MICROBIOLOGY AND MOLECULAR BIOLOGY REVIEWS, June 1997, p. 193–211 Vol. 61, No. 2
1092-2172/97/$04.00+0

Nucleocytoplasmic Transport of Macromolecules

ANITA H. CORBETT AND PAMELA A. SILVER*

Division of Cellular and Molecular Biology, Dana Farber Cancer Institute, and Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts

INTRODUCTION 193
NUCLEAR PORES: THE SITE OF TRANSLOCATION 194
Nuclear Pore Architecture 194
Components of the Nuclear Pore Complex 194
Functional Analysis of Nucleoporins 196
Interactions between Nucleoporins 197
Vertebrate Nucleoporins 197
PROTEIN IMPORT 197
Recognition 198
Nuclear localization signals 198
NLS receptor 198
Targeting to the Nuclear Pore 199
Translocation into the Nucleus 199
The GTP cycle 199
(i) Ran 199
(ii) Rna1p/RanGAP1 200
(iii) Prp20p/RCC1 200
(iv) NTF2 201
(v) Yrb1p/RanBP1 201
Candidate transport factors 201
(i) Proteins that contain Ran binding domains 201
(ii) Other GTPases 201
(iii) HSP70 202
Mechanism of translocation 202
Release into the Nucleus 202
Recycling of the Transport Factors 202
Models for Protein Import 202
Vectorial transport 202
NLS-containing proteins 203
Other transport pathways 203
NUCLEAR EXPORT 204
Nuclear Export Signals 204
RNA Export 204
Candidate Poly(A)$^+$ RNA Export Mediators 205
hnRNPs 205
Rev proteins and cofactors 205
Export of Other RNAs 205
U snRNAs 205
tRNA 206
CONCLUSIONS 206
ACKNOWLEDGMENTS 206
REFERENCES 206

INTRODUCTION

It is becoming increasingly apparent that the study of any biological process must take advantage of all available scientific methods. Not only is it important to examine the individual components in vitro by structural and biochemical means, but also it is critical that the entire process be studied in vivo through the use of genetic and cell biological approaches. These methods, in combination with the tools provided by molecular biology, complement one another and allow complex questions to be probed at all levels.

Our understanding of the bidirectional movement of macromolecules across the nuclear envelope has benefited from such a multifaceted approach. Nuclear trafficking is an exceedingly complex process consisting of numerous macromolecular highways with proteins and RNAs traveling back and forth through nuclear pores that serve as tunnels through the nuclear envelope. Although proteins are produced in the cytoplasm, those that participate in nuclear functions must be translocated into the nucleus, a process known as nuclear protein import. Some of these proteins are subsequently trans-

* Corresponding author. Mailing address: 849 Mayer Bldg., Dana Farber Cancer Institute, 44 Binney St., Boston, MA 02115. Phone: (617) 632-5101. Fax: (617) 632-5103. E-mail: pamela_silver@macmailgw.dfci.harvard.edu.

ported back into the cytoplasm via a specific process termed nuclear protein export. In addition to proteins, a variety of RNA species move back and forth across the nuclear envelope. All of these processes considered in concert comprise the general phenomenon described as nuclear transport.

Classical studies of nuclear protein import have generally examined the import of proteins that contain canonical nuclear localization signals (NLSs). Many RNA export studies have focused on the movement of poly(A)$^+$ RNA. Recent advances have shown that these views of protein import and RNA export are simplistic. Evidence suggesting that there are distinct mechanisms for the transport of different classes of proteins (5, 205) and RNAs (91, 227) into and out of the nucleus is emerging. Thus, the transport processes that have been studied most extensively may represent only the tip of the mechanistic iceberg.

The physical separation of nuclear and cytoplasmic functions bestows upon the eukaryotic cell a mechanism for regulation of cellular processes that is not available to prokaryotes. Compartmentalization is a regulatory mechanism where, for example, an activator may be sequestered from its activation target. There are many examples of transcription factors that are sequestered in the cytoplasm and are transported to the nucleus only in response to a cellular signal (17, 33, 174, 213, 242). In light of these considerations, it is clear that the macromolecular traffic crossing the nuclear envelope must be meticulously regulated both to maintain the normal state of the cell and to respond to intracellular signals that mediate cell growth and other essential processes.

