim stain by immunofluorescence on rat cells. Different monoclonal antibodies crossreacted with subsets of a family of 8–12 proteins (see Miller et al 1991a for complete references). To date only a few of the 8–12 glycoproteins have been definitively proven to be pore proteins or *nucleoporins*, since this requires production of monospecific antisera to each

protein. If all eventually prove to be pore proteins, it has been estimated that 80–96% of the proteins of the pore are still undiscovered. Elegant work by Hart and colleagues (Holt & Hart 1986; Holt et al 1987; see Hart et al 1988, 1989 for other references; Schindler et al 1987) demonstrated that the 8–12 are members of a novel class of glycoproteins containing many single N-acetylglucosamine residues. The most prominent of such proteins is the 62-kd pore protein (Davis & Blobel 1986, 1987). Other proteins include ones of 210, 180, 145, 100, 58, 54, and 45 kd (Snow et al 1987). An almost identical family of proteins, with p62 predominant, was found when the transport inhibitor WGA was used to probe rat liver nuclei protein blots (Finlay et al 1987). The monoclonal antibody RL1, which crossreacts with p62 and the family of nucleoporins, also inhibits protein import in *Xenopus* oocytes (Featherstone et al 1988). Both monoclonal and polyclonal antibodies specific for p62 bind to the pore and block import in vivo (Dabauvalle et al 1988a) and in vitro (E. Meier & D. Forbes, unpublished results), thus demonstrating that p62 is a pore protein and is essential to the import process.

In consequence, much attention has been focused on p62. The p62 gene has been cloned and sequenced from rat, frog, and human (D'Onofrio et al 1988; Starr et al 1990; Cordes et al 1991; Carmo-Fonseca et al 1991). In rat, it is present in a single copy in the genome and is without introns (D'Onofrio et al 1991; Starr et al 1990). The p62 protein consists of three domains: (a) an N-terminal domain containing 12 imperfect repeats of a 7 aa sequence (179aa); (b) a central domain rich in Ser, Thr, Pro, and Ala residues (157aa); and (c) a unique C-terminal domain (189aa). The interesting 7aa repeats do not appear to be the site of N-acetylglucosamine addition, as might be suspected. Instead, glycosylation has been shown to occur in the middle domain of p62 (Cordes et al 1991). The repeats are, however, the epitope for the first anti-pore monoclonal antibody, mAb414, which recognizes multiple proteins (Davis & Blobel 1986, 1987; Davis & Fink 1990). On a separate note, studies designed to incorporate mutant p62 into pores using transfected mammalian cells in order to assess the functional consequence have not yet been successful, as the expressed p62 remains in cytoplasmic inclusion bodies (Starr & Hanover 1990; Carmo-Fonseca et al 1991).

Although it has been difficult to prove that individual glycoproteins are actually pore proteins, since many of the original anti-pore antibodies are multispecific, a short list of likely candidates for pore proteins can be made. One of the anti-pore monoclonal antibodies, RL11, is monospecific and recognizes a single protein of 180 kd, staining only the nucleoplasmic side of the pore (Snow et al 1987). Other recent work examining p62 in rat nuclear pores found that it exists in a tight 550–600 kd complex with proteins of 58 and 54 kd, which indicates that these proteins are also pore proteins (Finlay et al 1991). The identification of complexes is a promising approach to es-

establishing a molecular structure of the pore. In separate studies, a human anti-pore antiserum, which recognizes proteins of 200 and 130 kd, has been found, which suggests that one or both of these are pore proteins (Dagenais et al 1988). It has also been suggested that myosin is a component of the nuclear pore, a contention that is being actively investigated; antisera to myosin seems to stain the pore in immunoelectron micrographs (Berrios & Fisher 1986; Berrios et al 1991). Additional monospecific reagents are sorely needed to delineate pore proteins, to determine their interactions, to ask whether such antisera block the transport of RNAs and/or proteins, and to create pores depleted of individual proteins, as discussed below.

### *Proteins of the Yeast Nuclear Pore*

Several monoclonal antibodies that give an immunofluorescent staining of rat nuclear pores also recognize multiple yeast proteins upon Western blotting. Some are proteins of the pore, while some are cytoplasmic. The results argue for conserved structural elements between yeast and vertebrate pore proteins, but also demonstrate that not every protein reacting with an antibody that stains the pore is in fact a pore protein (Aris & Blobel 1989; Davis & Fink 1990). Two studies have identified individual nuclear pore proteins of yeast. Hurt (1988) raised a polyclonal antisera against a yeast nucleoskeletal preparation and identified a nuclear envelope protein, NSP1. The essential NSP1 gene, which has been cloned and sequenced, encodes an 86-kd protein (100 kd on gels; Hurt 1988). The protein contains an N-terminal hydrophilic domain, a middle domain characterized by the presence of 22 nine amino acid repeats, very similar to those in p62, and a unique C-terminal domain (195 aa). This latter region has 31% identity and 50% similarity with the C-terminus of human p62 (Carmo-Fonseca et al 1991). These data suggest that NSP1 is most likely the yeast homologue of vertebrate p62. Although the sequence of the entire C-terminal region can be arranged into heptad repeats of the type often found in fibrous proteins, such as the intermediate filaments (Carmo-Fonseca et al 1991; Cordes et al 1991), the most interesting stretch of homology between NSP1 and p62 in this region is a 23 aa sequence with high homology to a  $\text{Ca}^{2+}$ -binding region found in the  $\text{Ca}^{2+}$ -binding proteins, parvalbumin and onco-albumin (Hurt 1988). Mutation of a single amino acid central to the putative  $\text{Ca}^{2+}$ -binding domain of NSP1 (Glu706→Pro706) renders the yeast temperature-sensitive for growth and appears to block nuclear transport (Nehrbass et al 1990). Remarkably only the C-terminal domain, which contains this putative  $\text{Ca}^{2+}$ -binding region, is needed for yeast viability, targeting of the protein to the pore, and nuclear transport (Nehrbass et al 1990; Hurt 1990).

In a separate hunt, Davis & Fink (1990) used anti-pore monoclonal antibodies raised to rat nuclei to screen a yeast cDNA expression library. They

cloned a gene, NUP1, encoding a crossreacting yeast protein, and subsequently proved it to encode a pore protein. NUP1, a 130-kd protein, is essential to yeast viability and is also composed of three domains. The middle domain contains 28 degenerate copies of the nine amino acid repeat found in NSP1. This repeat, which is the only similarity between NUP1 and NSP1, is now proposed to be present most often in proteins of the nuclear pore. Initial indications are that many repeat-containing pore proteins will be found, since yeast pore proteins of 100 kd (NUP2; Loeb et al 1991) and 49 kd (Wente et al 1991) have also recently been found to contain such repeats.

## NUCLEAR PORE ASSEMBLY AND DISASSEMBLY

### *Nuclear Pore Assembly and Reconstitution*

The nuclear pore must be built anew at the end of each mitosis (see, for example, Maul 1977; Benavente et al 1989a,b). Little is known of how the two nuclear membranes are fused to form a channel or how the nuclear pore is assembled within that channel. It has been possible, however, to reconstitute the nucleus, using either an extract of *Xenopus* eggs, where all the components necessary for assembly of the nucleus are stored in a disassembled state, or an extract of mitotic cultured cells (reviewed by Laskey & Leno 1990). Nuclear reconstitution is also possible in extracts of *Drosophila* embryos (Ulitzur & Gruenbaum 1989; Berrios & Avilion 1990). The nuclei formed contain double nuclear membranes, a nuclear lamina and, most relevant to the subject at hand, numerous nuclear pores.