The basic components of the nuclear transport system have been exceedingly well conserved throughout evolution. It has been known for some time that the general architecture of the transport channel, the nuclear pore, has been conserved among diverse species (166). This conservation has now expanded to the molecular level and includes many of the soluble transport factors and at least one component of the nuclear pore (4, 7, 51, 170). This evolutionary conservation of components comprising the nuclear transport machinery broadens the arena in which studies of this process can occur. Consequently, studies carried out in a number of different experimental systems have contributed to our current understanding of macromolecular transport. There are, however, several experimental systems that have contributed the most to our knowledge. In vitro transport assays with either *Xenopus* egg extracts or permeabilized mammalian cells have provided a great deal of information. These studies have been complemented by in vivo studies carried out in genetic systems such as the budding yeast, *Saccharomyces cerevisiae*, and the fission yeast, *Schizosaccharomyces pombe*. A number of recent reviews have neglected many of the contributions that have been made as a result of studies in these genetically amenable organisms. For this reason, the present review will attempt to present our current understanding of nuclear transport in an integrated manner while highlighting the contributions that have been made through studies in yeast.

NUCLEAR PORES: THE SITE OF TRANSLOCATION

Macromolecular communication between the nucleus and the cytoplasm is mediated by nuclear pores that serve as the physical connection between these two cellular compartments. Movement of all proteins and RNAs across the nuclear envelope occurs through these nuclear pores that form channels in the double membrane. Several excellent reviews have recently addressed the structure and function of the nuclear pore complex (59, 196, 223). The nuclear pore complex has been studied primarily by two different approaches. Microscopy has been used to examine the overall architecture of the pore complex (196), while the individual protein components, termed nucleoporins, have been defined through a combination of biochemical and genetic approaches (66, 222). These studies have demonstrated that the nuclear pore complex is extremely large and is currently estimated to contain between 40 and 100 distinct proteins (14, 222). Yeast nuclear pores are estimated to be approximately 66 MDa (204), whereas mammalian nuclear pores are on the order of 125 MDa (196, 198). Recent developments in biochemical (222) and genetic (66) methodologies, as well as the recent completion of the sequencing of the entire *S. cerevisiae* genome (70), are indications that the identification of the individual components of the pore will be completed in the foreseeable future. The challenge that remains is to meld these two approaches and determine the arrangement of each individual component in this large complex, an endeavor that is presently being undertaken through the use of immunoelectron microscopy (196).

Nuclear Pore Architecture

Briefly, the nuclear pore complex resembles a rivet embedded in the nuclear membrane (88, 196, 198). Studies of amphibian nuclear envelopes have revealed that the complex is asymmetric with respect to the nuclear envelope. Both the cytoplasmic and nuclear faces have rings with eightfold rotational symmetry that are connected by central spokes that may traverse the nuclear envelope (197). In vertebrate cells, the cytoplasmic face has filaments of approximately 50 to 100 nm that emanate away from the face of the nucleus whereas the nucleoplasmic face contains a basket-like structure that projects into the nucleus (88). In addition, the center of the pore complex contains a "transporter" or "central plug," which has been the point of some controversy. It is not yet clear whether this electron-dense material represents a structural element of the nuclear pore complex or perhaps merely a macromolecule in transit. At least one nucleoporin has been mapped to this domain of the nuclear pore (101, 196, 199), suggesting that it is truly a structural element of the pore complex.

Components of the Nuclear Pore Complex

Individual components of the nuclear pore complex have been identified by both biochemical and genetic approaches. Although a number of different systems have been used to isolate nucleoporins, by far the most success has been achieved with the budding yeast, *S. cerevisiae*. The first yeast nucleoporins were identified by purification of yeast nuclear envelopes (122), isolation of proteins by immunological methods, and reverse genetics to identify the corresponding genes (60, 121). Genetic screens that were originally based on the first two genes identified in this manner, *NSP1* (121) and *NUP1* (60), have been invaluable in the identification of new nucleoporins. In particular, synthetic lethal screens that identify genetic interactions between genes encoding nucleoporins have led to the identification of more than half of the known genes (66). These screens take advantage of situations in which a conditional mutant is viable on its own but is lethal in combination with a second mutation, presumably in a functionally related gene (20). Another method that has been quite useful in the identification of novel nucleoporins has been to screen for conditional yeast mutants with defects in macromolecular transport (9, 89, 96, 110, 151). These genetic approaches have been complemented by the original biochemical approach, purifying nuclear envelope fractions enriched in pore complexes

TABLE 1. Nucleoporins identified in *S. cerevisiae*

Nucleoporin	Class	Interactions[a]	Phenotype	Reference(s)
NUP49	GLFG	*NSP1*, Nsp1p, Nup57p, Nic96p	Essential; protein import and RNA export defects	98, 269, 273
NUP57	GLFG	*NSP1*, Nsp1p, Nup49p, Nic96p	Essential	99
NUP82		*NSP1*, *NUP120*, *NUP85*	Essential; RNA export defects	98, 123
NUP84		*NSP1*, Nup85p, Nup120p, Sec13p, Seh1p	Not essential; RNA export defects	245
NUP85		*NSP1*, Nup84p, Sec13p, Seh13p, Nup120p	Not essential; RNA export defects	89, 245
NIC96 (90 kDa)		*NSP1*, *POM152*, Nsp1p, Nup49p, Nup57p	Essential	97, 98
NUP2 (95 kDa)	FXFG	*NSP1*, *NUP2*	Not essential	153
NUP100	GLFG	*NUP116*, *NUP145*	Not essential	269
NSP1 (~100 kDa)	GLFG/FXFG	*NUP1*, *NUP2*, *NUP49*, *NUP57*, *NUP82*, *NUP84*, *NUP85*, *NIC96*, *NUP116*, *NUP133*, *NUP145*, Nup49p, Nup57p, Nic96p	Essential; protein import defects, tRNA export defects	97, 121, 186, 273
NUP116	GLFG	*NSP1*, *NUP100*, *NUP145*	Not essential	238, 269, 273
NUP120		*NUP133*, *NUP159*	Not essential; RNA export defects	6, 110
NUP1 (130 kDa)	FXFG/GLFG	*NSP1*, *NUP2*, *NUP133*	Essential; RNA export defects	26, 60, 232, 269
NUP133		*NUP1*, *NUP120*	Not essential; RNA export defects	67, 151, 238
NUP145	GLFG	*NSP1*, *NUP100*, *NUP116*	Essential; RNA export defects	72, 267
POM152		*NIC96*, *NUP170*, *NUP188*	Not essential	7, 222
NUP157		*NUP170*, *NUP188*	Not essential	7
NUP159	XXFG/FXFG		Essential; RNA export defects	96, 144
NUP170		*POM152*, *NUP157*, *NUP188*	Not essential	7, 136
NUP188		*POM152*, *NUP170*	Not essential	7, 188

[a] Interactions are either genetic or biochemical. Genetic interactions are indicated by listing the gene encoding the protein. Biochemical interactions are indicated by listing the relevant proteins.

and using microsequencing to identify proteins corresponding to bands on a protein gel, a technique that has proved useful for mammalian systems (210, 211) as well as yeast (7, 136, 222). Finally, with the release of the complete sequence of the *S. cerevisiae* genome, it has been possible to identify gene classes simply on the basis of sequence homology (7, 70). The combination of these methods has made yeast the premier organism for the molecular analysis of the individual components of the nuclear pore. The sequence of the entire genome also facilitates the characterization of components at the molecular level. Since genes encoding all nucleoporins have been sequenced (as have all *S. cerevisiae* genes), albeit not identified, the nuclear pore components are all defined by specific genetic loci rather than by function or protein molecular weight. This is in contrast to some nucleoporins described in higher eukaryotes, where the protein of interest corresponds to a band with unknown molecular identity on a gel.

Table 1 lists the nucleoporins that have been identified in *S. cerevisiae*. Conventionally, each component of the nuclear pore is named by the three-letter code *NUP* followed by the molecular mass of the protein. For historical reasons, there are several nucleoporins that do not conform to this convention, including *NUP1* (60), *NUP2* (153), *NIC96* (97), and *NSP1* (121). The molecular masses of these proteins are listed in Table 1. Pore components that are thought to be membrane-spanning proteins based on sequence analysis are referred to as POMs, for pore membrane proteins (276).