A single *Xenopus* egg contains  $\sim 3 \times 10^7$  disassembled pores. In *Xenopus*, Vigers & Lohka (1991) found a vesicle population that is involved in pore formation, although at present it remains hard to purify this class of vesicles. Also addressing the mechanism of nuclear pore formation, Sheehan et al (1988) fractionated a *Xenopus* assembly extract into cytosolic and membrane vesicle fractions. They found that the cytosolic fraction, when added to chromatin in the presence of very limiting amounts of membranes, formed ring-like structures, which they termed prepores, on the chromatin. They hypothesize that these membrane-free half pores are an intermediate step in the formation of pores on a chromatin scaffold at the end of mitosis. Subsequent binding of membrane vesicles, followed by membrane fusion and riveting of the two membranes together with the second half of the pore, would then construct the finished nuclear envelope. Although an attractive hypothesis, this model cannot explain pore formation at S phase in mammalian cells, where the pore number is known to double in the intact nucleus (Maul 1977). Chromatin is not accessible to take part in pore formation in this instance. Thus either the Sheehan et al (1988) model is incorrect, or there

must be a completely different second mechanism for forming pores that occurs at times other than mitosis.

The study of nuclear transport has advanced to the point where one would like to assign specific functions to individual pore proteins. One attractive approach would be to modify or remove a single protein from the pore and then determine the consequence to pore function. This strategy is virtually impossible with the preexisting pores of rat liver nuclei or other fully formed nuclei, but is possible with nuclear reconstitution systems. Specifically, using the *Xenopus* egg nuclear reconstitution extract it is possible to assemble nuclei lacking the pore glycoproteins (Finlay & Forbes 1990). The *Xenopus* egg contains three major WGA-binding proteins of 62, 97, and 200 kd, as well as WGA-binding proteins of lesser abundance or glycosylation, all of which are present in a soluble form, presumably stored for use in the later assembly of pores. Polyclonal antisera to p60, p97, and p200 each give a nuclear pore stain (C. Macaulay & D. Forbes, unpublished). Nuclei reconstituted in an extract depleted of all these proteins are unable to transport, but are still capable of diffusion, which indicates that these proteins are required for active transport (Finlay & Forbes 1990). Addition of the pore glycoproteins from either *Xenopus* or rat back to a depleted reconstitution extract results in nuclei that are now fully active in transport. This indicates that the pore can be reconstituted with exogenously added proteins and, furthermore, that rat pore glycoproteins can substitute functionally for their *Xenopus* homologues. When electron microscopy was performed on the original depleted nuclei, the nuclei were found to contain fairly normal-looking nuclear pores, albeit less densely staining. Thus the pore glycoproteins do not make up the major structures of the nuclear pore, such as the scaffold, but are required for the active transport of signal sequence-bearing proteins. Indeed, work by Akey & Goldfarb (1989) and Stewart et al (1990) shows that nucleoplasmin-coated gold particles bind to the central transporter of the pore in identical locations to the sites of WGA-gold binding, which suggests that these proteins are among the first proteins an incoming nuclear protein meets. It must be noted, however, that Dabauvalle et al (1990) find that in their hands the removal of the WGA-binding pore glycoproteins results in nuclei with no pores. This may reflect the inherent difficulty of the reconstitution experiments, but it is as yet an unresolved difference.

Reconstitution of nuclei was also performed in extracts depleted with anti-pore antibodies so that the extracts contained varying levels of a 550–600 kd complex of three pore proteins, p62, p58, and p54. In nuclei formed in such extracts, transport was found to show a strict linear dependence on the amount of complex present at the time of pore formation (Finlay et al 1991). A similar multiprotein complex exists in soluble form in the *Xenopus* egg,

although different molecular masses of the *Xenopus* complex have been seen by different investigators (550–600 kd and 254 kd; Finlay et al 1991; Dabauvalle et al 1990, respectively). It is clear that the reconstitution approach to the assembly of the pore should continue to be productive.

### *Disassembly at Mitosis*

In classical electron microscopic studies of mitotic cells, the nuclear pore can be seen to disappear gradually, eventually leaving a visible hole in the nuclear membranes (Maul 1977). Nuclear pore disassembly thus may involve an ordered release of pore subunits. Disappearance of the pore structure may then be followed by a selective vesicularization of the nuclear membrane in the vicinity of the pore (Harel et al 1989; Courvalin et al 1991), followed by eventual detachment and disappearance of the entire nuclear envelope (in vertebrates). A likely possibility is that the covalent modification of one or more pore proteins initiates this cascade of disassembly at mitosis. Recent findings with nuclear lamins, intermediate filaments, and the nuclear membranes have implicated the kinase, mitosis-promoting factor (MPF), either directly or indirectly, as the cause of disassembly at mitosis (see references in Chou et al 1990). Relevant to this, it has recently been found that two of the three major *Xenopus* pore glycoproteins, p200 and p97, when monitored in an egg extract that cycles between mitosis and interphase, become hyperphosphorylated at mitosis (Macaulay & Forbes 1991). When phosphorylated, the pore glycoproteins are unable to assemble into new pores (C. Macaulay, unpublished results). The processes of pore assembly and disassembly should be of increasing interest, not in the least for their potential to shed light on overall pore structure and subsequent implications for function.

### *Annulate Lamellae*

An interesting cytoplasmic structure, termed annulate lamellae, has long been observed in oocytes and transformed cells (Kessel 1989; Merisko 1989). Annulate lamellae consist of very closely packed pores arranged in 1–100 double-membrane stacks. They most likely represent a storage form of nuclear pores (Stafstrom & Staehelin 1984). The pores of annulate lamellae bind nucleoplasmin-coated gold particles, WGA-ferritin, and anti-pore antibodies in a manner identical to pores of the nucleus (Feldherr et al 1984; Allen 1990). A human autoimmune antiserum specific only to annulate lamellae has been identified by Chen & Merisko (1988), which suggests that distinct proteins can exist, perhaps in the glue holding the stacks together. These authors have also found that the nuclear lamins are not part of the annulate lamellae structure. Very recently, it became possible to form large quantities of annulate lamellae in vitro (Dabauvalle et al 1991), using the same extracts of



*Xenopus* eggs capable of nuclear assembly (Lohka & Masui 1983; reviewed in Laskey & Leno 1990). Interestingly, annulate lamellae formed in extracts depleted with WGA-Sepharose do contain nuclear pores (E. Meier & D. Forbes, unpublished results), consistent with previous findings (Finlay et al 1991). Once isolated, in vitro assembled annulate lamellae should greatly aid in the purification of pores and the study of pore assembly.