Identification of approximately 20 genes encoding nucleoporins in yeast and a handful of others in higher eukaryotes has led to the classification of these proteins into several different groups based on sequence motifs contained within their primary amino acid sequences. Two classes of phenylalanine/glycine (FG) repeats, GLFG and FXFG subtypes, have been identified. A number of nucleoporins contain one or the other or a combination of these repeats (Table 1). Since the sequencing of the yeast genome is complete, it is clear that there are no other nucleoporins that fall into the FG repeat family. It is possible, even likely, that there is an as yet unidentified class of nucleoporins containing a different motif or that the remaining nucleoporins are distinct from known nucleoporins and from one another.

Thus far, the in vivo function of the FG repeat regions has not been elucidated. It has, however, been shown that some soluble nuclear transport factors interact with these FG repeat domains in vitro (127, 188, 216). Thus, the FG repeat domains may play a role in targeting the soluble transport factors to the nuclear pore. It has been demonstrated for several nucleoporins that the repeat regions are not the essential regions of the proteins (73, 187). This suggests that the repeats of other nucleoporins can substitute for those deleted regions and implies a redundancy of function among related components of the nuclear pore complex. This is also consistent with the fact that many nucleoporins are not themselves essential, again implying a redundancy of function.

Several nucleoporins do not contain FG repeats but contain other sequence motifs. These include the coiled-coil domains found in Nsp1p, Nup49p, Nup57p, Nic96p, CAN/NUP214, and p62 (59, 97, 99, 143, 273); the leucine zipper found in Nup107 and CAN/NUP214 (143, 210); and the zinc finger domain found in Nup153 (252). In addition, several of the yeast nucleoporins have sequences that are consistent with RNA binding domains. These include Nup100p (269), Nup116p (269, 273), and Nup145p (267).

At least two screens carried out in yeast have identified other FG-containing candidate nucleoporins or nucleoporin-associated proteins. A two-hybrid/interaction trap (74, 103) screen carried out with the human immunodeficiency virus (HIV) Rev protein identified yeast (251) and human (28, 84) Rev inter-

acting proteins (Rips). These Rips contain FG repeats that are diagnostic of nucleoporins. Yeast Rip1p is concentrated at the nuclear rim (251). A second screen carried out in *S. cerevisiae* (31) identified a protein called Np14p that is required for nuclear membrane integrity and nuclear transport (61). Np14p contains several FG repeat motifs and is localized to the nuclear periphery. In addition, overexpression of Np14p is able to partially rescue the structural defects observed in nuclear pores (268) when the *NUP116* gene is deleted.

One dilemma that arises with regard to components of the nuclear pore is the question of what constitutes a nucleoporin. Are nucleoporins structural components of the pore complex, or are they also dynamic transport mediators that function primarily at the pore? The current definition of a nucleoporin is that it is localized to the nuclear pore. In many cases, proteins are also designated nucleoporins if they contain FG repeat motifs. This raises the question whether importin-β, for example, should be designated a nucleoporin. Importin-β contains several FG repeats, performs its function at the nuclear rim, and spends at least a portion of its time at the nuclear rim (95, 141, 209). Obviously, this point will not be resolved until a model of the nuclear pore complex has been established.

Functional Analysis of Nucleoporins

Not only is budding yeast the system of choice for the identification of nucleoporins, but also there are significant advantages to this organism for the subsequent analysis of nucleoporin function. The genes can be deleted to determine whether they encode essential proteins, and the phenotypes of either deletions or conditional alleles of the genes can be examined. Results of these types of studies demonstrate that some nucleoporins are essential for viability whereas others are not (Table 1). Again, this probably reflects redundant functions of some of the pore components, a hypothesis that is supported by the fact that many mutations in genes encoding nucleoporins display synthetic lethal interactions with conditional alleles of other nucleoporin genes (66).

Since the nucleoporins are both the structural and functional components of the nuclear pore complex, it seems likely that many of them will serve general functions (perhaps those that are primarily structural) and that some may play more specific roles in the transport of different classes of macromolecules. Functional analysis is complicated by the fact that nuclear pores serve as the transit site for macromolecules both entering and exiting the nucleus. Since the transport process is bidirectional, it is likely that an alteration that affects import may also affect export either directly or indirectly and vice versa. Thus, it is somewhat complicated to assign in vivo functions to individual nucleoporins. Nevertheless, a number of genes encoding nucleoporins have been identified in a screen (9) for *S. cerevisiae* mutants defective in the export of poly(A)$^+$ RNA from the nucleus. These nucleoporins were originally referred to as *RAT* genes to designate RNA trafficking, but they have now been renamed to follow the *NUP* convention. They include *NUP85*/*RAT9* (89), *NUP120*/*RAT2* (110), *NUP133*/*RAT3* (151), and *NUP159*/*RAT7* (96). Furthermore, the nucleoporins Nup100p (269), Nup116p (269, 273), and Nup145p (267) contain putative RNA binding domains. Subsequent experimentation has suggested that at least some of these nucleoporins play a more direct role in the export of poly(A)$^+$ RNA from the nucleus than in protein import. For example, mutations in the essential gene *NUP159* cause defects in poly(A)$^+$ RNA export, but no defect in protein import has been detected (96).

Although genetic screens for mutants defective in nuclear protein import have not identified nucleoporins in the same abundance as have screens for mutants defective in poly(A)$^+$ RNA export, some nucleoporins are directly implicated in protein import. The nucleoporin that falls most readily into this class is Nsp1p. Several *NSP1* mutants have been characterized, and thus far only protein import defects have been observed (98, 186, 187). In vitro studies suggest that the protein import defect observed in *NSP1* mutants arises from a decrease in the docking of substrate to the nuclear envelope as well as from an inability to translocate substrate across the pore (233). These findings could explain why mutations in *NSP1* directly affect protein import but do not seem to affect RNA export.

There is at least one case where different mutations in a single nucleoporin delineate distinct roles for that protein in both protein import and poly(A)$^+$ RNA export. Two different alleles of the nucleoporin *NUP49* have defects in one process but not in the other. The *nup49-313* allele causes defects in the import of proteins to the nucleus (67, 233) but not in poly(A)$^+$ RNA export. In vitro assays demonstrate that this *NUP49* allele causes defects in docking proteins to the nuclear rim (233). In contrast, the *nup49-316* allele causes defects in poly(A)$^+$ RNA export but not in protein import (67). These findings suggest that Nup49p is essential for both protein import to the nucleus and poly(A)$^+$ RNA export.

Understanding precisely what role each nucleoporin plays in macromolecular transport will probably require a model of the entire pore complex, but the phenotypes of the different mutants may contribute to our construction of an overall model. One point to consider is that many of the relevant phenotypes have not been examined for each nucleoporin, and in the case of some essential genes, no conditional alleles are yet available.

A number of other in vivo defects have been attributed to conditional mutations in different nucleoporin genes. Nontransport phenotypes that have been reported include defects in nuclear envelope and pore structure, clustering of nuclear pores, and nucleolar fragmentation (6, 59, 67, 89, 188, 203, 223). The prevailing theory is that the pore-clustering phenotype results from a role for nucleoporins in anchoring the nuclear pores within the membrane and possibly in forming a matrix of nuclear pores embedded in the nuclear envelope, analogous to a scaffold for the membrane (1). The nucleolar fragmentation phenotype may result from a backlog in RNA processing and transport, but this is a controversial issue that has yet to be resolved. It has also been demonstrated that tRNA processing is compromised in some nucleoporin mutants but not in others (238, 244).

There are some limited approaches to assigning in vivo function to particular classes of nucleoporins in eukaryotes other than budding yeast. For example, one method consists of depletion of *Xenopus* egg extracts of nuclear pore components with wheat germ agglutinin or antibodies prior to nuclear envelope assembly (75, 167, 190, 207). Such an approach has been used to demonstrate that the vertebrate nucleoporin complex p62-p58-p54 is required for docking of proteins at the nuclear pore (75) and also to examine the steps in nuclear pore assembly (157). These experiments are, however, restricted by the availability of specific tools to deplete each nucleoporin.

Recently, the first mouse knockout of a nucleoporin gene was described (260). This study demonstrated that the CAN/NUP214 gene is essential for viability in mice. The results also suggest that the CAN/NUP214 gene product is required for the import of NLS-containing proteins to and the export of poly(A)$^+$ RNA from the nucleus. No gross morphological defects were observed in the nuclear envelopes or the nuclear pores. This study provides the first genetic analysis of nucleoporin function in higher eukaryotes.