## FUTURE PROSPECTS

The next few years should be ones of exceptional progress in elucidating the structure and function of the nuclear pore. Until now, molecular analysis of the pore has been greatly hindered by the dearth of pore proteins. In yeast, the recent purification of the pore should remedy this and provide many candidates for sequence analysis. In *Xenopus* and rat, analysis of the subunits into which the nuclear pore can be disassembled, whether biochemically or at mitosis, should indicate not only what those subunits are, but which proteins are nearest neighbors within the assembled pore. These subunits can then be correlated with the three-dimensional structure of the pore derived from increasingly sophisticated electron microscopy and provide molecular tenants for the major scaffold of the pore, the transporter, and the intriguing cytoplasmic filaments and nucleoplasmic basket. The molecular cloning of nucleoporins will continue to be of great interest. Having in hand the sequence of any pore protein could potentially be just the clue that suggests a mechanism of action for nuclear import. If, for example, a newly discovered pore protein is found to have homology to kinesin or actin, immediate insights into the mechanism of transport may be gained. Reconstitution systems, together with depletion by antisera to the new pore proteins identified, should allow the formation of nuclei lacking individual proteins and the determination of the structural and functional consequences. In addition, it should be possible to produce mutant pore proteins for in vitro reconstitution of mutant nuclear pores and similar delineation of function. In a separate approach, yeast genetics may prove especially profitable in the hunt for new proteins of the pore, as well as for cytoplasmic proteins, such as signal sequence receptors, potential recyclable kinesin-type carriers, etc, although this strategy may be one for the long term. Examination of the nuclear signal sequence receptor(s) should prove interesting for determining what accessory proteins the receptor needs for interaction with a signal-sequence bearing protein or for interaction with the nuclear pore, thereby elucidating the initial steps in protein import. These initial steps will be most likely those at which developmental and cellular control of transport is exerted. In the reverse direction, both genetic and biochemical approaches to RNA export may provide us with many unexpected insights into what is a very murky area at

present. In summary, a many-pronged attack is in progress on the structure of the nuclear pore and the mechanism of transport of its many biological substrates. The results should prove fruitful and interesting.

#### ACKNOWLEDGMENTS

The author thanks Marc Melnik and Shirley Allen for excellent assistance with the Literature Cited. She thanks John Newport, Maureen Powers, Colin Macaulay, Eva Meier, and David Goldfarb for critical reading of the manuscript. She apologizes for the omission of many relevant references due primarily to a lack of space (and, most likely, for a few omitted due to exhaustion).

#### Literature Cited

- Adam, S. A., Gerace, L. 1991. Cytosolic proteins that specifically bind nuclear location signals are receptors for nuclear import. *Cell* 66:837-47
- Adam, S. A., Lobl, T. J., Mitchell, M. A., Gerace, L. 1989. Identification of specific binding proteins for a nuclear location sequence. *Nature* 337:276-79
- Adam, S. A., Sterne-Marr, R., Gerace, L. 1990. Nuclear protein import in permeabilized mammalian cells requires soluble cytoplasmic factors. *J. Cell Biol.* 111:807-16
- Agutter, P. S. 1991. *Between Nucleus and Cytoplasm*. London/New York: Chapman & Hall. 1st ed. 148 pp.
- Akey, C. W. 1989. Interactions and structure of the nuclear pore complexes revealed by cryo-electron microscopy. *J. Cell Biol.* 109:955-70
- Akey, C. W. 1990. Visualization of transport-related configurations of the nuclear pore transporter. *Biophys. J.* 58:341-55
- Akey, C. W. 1991. Probing the structure and function of the nuclear pore complex. *Sem. Cell Biol.* 2:167-77
- Akey, C. W. 1992. The nuclear pore complex: A macromolecular transport assembly. In *Nuclear Trafficking*, ed. C. Felherr. New York: Academic 370 pp.
- Akey, C. W., Goldfarb, D. S. 1989. Protein import through the nuclear pore complex is a multistep process. *J. Cell Biol.* 109:971-82
- Allen, E. 1990. Pores of annulate lamellae and nuclei bind wheat germ agglutinin and monoclonal antibody similarly. *J. Struct. Biol.* 103:140-51
- Allen, J. L., Douglas, M. G. 1989. Organization of the nuclear pore complex in *Saccharomyces cerevisiae*. *J. Ultrastruct. Mol. Struct. Res.* 102:95-108
- Amberg, D. C., Copeland, C. S., Cole, C. N. 1991. mRNA trafficking mutants of *S. cerevisiae*. *J. Cell Biol.* 115:248a
- Aris, J. P., Blobel, G. 1989. Yeast nuclear envelope proteins cross react with an antibody against mammalian pore complex proteins. *J. Cell Biol.* 108:2059-67
- Bataille, N., Helser, T., Fried, H. M. 1990. Cytoplasmic transport of ribosomal subunits microinjected into the *Xenopus laevis* oocyte nucleus: A generalized, facilitated process. *J. Cell Biol.* 111:1571-82
- Benavente, R., Dabauvalle, M.-C., Scheer, U., Chaly, N. 1989a. Functional role of newly formed pore complexes in postmitotic nuclear reorganization. *Chromosoma* 98:233-41
- Benavente, R., Scheer, U., Chaly, N. 1989b. Nucleocytoplasmic sorting of macromolecules following mitosis: Fate of nuclear constituents after inhibition of pore complex function. *Eur. J. Cell Biol.* 50:209-19
- Benditt, J. O., Meyer, C., Fasold, H., Barnard, F. C., Riedel, N. 1989. Interaction of a nuclear location signal with isolated nuclear envelopes and identification of signal-binding proteins by photoaffinity labeling. *Proc. Natl. Acad. Sci. USA* 86:9327-31
- Berrios, M., Fisher, P. A. 1986. A myosin heavy chain-like polypeptide is associated with the nuclear envelope in higher eukaryotic cells. *J. Cell Biol.* 103:711-24
- Berrios, M., Fisher, P. A., Matz, E. C. 1991. Localization of a myosin heavy chain-like polypeptide to *Drosophila* nuclear pore complexes. *Proc. Natl. Acad. Sci. USA* 88:219-23
- Berrios, M., Avilion, A. A. 1990. Nuclear formation in a *Drosophila* cell-free system. *Exp. Cell Res.* 191:64-70
- Blumberg, H., Silver, P. A. 1991. A homologue of the bacterial heat-shock gene *DnaJ*