TABLE 2. Vertebrate nucleoporins

Nucleoporin	Motif or comment	Localization	Reference(s)
p54	Not cloned	Central plug, nuclear and cytoplasmic rings	75, 199
p58	Not cloned	Central plug, nuclear and cytoplasmic rings	75, 199
p62	XFXFG; coiled coil	Central plug, nuclear and cytoplasmic rings	75, 199
p75	Not cloned	Cytoplasmic ring and filaments	199
NUP98	GLFG/FXFG/FG	Nucleoplasmic face	212
NUP107	Leucine zipper		210
POM121	FG	Integral membrane	105, 247
NUP153	Zn^{2+} fingers	Nuclear basket	252
NUP155	Functional conservation with yeast Nup170p	Nucleoplasmic and cytoplasmic faces	7, 136, 211
NUP180		Cytoplasmic ring and filaments	270
gp210	Glycoprotein	Membrane protein	100, 275
CAN/NUP214/p250	XFXFG; coiled coil, leucine zipper	Cytoplasmic rings and filaments	143, 199
p265/Tpr		Cytoplasmic face	36
NUP358/RanBP2	Ran binding domain	Cytoplasmic filaments	271, 277, 280

Interactions between Nucleoporins

Several of the nucleoporins can be grouped into substructures based on biochemical copurification of complexes and on genetic interactions (summarized in Table 1). For example, since conditional alleles of *NSP1* have been the basis for numerous genetic studies, a number of nucleoporin genes that interact with *NSP1* have been identified. These include *NUP1*, *NUP2*, *NUP49*, *NUP57*, *NUP82*, *NUP84*, *NUP85*, *NIC96*, *NUP116*, *NUP133*, and *NUP145* (66, 72, 97–99, 273). There are several interpretations of the extensive genetic interactions between nuclear pore components. It is possible that many of these components physically interact with one another or that they perform similar functions. It is also possible that a pore complex can survive in the absence of one structural building block but collapses when a second block is removed. Thus, while the genetic studies have been most lucrative in terms of the identification of nucleoporins and other transport components, it is necessary to carry out the complementary biochemical studies in order to begin to identify direct physical functions for the different pore components. The biochemical studies suggest that at least some of these genetic interactions represent true physical association, because Nsp1p can be purified in a complex with Nup49p, Nup57p, and Nic96p (97). Further support for the existence of subcomplexes of nucleoporins comes from a second study in which a tagged version of Nup84p was used to identify a complex of interacting proteins including Nup120p, Nup85p, Sec13p, and a Sec13p homolog (245). These experiments have begun to identify direct physical interactions between pore components and thus to dissect the nuclear pore into its distinct subcomplexes. These studies confirm that the convergence of genetic and biochemical data in combination with increasingly detailed microscopic analysis will ultimately result in the construction of a model of the entire nuclear pore complex.

Vertebrate Nucleoporins

Several vertebrate nucleoporins have been identified by a number of different approaches (Table 2). The studies on these nucleoporins have been limited by the lack of genetic approaches available in higher eukaryotes. In some cases, the vertebrate proteins appear to have yeast homologs, but the connections are not always clear. For example, on the basis of structural homology and cross-reactivity of antibodies, it has been proposed that vertebrate p62 is the homolog of yeast Nsp1p (38). In contrast to the soluble transport factors, only in a single case has sequence homology been tested to determine whether it translates to conservation of function. This particular study demonstrated that the mammalian NUP155 gene under the control of a constitutive promoter complements the synthetic lethality of a *nup170*Δ*pom152*Δ double mutant, suggesting that mammalian Nup155 is functionally homologous to yeast Nup170p (7, 136).

Although yeast may be the system of choice for identifying individual components of the nuclear pore complex, the balance currently shifts to the study of higher eukaryotes for the localization of individual nucleoporins within the three-dimensional structure of the nuclear pore. Immunolocalization has been used to demonstrate that a number of the known vertebrate nucleoporins localize to the cytoplasmic filaments (Table 2). These nucleoporins include CAN/NUP214/p250 (199), Tpr/p265 (36), and Nup358/RanBP2 (271, 280). In addition, p62 has been localized to both the internal and external faces of the central plug (102) and NUP153 has been localized to the face of the nuclear basket (252).

PROTEIN IMPORT

In recent years, the convergence of biochemical, genetic, and cell biological approaches in the study of nuclear trafficking has led to significant advances in our understanding of the mechanism of protein transport (92, 206, 254). Historically, nuclear protein import has been described as a two-step process: an energy-independent binding at the nuclear pore followed by an energy-dependent translocation into the nucleus (191). Advances in our understanding of the import mechanism as well as the identification of transport factors have led to the realization that this process can actually be divided into more distinct and specialized steps: (i) recognition of the transport substrate in the cytoplasm, (ii) targeting to the nuclear pore complex, (iii) translocation through the nuclear pore, (iv) release of the transport substrate at the nucleoplasmic face of the pore complex, and (v) recycling of the transport factors. While none of these steps are completely understood, some molecular detail (discussed below) is beginning to emerge.

Much of our insight into the mechanism of nuclear transport has come from the realization that, like many other cellular processes (29, 39), it depends on a cycle of GTP hydrolysis. In the case of nuclear transport, this GTPase cycle is mediated by the small GTP-binding protein Ran (133, 168, 176, 177). Other essential factors are those that regulate the activity of Ran and target transport substrates to the nuclear pore.

TABLE 3. Protein import factors

Transport factor	Function	Localization	References
Importin-α, *SRP1*, *KAP60*, *NBP70*, karyopherin-α, p54/56, NPI-1, pendulin, oho31, PTAC p54, Rch1	NLS binding	Cytoplasmic, nuclear, nuclear Rim	56, 94, 126, 146, 181, 249, 257, 279
Importin-β, *KAP95*, *RSL1*, karyopherin-β, p97	Interacts with α to target NLS-containing proteins to the nuclear pore	Cytoplasmic nuclear im	42, 95, 141, 209
GSP1&GSP2/Ran/*spi1* (*S. pombe*)	GTP hydrolysis	Nuclear, cytoplasmic	19, 133, 163, 165, 168, 177
NTF2/p10/PP15	Binds to Ran-GDP and nucleoporins with FG repeats	Nuclear rim	51, 178, 185, 201
RNA1/RanGAP1/*rna1* (*S. pombe*)	GTPase-activating protein (GAP)	Cytoplasmic, nuclear rim	22, 115, 117, 165
PRP20/*RCC1*/*pim1* (*S. pombe*), *BJ1* (*Drosophila*)	Nucleotide exchange factor	Nuclear	24, 57, 134, 230
YRB1/*RanBP1*/*spb1* (*S. pombe*)	GTPase-activating protein activator	Cytoplasmic, nuclear rim	23, 109, 195, 235

Recognition

Nuclear localization signals. Proteins destined to be targeted into the nucleus following translation in the cytoplasm contain specific signals in their primary sequences that dictate that destiny (65). The most extensively studied signals are termed nuclear localization signals (NLSs) and, unlike other signal sequences, can be located anywhere in the primary sequence of the protein. No true consensus has emerged for NLSs, although numerous signals that target proteins to the nucleus have been identified. The canonical short NLS is the 7-amino-acid stretch from the simian virus 40 large T antigen, PKKKRKV (135). Mutation of the underlined amino acid renders the sequence nonfunctional (135, 147). This short NLS sequence has been joined by the bipartite NLS, which consists of two runs of basic amino acids (similar to the simian virus 40 NLS) separated by a spacer region (65). The classic example of this type of NLS is found in nucleoplasmin (221). Furthermore, other longer, more complex sequences that target proteins to the nucleus have been identified (246). Interestingly, at least one of these complex signals, found in the heteronuclear RNA binding protein hnRNPA1, is sufficient not only for targeting into the nucleus but also for subsequent export out of the nucleus (173).

The context of the NLS can influence the rate of transport to the nucleus (189). In particular, it is possible to regulate transport to the nucleus by modification of the amino acids flanking the NLS. One example of this regulation is the reversible phosphorylation of amino acid residues proximal to the NLS (130, 220). This mechanism is used to modulate the localization of transcription factors in response to a signal transduction cascade. In yeast, several transcription factors move into the nucleus in a manner that is regulated by their phosphorylation state (130). For example, the Swi5p and Swi6p transcription factors are cytoplasmic when phosphorylated on a specific serine residue but move into the nucleus during the stage in the cell cycle where they are dephosphorylated (174, 242).