- that alters protein sorting in yeast. *Nature* 349:627-30
- Borer, R. A., Lehner, C. F., Eppenberger, H. M., Nigg, E. A. 1989. Major nucleolar proteins shuttle between nucleus and cytoplasm. *Cell* 56:379-90
- Breeuwer, M., Goldfarb, D. S. 1990. Facilitated nuclear transport of histone H1 and other small nucleophilic proteins. *Cell* 60:999-1008
- Burglin, T. R., De Robertis, E. M. 1987. The nuclear migration signal of *Xenopus laevis* nucleoplasm. *EMBO J.* 6:2617-25
- Carmo-Fonseca, M., Kern, H., Hurt, E. C. 1991. Human nucleoporin p62 and essential yeast nuclear pore protein NSP1 show sequence homology and a similar domain organization. *Eur. J. Cell Biol.* 55:17-30
- Chang, D. D., Sharp, P. A. 1989. Regulation by HIV Rev depends upon recognition of splice sites. *Cell* 59:789-95
- Chang, D. D., Sharp, P. A. 1990. Messenger RNA transport and HIV rev regulation. *Science* 249:614-15
- Chelsky, D., Ralph, R., Jonak, G. 1989. Sequence requirements for synthetic peptide-mediated translocation to the nucleus. *Mol. Cell. Biol.* 9:2487-92
- Chen, T.-Y., Merisko, E. M. 1988. Annulate lamellae: Comparison of antigenic epitopes of annulate lamellae membranes with the nuclear envelope. *J. Cell Biol.* 107:1299-1306
- Chin, D. J., Green, G. A., Zon, G., Szoka, F. C. Jr., Straubinger, R. M. 1990. Rapid nuclear accumulation of injected oligodeoxyribonucleotides. *New Biologist* 2:1091-1100
- Chou, Y. H., Bischoff, J. R., Beach, D., Goldman, R. D. 1990. Intermediate filament reorganization during mitosis is mediated by p34cdc2 phosphorylation of vimentin. *Cell* 62:1063-71
- Citovsky, V., Zupan, J., Warnick, D., Zambryski, P. 1992. Nuclear localization of Agrobacterium VirE2 protein in plant cells. *Science*. 256:1802-5
- Clever, J., Yamada, M., Kasamatsu, H. 1991. Import of simian virus 40 virions through nuclear pore complexes. *Proc. Natl. Acad. Sci. USA* 88:7333-37
- Copeland, C. S., Amberg, D. C., Cole, C. N., Snyder, M. 1991. Clustering of nuclear pore complexes in mRNA trafficking mutants of *Saccharomyces cerevisiae*. *J. Cell Biol.* 115:317a
- Cordes, V., Waizenegger, I., Krohne, G. 1991. Nuclear pore complex glycoprotein p62 of *Xenopus laevis* and mouse: cDNA cloning and identification of its glycosylated region. *Eur. J. Cell Biol.* 55:31-47
- Courvalin, J.-C., Chaudhary, N., Worman, H. J., Wozniak, R. W., Blobel, G. 1991. Ordered disassembly and reassembly of the nuclear envelope during mitosis. *J. Cell Biol.* 115:373a
- Dabauvalle, M.-C., Benevente, R., Chaly, N. 1988a. Monoclonal antibodies to a  $M_r$  68,000 pore complex glycoprotein interfere with nuclear protein uptake in *Xenopus* oocytes. *Chromosoma* 97:193-97
- Dabauvalle, M.-C., Loos, K., Merkert, H., Scheer, U. 1991. Spontaneous assembly of pore complex-containing membranes ("annulate lamellae") in *Xenopus* egg extract in the absence of chromatin. *J. Cell Biol.* 112:1073-82
- Dabauvalle, M.-C., Loos, K., Scheer, U. 1990. Identification of a soluble precursor complex essential for nuclear pore assembly in vitro. *Chromosoma* 100:56-66
- Dabauvalle, M.-C., Schulz, B., Scheer, U., Peters, R. 1988b. Inhibition of nuclear accumulation of karyophilic proteins in living cells by microinjection of the lectin wheat germ agglutinin. *Exp. Cell Res.* 174:291-96
- Dagenais, A., Bibor-Hardy, V., Senecal, J.-L. 1988. A novel autoantibody causing a peripheral fluorescent antinuclear antibody pattern is specific for nuclear pore complexes. *Arthritis Rheumatism* 31:1322-27
- Dang, C. V., Lee, W. M. F. 1988. Identification of the human c-myc protein nuclear translocation signal. *Mol. Cell. Biol.* 8:4048-54
- Dargemont, C., Kuhn, L. C. 1992. Export of mRNA from microinjected nuclei of *Xenopus laevis* oocytes. *J. Cell Biol.* In press
- Davis, L. I., Blobel, G. 1986. Identification and characterization of a nuclear pore protein. *Cell* 45:699-709
- Davis, L. I., Blobel, G. 1987. Nuclear pore complex contains a family of glycoproteins that includes p62: Glycosylation through a previously unidentified cellular pathway. *Proc. Natl. Acad. Sci. USA* 84:7552-56
- Davis, L. I., Fink, G. R. 1990. The NUP1 gene encodes an essential component of the yeast nuclear pore complex. *Cell* 61:965-78
- Dingwall, C. 1991. Transport across the nuclear envelope: Enigmas and explanations. *BioEssays* 13:213-18
- Dingwall, C., Dilworth, S. M., Black, S. J., Kearsy, S. E., Cox, L. S., Laskey, R. A. 1987. Nucleoplasmic cDNA sequence reveals polyglutamic acid tracts and a cluster of sequences homologous to putative nuclear localization signals. *EMBO J.* 6:69-74
- Dingwall, C., Laskey, R. A. 1991. Nuclear targeting sequences—a consensus? *Trends Biochem. Sci.* 16:478-81
- Dingwall, C., Robbins, J., Dilworth, S. M., Roberts, B., Richardson, W. D. 1988. The nucleoplasmic nuclear location sequence is larger and more complex than that of SV-40 large T antigen. *J. Cell Biol.* 107:841-49

- Dingwall, C., Sharnick, S. V., Laskey, R. A. 1982. A polypeptide domain that specifies migration of nucleoplasmin into the nucleus. *Cell* 30:449-58
- D'Onofrio, M., Lee, M. D., Starr, C. M., Miller, M., Hanover, J. A. 1991. The gene encoding rat nuclear pore glycoprotein p62 is intronless. *J. Biol. Chem.* 266:11980-85
- D'Onofrio, M., Starr, C. M., Park, M. K., Holt, G. D., Haltiwanger, R. S., et al. 1988. Partial cDNA sequence encoding a nuclear pore protein modified by O-linked N-acetylglucosamine. *Proc. Natl. Acad. Sci. USA* 85:9595-99
- Dreyer, C. 1987. Differential accumulation of oocyte nuclear proteins by embryonic nuclei of *Xenopus*. *Development* 101:829-46
- Dreyer, C., Scholz, E., Hausen, P. 1982. The fate of oocyte nuclear proteins during early development of *Xenopus laevis*. *Wilhelm Roux's Arch. Entwicklungsmech. Org.* 191:228-33
- Dworetzky, S. I., Feldherr, C. M. 1988. Translocation of RNA-coated gold particles through the nuclear pores of oocytes. *J. Cell Biol.* 106:575-84
- Dworetzky, S. I., Lanford, R. E., Feldherr, C. M. 1988. The effects of variations in the number and sequence of targeting signals on nuclear uptake. *J. Cell Biol.* 107:1279-87
- Featherstone, C., Darby, M. K., Gerace, L. 1988. A monoclonal antibody against the nuclear pore complex inhibits nucleocytoplasmic transport of protein and RNA in vivo. *J. Cell Biol.* 107:1289-97
- Feldherr, C. 1992. *Nuclear Trafficking*, ed. C. Feldherr. New York: Academic 370 pp.
- Feldherr, C. M., Akin, D. 1990. The permeability of the nuclear envelope in dividing and nondividing cell cultures. *J. Cell Biol.* 111:1-8
- Feldherr, C. M., Akin, D. 1991. Signal-mediated nuclear transport in proliferating and growth-arrested BALB/c 3T3 cells. *J. Cell Biol.* 115:933-39
- Feldherr, C. M., Kallenbach, E., Schultz, N. 1984. Movement of a karyophilic protein through the nuclear pores of oocytes. *J. Cell Biol.* 99:2216-22
- Filson, A. J., Lewis, A., Blobel, G., Fisher, P. A. 1985. Monoclonal antibodies prepared against the major *Drosophila* nuclear matrix-pore complex-lamina glycoprotein bind specifically to the nuclear envelope in situ. *J. Biol. Chem.* 260:3164-72
- Finlay, D. R., Newmeyer, D. D., Price, T. M., Forbes, D. J. 1987. Inhibition of in vitro nuclear transport by a lectin that binds to nuclear pores. *J. Cell Biol.* 104:189-200
- Finlay, D. R., Newmeyer, D. D., Hartl, P. M., Horecka, J., Forbes, D. J. 1989. Nuclear transport in vitro. *J. Cell Sci. Suppl.* 11:225-42
- Finlay, D. R., Forbes, D. J. 1990. Reconstitution of biochemically altered nuclear pores: Transport can be eliminated and restored. *Cell* 60:17-29
- Finlay, D. R., Meier, E., Bradley, P., Horecka, J., Forbes, D. J. 1991. A complex of nuclear pore proteins required for pore function. *J. Cell Biol.* 114:169-83
- Fischer, U., Darzynkiewicz, E., Tahara, S. M., Dathan, N. A., Luhrmann, R., Mattaj, I. W. 1991. Diversity in the signals required for nuclear accumulation of U snRNPs and variety in the pathways of nuclear transport. *J. Cell Biol.* 113:705-14
- Fischer, U., Luhrmann, R. 1990. An essential signaling role for the m3G cap in the transport of U1 snRNP to the nucleus. *Science* 249:786-90
- Franke, W. W. 1974. Structure, biochemistry, and functions of the nuclear envelope. *Int. Rev. Cytol.* 4:71-236
- Gall, J. G. 1954. Observations on the nuclear membrane with the electron microscope. *Exp. Cell. Res.* 7:197-200
- Garcia-Bustos, J., Heitman, J., Hall, M. N. 1991. Nuclear protein localization. *Biochem. Biophys. Acta* 1071:83-101
- Gerace, L., Ottaviano, Y., Kondor-Koch, C. 1982. Identification of a major polypeptide of the nuclear pore complex. *J. Cell Biol.* 95:826-37
- Gilmore, T. D. 1990. NF-KB, KBF1, dorsal and related matters. *Cell* 62:841-43
- Goldfarb, D. 1992. Are the cytosolic components of the nuclear, ER, and mitochondrial import apparatus functionally related? *Cell* 70:185-88
- Goldfarb, D. S., Garipey, J., Schoolnik, G., Kornberg, R. D. 1986. Synthetic peptides as nuclear localization signals. *Nature* 332:641-44
- Goldfarb, D., Michaud, N. 1991. Pathways for the nuclear transport of proteins and RNAs. *Trends Cell Biol.* 1:20-24
- Govind, S., Steward, R. 1991. Dorsoventral pattern formation in *Drosophila*: Signal transduction and nuclear targeting. *Trends Genet.* 7:119-25
- Greber, U. F., Gerace, L. 1992. Nuclear protein import is inhibited by an antibody to a luminal epitope of a nuclear pore complex glycoprotein. *J. Cell Biol.* 116:15-30
- Greber, U. F., Senior, A., Gerace, L. 1990. A major glycoprotein of the nuclear pore complex is a membrane-spanning polypeptide with a large luminal domain and a small cytoplasmic tail. *EMBO J.* 9:1495-1502
- Guddat, U., Bakken, A. H., Pieler, T. 1990. Protein-mediated nuclear export of RNA: 5S rRNA containing small RNPs in *Xenopus* oocytes. *Cell* 60:619-28
- Guiochon-Mantel, A., Loosfelt, H., Lescop, P., Sar, S., Atger, M., et al. 1989. Mech-

- anisms of nuclear localization of the progesterone receptor: Evidence for interaction between monomers. *Cell* 57:1147-54
- Gupta, A. K., Ware, V. 1989. Nucleocytoplasmic transport of ribosomes in a eukaryotic system: is there a facilitated transport process? *Proc. Natl. Acad. Sci. USA* 86:1791-95
- Hall, M. N., Hereford, L., and Herskowitz, I., 1984. Targeting of *E. coli*  $\beta$ -galactosidase to the nucleus in yeast. *Cell* 36:1057-65
- Hamm, J., Darzynkiewicz, E., Tahara, S. M., Mattaj, I. W. 1990. The trimethylguanosine cap structure of U1 snRNA is a component of a bipartite nuclear targeting signal. *Cell* 62:569-77
- Hamm, J., Mattaj, I. W. 1990. Monomethylated cap structures facilitate RNA export from the nucleus. *Cell* 63:109-18
- Harel, A., Zlotkin, E., Nainudel-Epszteyn, S., Feinstein, N., Fisher, P. A., Gruenbaum, Y. 1989. Persistence of major nuclear envelope antigens in an envelope-like structure during mitosis in *Drosophila melanogaster* embryos. *J. Cell Sci.* 94:463-70
- Hart, G. W., Haltiwanger, R. S., Holt, G. D., Kelly, W. G. 1989. Glycosylation in the nucleus and cytoplasm. *Annu Rev. Biochem.* 58:841-74
- Hart, G. W., Holt, G. D., Haltiwanger, R. S. 1988. Nuclear and cytoplasmic glycosylation: novel saccharide linkages in unexpected places. *Trends Biochem. Sci.* 13:380-84
- Hinshaw, J. E., Carragher, B. O., Milligan, R. A. 1992. Architecture and design of the nuclear pore complex. *Cell.* 69:1133-41
- Holt, G. D., Hart, G. W. 1986. The subcellular distribution of terminal N-acetylglucosamine moieties: Localization of a novel protein-saccharide linkage, O-linked GlcNAc. *J. Biol. Chem.* 261:8049-57
- Holt, G. D., Snow, C. M., Senior, A., Haltiwanger, R. S., Gerace, L., Hart, G. W. 1987. Nuclear pore glycoproteins contain cytoplasmically disposed O-linked N-acetylglucosamine. *J. Cell Biol.* 104:1157-64
- Hopper, A. K., Traglia, H. M., Dunst, R. W. 1990. The yeast RNA1 gene product necessary for RNA processing is located in the cytosol and apparently excluded from the nucleus. *J. Cell Biol.* 111:309-21
- Howard, E. A., Zupan, J. R., Citovsky, V., and Zambryski, P. C. 1992. The VirD2 protein of *Agrobacterium tumefaciens* contains a C-terminal bipartite nuclear localization signal: Implications for nuclear uptake of DNA in plant cells. *Cell* 68:109-18
- Hunt, T. 1989. Cytoplasmic anchoring proteins and the control of nuclear localization. *Cell* 59:949-51
- Hurt, E. C. 1988. A novel nucleoskeletal-like protein located at the nuclear periphery is required for the life cycle of *Saccharomyces cerevisiae*. *EMBO J.* 7:4323-34
- Hurt, E. C. 1990. Targeting of a cytosolic protein to the nuclear periphery. *J. Cell Biol.* 111:2829-37
- Imamoto-Sonobe, N. I., Matsuoka, Y., Samba, T., Okada, Y., Uchida, T., Yoneda, Y. 1990. A protein recognized by antibodies to Asp-Asp-Asp-Glu-Asp shows specific binding activity to heterogeneous nuclear transport signals. *J. Biol. Chem.* 265:16504-08
- Izaurralde, E., Mattaj, I. W. 1992. Transport of RNA between nucleus and cytoplasm. *Sem. Cell Biol.* 3:In press
- Jans, D. A., Ackermann, M. J., Bischoff, J. R., Beach, D. H., Peters, R. 1991. p34cdc2-mediated phosphorylation at T124 inhibits nuclear import of SV-40 T antigen proteins. *J. Cell Biol.* 115:1203-12
- Jarnik, M., Aebi, U. 1991. Toward a more 3-D structure of the nuclear pore. *J. Struct. Biol.* 107:291-308
- Jarnik, M., Aebi, U., Ris, H. 1991. Towards a complete 3-D model of the nuclear pore complex (NPC). *J. Cell Biol.* 115:458a
- Kadowaki, T., Zhao, Y., Tartakoff, A. M. 1992. *mtr1*, a conditional yeast mutant deficient in mRNA transport from nucleus to cytoplasm. *Proc. Natl. Acad. Sci. USA* 89:2312-316
- Kalderon, D., Richardson, W. D., Markham, A. F., Smith, A. E. 1984a. Sequence requirements for nuclear location of simian virus 40 large-T antigen. *Nature* 311:33-38
- Kalderon, D., Roberts, B. L., Richardson, W. D., Smith, A. E. 1984b. A short amino acid sequence able to specify nuclear location. *Cell* 39:499-509
- Kalinich, J. F., Douglas, M. G. 1989. In vitro translocation through the yeast nuclear envelope. *J. Biol. Chem.* 264:17979-89
- Kessel, R. G. 1989. The annulate lamellae—from obscurity to spotlight. *Elect. Microsc. Rev.* 2:257-348
- Lanford, R. E., Butel, J. S. 1984. Construction and characterization of an SV40 mutant defective in nuclear transport of T antigen. *Cell* 37:801-13
- Lanford, R. E., Feldherr, C. M., White, R. G., Dunham, R. G., Kanda, P. 1990. Comparison of diverse transport signals in synthetic peptide-induced nuclear transport. *Exp. Cell Res.* 186:32-38
- Lanford, R. E., Kanda, P., Kennedy, R. C. 1986. Induction of nuclear transport with a synthetic peptide homologous to the SV40 T antigen transport signal. *Cell* 46:575-82
- Lang, I., Peters, R. 1984. Nuclear envelope

- permeability: A sensitive indicator of pore complex integrity. In *Information and Energy Transduction in Biological Membranes*, pp. 377-86. New York: Liss
- Lang, I., Scholz, M., Peters, R. 1986. Molecular mobility and nucleocytoplasmic flux in hepatoma cells. *J. Cell Biol.* 102:1183-90
- Laskey, R. A., Leno, G. H. 1990. Assembly of the cell nucleus. *Trends Genet.* 6:406-10
- Lawrence, J. B., Singer, R. H., Marselle, L. M. 1989. Highly localized tracks of specific transcripts within interphase nuclei visualized by in situ hybridization. *Cell* 57:493-502
- Lee, W.-C., Melese, T. 1989. Identification and characterization of a nuclear localization sequence-binding protein in yeast. *Proc. Natl. Acad. Sci. USA* 86:8808-12
- Lee, W.-C., Xue, Z., Melese, T. 1991. The NSR1 gene encodes a protein that specifically binds nuclear localization sequences and has two RNA recognition motifs. *J. Cell Biol.* 113:1-12
- Li, R., Thomas, J. O. 1989. Identification of a human protein that interacts with nuclear localization signals. *J. Cell Biol.* 109:2623-32
- Loeb, J. D. J., Davis, L. I., Fink, G. R. 1991. Characterization of the yeast nucleoporin gene family. *J. Cell Biol.* 115:318a
- Lohka, M. J., Masui, Y. 1983. Formation in vitro of sperm pronuclei and mitotic chromosomes induced by amphibian ooplasmic components. *Science* 222:719-21
- Macaulay, C., Forbes, D. 1991. Cell cycle dependent phosphorylation of nuclear pore glycoproteins. *J. Cell Biol.* 115:373a
- Mandell, R. B., Feldherr, C. M. 1990. Identification of two HSP70-related *Xenopus* oocyte proteins that are capable of recycling across the nuclear envelope. *J. Cell Biol.* 111:1775-83
- Maquat, L. E. 1991. Nuclear mRNA export. *Curr. Opin. Cell Biol.* 3:1004-12
- Martin, K., Helenius, A. 1991a. Transport of incoming influenza virus nucleocapsids into the nucleus. *J. Virol.* 65:232-44
- Martin, K., Helenius, A. 1991b. Nuclear transport of influenza virus ribonucleoproteins: The viral matrix protein (M1) promotes export and inhibits import. *Cell* 67:117-30
- Mattaj, I. W. 1990. Splicing stories and poly(A) tales: An update on RNA processing and transport. *Curr. Opin. Cell Biol.* 2:528-38
- Maul, G. G. 1977. The nuclear and cytoplasmic pore complex: Structure, dynamics, distribution and evolution. *Int. Rev. Cytol. Suppl.* 6:75-186
- Mehlin, H., Skoglund, U., Daneholt, B. 1991. Transport of Balbiani ring granules through nuclear pores in *Chironomus tentans*. *Exp. Cell Res.* 193:72-77
- Meier, U. T., Blobel, G. 1990. A nuclear localization signal binding protein in the nucleolus. *J. Cell Biol.* 111:2235-45
- Meinkoth, J. L., Ji, Y., Taylor, S. S., Feramisco, J. R. 1990. Dynamics of the distribution of cyclic AMP-dependent protein kinase in living cells. *Proc. Natl. Acad. Sci. USA* 87:9595-99
- Merisko, E. M. 1989. Annulate lamellae: An organelle in search of a function. *Tissue Cell* 3:343-54
- Michaud, N., Goldfarb, D. S. 1991. Multiple pathways in nuclear transport; The import of U2 snRNP occurs by a novel kinetic pathway. *J. Cell Biol.* 112:215-23
- Michaud, N., Goldfarb, D. 1992. Microinjected U snRNAs are imported to oocyte nuclei via the nuclear pore complex by three distinguishable targeting pathways. *J. Cell Biol.* 116:851-62
- Miller, M., Park, M. K., Hanover, J. A. 1991a. Nuclear pore complex: Structure, function and regulation. *Phys. Rev.* 7:909-49
- Miller, M., Reddy, B. A., Kloc, M., Li, X. X., Dreyer, C., Etkin, L. D. 1991b. The nuclear-cytoplasmic distribution of the *Xenopus* nuclear factor, xnf7, coincides with its state of phosphorylation during early development. *Development* 113:569-75
- Milligan, R. A. 1986. A structural model for the nuclear pore complex. In *Nucleocytoplasmic Transport*, ed. R. Peters, M. Trendelenburg, pp. 113-122. Berlin/Heidelberg: Springer-Verlag
- Moll, T., Tebb, G., Surana, U., Robitsch, H., Nasmyth, K. 1991. The role of phosphorylation and the CDC28 protein kinase in cell cycle-regulated nuclear import of the *S. cerevisiae* transcription factor SW15. *Cell* 66:743-58
- Moore, M., Blobel, G. 1992. The two steps of nuclear import, targeting to the nuclear envelope and translocation through the nuclear pore, require different cytosolic factors. *Cell* 69:939-50
- Morin, N., Delsert, C., Klessig, D. F. 1989. Nuclear localization of the adenovirus DNA binding protein: Requirement for two signals and complementation during viral infection. *Mol. Cell Biol.* 9:4372-80
- Nasmyth, K., Adolf, G., Lydall, D., Seddon, A. 1990. The identification of a second cell cycle control on the HO promoter in yeast: Cell cycle regulation of SW15 nuclear entry. *Cell* 62:631-47
- Nehrbass, U., Kern, H., Mutvei, A., Horstmann, H., Marshallsay, B., Hurt, E. C. 1990. NSP1: A yeast envelope protein

- localized at the nuclear pores exerts its essential function by its carboxy-terminal domain. *Cell* 61:979-89
- Neuman de Vegvar, H. E., Dahlberg, J. E. 1990. Nucleocytoplasmic transport and processing of small nuclear RNA precursors. *Mol. Cell. Biol.* 10:3365-75
- Nelson, M., Silver, P. 1989. Context affects nuclear protein localization in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 9:384-89
- Newmeyer, D. D. 1990. Nuclear import in vitro. *Prog. Mol. Subcell. Biol.* 11:12-50
- Newmeyer, D. D., Finlay, D. R., Forbes, D. J. 1986a. In vitro transport of a fluorescent nuclear protein and exclusion of non-nuclear proteins. *J. Cell Biol.* 103:2091-02
- Newmeyer, D. D., Forbes, D. J. 1988. Nuclear import can be separated into distinct steps in vitro: Nuclear pore binding and translocation. *Cell* 52:641-53
- Newmeyer, D. D., Forbes, D. J. 1990. An N-ethylmaleimide-sensitive cytosolic factor necessary for nuclear protein import: Requirement in signal-mediated binding to the nuclear pore. *J. Cell Biol.* 110:547-57
- Newmeyer, D. D., Lucocq, J. M., Burglin, T. R., De Robertis, E. M. 1986b. Assembly in vitro of nuclei active in nuclear protein transport: ATP is required for nucleoplasmic accumulation. *EMBO J.* 5:501-10
- Newmeyer, D. D., Wilson, K. L. 1991. Egg extracts for nuclear import and nuclear assembly reactions. *Methods Cell Biol.* 36:599-626
- Nigg, E. A. 1992. Signal transduction to the cell nucleus. In *Advances in Molecular and Cell Biology*, pp. 103-31. Greenwich, Conn.: JAI Press
- Nigg, E. A., Baeuerle, P. A., Luhrmann, R. 1991. Nuclear import-export: In search of signals and mechanisms. *Cell* 66:15-22
- Paine, P. L., Horowitz, S. B. 1980. The movement of material between nucleus and cytoplasm. In *Cell Biology: A Comprehensive Treatise*, ed. D. M. Prescott, L. Goldstein, 4:299-338. New York: Academic
- Pandey, S., Parnaik, V. K. 1991. Identification and characterization of nuclear location signal-binding proteins in nuclear envelopes. *Biochim. Biophys. Acta* 1063:81-89
- Parnaik, V. K., Kennady, P. K. 1990. Nuclear transport of proteins translated in vitro from SP6 plasmid-generated mRNAs. *Mol. Cell. Biol.* 10:1287-92
- Peters, R., 1986. Fluorescence microphotolysis to measure nucleocytoplasmic transport and intracellular mobility. *Biochim. Biophys. Acta* 864:305-59
- Peters, R., Lang, I., Scholz, M., Schulz, B., Kayne, F. 1986. Fluorescence microphotolysis to measure nucleocytoplasmic transport in vivo et vitro. *Biochem. Soc. Trans.* 14:821-22
- Picard, D., Kumart, V., Chambont, P., Yamamoto, K. R. 1990. Signal transduction by steroid hormones: Nuclear localization is differentially regulated in estrogen and glucocorticoid receptors. *Cell Reg.* 1:291-299
- Picard, D., Yamamoto, K. R. 1987. Two signals mediate hormone-dependent nuclear localization of the glucocorticoid receptor. *EMBO J.* 6:3333-40
- Pines, J., Hunter, T. 1991. Human cyclins A and B1 are differentially located in the cell and undergo cell cycle-dependent nuclear transport. *J. Cell Biol.* 115:1-17
- Pinol-Roma, S., Dreyfuss, G. 1991. Transcription-dependent and transcription-independent nuclear transport of hnRNP proteins. *Science* 253:312-14
- Pinol-Roma, S., Dreyfuss, G. 1992. Shuttling of pre-mRNA binding proteins between nucleus and cytoplasm. *Nature* 355:730-32
- Reichelt, R., Holzenbury, A., Buhle, E. L. Jr., Jarnik, M., Engel, A., Aebi, U. 1990. Correlation between structure and mass distribution of the nuclear pore complex and of distinct pore complex components. *J. Cell Biol.* 110:883-94
- Richardson, W. D., Mills, A. D., Dilworth, S. M., Laskey, R. A., Dingwall, C. 1988. Nuclear protein migration involves two steps: Rapid binding at the nuclear envelope followed by slower translocation through nuclear pores. *Cell* 52:655-664
- Richardson, W. D., Roberts, B. L., Smith, A. E. 1986. Nuclear location signals in polyoma virus large-T. *Cell* 44:77-85
- Richter, J. D., Standiford, D. 1992. Structure and regulation of nuclear localization signals. See Feldherr 1992
- Rihs, H. P., Peters, R. 1989. Nuclear transport kinetics depend on phosphorylation-site-containing sequences flanking the karyophilic signal of the simian virus 40 T-antigen. *EMBO J.* 8:1479-84
- Rihs, H.-P., Jans, D. A., Fan, H., Peters, R. 1991. The rate of nuclear cytoplasmic protein transport is determined by the casein kinase II site flanking the nuclear localization sequence of the SV40 T-antigen. *EMBO J.* 10:633-39
- Ris, 1990. Application of low voltage high resolution SEM in the study of complex intracellular structures. In *Proc. XII Internat. Congr. Electron Microsc.* p. 18-19. San Francisco: San Francisco Press
- Ris, H. 1991. The three-dimensional structure of the nuclear pore complex as seen by high voltage electron microscopy and high resolution low voltage scanning electron microscopy. *EMSA Bull.* 21:54-56

- Robbins, J., Dilworth, S. M., Laskey, R. A., Dingwall, C. 1991. Two interdependent basic domains in nucleoplasmin nuclear targeting sequence: Identification of a class of bipartite nuclear targeting sequences. *Cell* 64:615-23
- Roberts, B. L., Richardson, W.D., Smith, A. E. 1987. The effect of protein context on nuclear location signal function. *Cell* 50:466-75
- Rome, L., Kedersha, N., Chugani, D. 1991. Unlocking vaults: Organelles in search of a function. *Trends Cell Biol.* 1:47-50
- Rout, M. P., Blobel, G. 1991. A biochemical enrichment procedure for components of the yeast nuclear pore complex. *J. Cell Biol.* 115:458a
- Sadler, I., Chiang, A., Kurihara, T., Rothblatt, J., Way, J., Silver, P. 1989. A yeast gene important for protein assembly into the endoplasmic reticulum and the nucleus has homology to *DnaJ*, and *Escherichia coli* heat shock protein. *J. Cell Biol.* 109:2665-75
- Scheer, U., Dabauvalle, M.-C., Merkert, H., Benevente, R. 1988. The nuclear envelope and the organization of the pore complexes. *Cell Biol. Int. Rep.* 12:669-89
- Schindler, M., Hogan, M., Miller, R., DeGaetano, D. 1987. A nuclear specific glycoprotein representative of a unique pattern of glycosylation. *J. Biol. Chem.* 262:1254-60
- Schneider, J., Schindewolf, C., van Zee, K., Fanning, E. 1988. A mutant SV40 large T antigen interferes with nuclear localization of a heterologous protein. *Cell* 54:117-25
- Sheehan, M. A., Mills, A. D., Sleeman, A. M., Laskey, R. A., Blow, J. J. 1988. Steps in the assembly of replication-competent nuclei in a cell-free system from *Xenopus* eggs. *J. Cell Biol.* 106:1-12
- Shi, Y., Thomas, J. O. 1992. The transport of proteins into the nucleus requires the 70-kilodalton heat shock protein or its cytosolic cognate. *Mol. Cell. Biol.* 12:2186-92
- Shirakawa, F., Mizel, S. B. 1989. In vitro activation and nuclear translocation of NF-K catalyzed by cyclic AMP-dependent protein kinase and protein kinase C. *Mol. Cell. Biol.* 9:2424-30
- Silver, P. A. 1991. How proteins enter the nucleus. *Cell* 64:489-97
- Silver, P., Sadler, I., Osborne, M. A. 1989. Yeast proteins that recognize nuclear localization sequences. *J. Cell Biol.* 109:983-89
- Simental, J. A., Sar, M., Lane, M. V., French, F. S., Wilson, E. M. 1991. Transcriptional activation and nuclear targeting signals of the human androgen receptor. *J. Biol. Chem.* 266:510-18
- Slavicek, J. M., Jones, N. C., Richter, J. D. 1989. A karyophilic signal sequence in adenovirus Type 5 E1A is functional in *Xenopus* oocytes but not in somatic cells. *J. Virol.* 63:4047-50
- Snow, C. M., Senior, A., Gerace, L. 1987. Monoclonal antibodies identify a group of nuclear pore complex glycoproteins. *J. Cell Biol.* 104:1143-56
- Stafstrom, J. P., Staehelin, L. A. 1984. Are annulate lamellae in the *Drosophila* embryo the result of overproduction of nuclear pore components? *J. Cell Biol.* 98:699-708
- Starr, C. M., D'Onofrio, M., Park, M. K., Hanover, J. A. 1990. Primary sequence and heterologous expression of nuclear pore glycoprotein p62. *J. Cell Biol.* 110:1861-71
- Starr, C. M., Hanover, J. A. 1990. Glycosylation of nuclear pore protein p62. *J. Biol. Chem.* 265:6868-73
- Sterne-Marr, R., Blevitt, J. M., Gerace, L. 1992. O-linked glycoproteins of the nuclear pore complex interact with a cytosolic factor required for nuclear protein import. *J. Cell Biol.* 116:271-80
- Stewart, M., Whytock, S. 1988. The structure and interactions of components of nuclear envelopes from *Xenopus* oocyte germinal vesicles observed by heavy metal shadowing. *J. Cell Sci.* 90:409-23
- Stewart, M., Whytock, S., Mills, A. D. 1990. Association of gold-labelled nucleoplasmin with the centres of ring components of *Xenopus* oocyte nuclear pore complexes. *J. Mol. Biol.* 213:575-82
- Stochaj, U., Osborne, M., Kurihara, T., Silver, P. 1991. A yeast protein that binds nuclear localization sequences: Purification, localization, and antibody inhibition of binding activity. *J. Cell Biol.* 113:1243-54
- Stochaj, U., Silver, P. 1992. A conserved phosphoprotein that specifically binds nuclear localization sequences is essential for nuclear import. *J. Cell Biol.* 117:473-82
- Tobian, J. A., Drinkard, L., Zasloff, M. 1985. tRNA nuclear transport: Defining the critical regions of human tRNA-met by point mutagenesis. *Cell* 43:415-22
- Ulitzur, N., Gruenbaum, Y. 1989. Nuclear envelope assembly around sperm chromatin in cell-free preparations from *Drosophila* embryos. *FEBS Lett.* 259:113-16
- Underwood, M. R., Fried, H. M. 1990. Characterization of nuclear localizing sequences derived from yeast ribosomal protein L29. *EMBO J.* 9:91-99
- Unwin, P. N. T., Milligan, R. A. 1982. A large particle associated with the perimeter of the nuclear pore complex. *J. Cell Biol.* 93:63-75
- Van Etten, R. A., Jackson, P., Baltimore, D. 1989. The mouse type IV *c-abl* gene product is a nuclear protein, and activation of



- transforming ability is associated with cytoplasmic localization. *Cell* 58:669-78
- Vigers, G. P. A., Lohka, M. J. 1991. A distinct vesicle population targets membranes and pore complexes to the nuclear envelope in *Xenopus* eggs. *J. Cell Biol.* 112:545-56
- Wente, S. R., Rout, M. P., Blobel, G. 1991. Identification of genes encoding novel nucleoporins of the yeast nuclear pore complex. *J. Cell Biol.* 115:319a
- Wolff, B., Willingham, M. C., Hanover, J. A. 1988. Nuclear protein import: Specificity for transport across the nuclear pore. *Exp. Cell Res.* 178:318-34
- Wozniak, R. W., Bartnik, E., Blobel, G. 1989. Primary structure analysis of an integral membrane glycoprotein of the nuclear pore. *J. Cell Biol.* 108:2083-92
- Wozniak, R. W., Blobel, G. 1991. The transmembrane segment and cyto/nucleoplasmic tail of gp210 are both necessary and sufficient for the targeting of a membrane protein to the nuclear pore. *J. Cell Biol.* 115:458a
- Xing, Y., Lawrence, J. B. 1991. Preservation of specific RNA distribution within the chromatin-depleted nuclear substructure demonstrated by in situ hybridization coupled with biochemical fractionation. *J. Cell Biol.* 112:1055-63
- Yamasaki, L., Kanda, P., Lanford, R. E. 1989. Identification of four nuclear transport signal-binding proteins that interact with diverse transport signals. *Mol. Cell Biol.* 9:3028-36
- Yamasaki, L., Lanford, R. E. 1991. Analysis of p55 and p70 nuclear transport signal binding activities from rat liver. *J. Cell Biol.* 115:317a
- Yamasaki, L., Lanford, R. E. 1992a. Nuclear transport receptors: Specificity amidst diversity. See Feldherr 1992
- Yamasaki, L., Lanford, R. E. 1992b. Nuclear transport: a guide to import receptors. *Trends Cell Biol.* 2:123-27
- Yoneda, Y., Arioka, T., Imamoto-Sonobe, N., Sugawa, H., Shimonishi, Y., Uchida, T. 1987a. Synthetic peptides containing a region of SV40 large T-antigen involved in nuclear localization direct the transport of proteins into the nucleus. *Exp. Cell Res.* 170:439-52
- Yoneda, Y., Imamoto-Sonobe, N., Matsuoka, Y., Iwamoto, R., Kiho, Y., Uchida, T. 1988. Antibodies to Asp-Asp-Glu-Asp can inhibit transport of nuclear proteins into the nucleus. *Science* 242:276-78
- Yoneda, Y., Imamoto-Sonobe, N., Yamaizumi, M., Uchida, T. 1987b. Reversible inhibition of protein import into the nucleus by wheat germ agglutinin injected into cultured cells. *Exp. Cell Res.* 173:586-95
- Zasloff, M. 1983. tRNA transport from the nucleus in a eukaryotic cell: Carrier-mediated translocation process. *Proc. Natl. Acad. Sci. USA* 80:6436-40
- Zimmer, F. J., Dreyer, C., Hausen, P. 1988. The function of the nuclear envelope in nuclear protein accumulation. *J. Cell Biol.* 106:1435-44

## NOTE ADDED IN PROOF

- Meier, U. T., Blobel, G. 1992. Nopp140 shuttles on tracks between nucleus and cytoplasm. *Cell* 70:127-38