NLS receptor. Proteins are targeted to the nuclear pore via an interaction in the cytoplasm between the NLS within the protein and a soluble NLS receptor. For a number of years, the molecular identification of the NLS receptor eluded researchers. However, recent advances in research in yeast and higher eukaryotes have led to the isolation of a heterodimeric complex required for targeting NLS-containing proteins to nuclear pores.

The first subunit of the NLS receptor was identified biochemically by fractionation of *Xenopus* cytosol in conjunction with an in vitro import assay. This subunit is a protein of approximately 60 kDa termed importin-α (94). When the cDNA corresponding to the protein was cloned and sequenced, it became apparent that the *S. cerevisiae* gene encoding yeast importin-α had already been cloned. The protein had been shown to be associated with nuclear pores (18, 278); however, its specific function remained unknown. In fact, the yeast gene was identified in a genetic screen for suppressors of a temperature-sensitive allele of RNA polymerase I, resulting in the designation of the gene as *SRP1* (suppressor of RNA polymerase). Several other studies (with a variety of organisms) have also identified this subunit of the NLS receptor (3, 126, 181, 194) (Table 3). The second subunit of the NLS receptor is a protein of approximately 95 kDa termed importin-β. Like importin-α, this protein was identified by a number of complementary approaches (42, 127, 141, 212). Since importin-α and importin-β have been isolated by a number of different groups, they have been given a number of different names (Table 3). The yeast genes have been referred to most frequently as *KAP60* (α) and *KAP95* (β), while the proteins have been referred to as either karyopherin-α and karyopherin-β or importin-α and importin-β.

While both subunits of the NLS receptor can interact with NLS sequences, the binding to importin-α is significantly tighter (95, 181). This finding, in combination with the observation that importin-β interacts with repeats contained in several nucleoporins (127, 212, 216), has led to the proposal of a model in which importin-α is primarily responsible for binding to NLS-containing proteins and importin-β then targets the complex to the nuclear pore (95, 181). There is also genetic data consistent with this model of NLS receptor function. Mutations in genes encoding either subunit of the NLS receptor cause defects in the import of NLS-containing proteins in vivo (141, 154). Furthermore, the intracellular localization of the two subunits is consistent with the model. Importin-β is found both in the cytoplasm and concentrated at nuclear pores on either side of the nuclear envelope (18, 95, 141). Importin-α is also found in these locations, but, in addition, a significant pool

is found in the nucleus (95). In fact, in some mutant yeast strains, importin-α actually accumulates in the nucleus under conditions where export from the nucleus is blocked (6, 141).

Structure-function analysis of the yeast importin-α protein has contributed to our understanding of the mechanism used by the NLS receptor to target proteins to the nuclear pore. Importin-α, as well as a number of other proteins, contains an internal region with hydrophobic repeats sometimes referred to as "armadillo" repeats (202, 279). This region can bind to NLS-containing sequences (53, 265). The N terminus of importin-α also contains a number of residues that are highly conserved. Two recent studies have demonstrated that it is this region that mediates binding to importin-β, an activity which is sufficient to direct importin-α (or a heterologous protein) into the nucleus (90, 265). It will be of interest to extend these structure-function analyses to in vivo studies by carrying out similar experiments with mutant proteins expressed in yeast.

Several other interesting observations have been made with respect to the in vivo function and regulation of the importin-α subunit. A mutant allele of the yeast importin-α gene, *srp1-31*, causes defects in progression through the cell cycle in addition to defects in nuclear import (154). Cells harboring this mutation were arrested at the G_2/M transition with a short bipolar spindle. This finding is one of several that suggests intriguing connections between nuclear transport and cell cycle progression (for reviews, see references 57 and 229). One observation that may also be relevant to the in vivo function of importin-α is the finding that the protein appears to be phosphorylated (13, 250). It is possible that phosphorylation modulates the interaction with the NLS-containing substrate.

Targeting to the Nuclear Pore

Very little is known about how the import complex is targeted to the nuclear pore. It is thought that the cytoplasmic filaments identified in higher eukaryotes may serve to concentrate the transport substrate at the face of the nuclear pore. Recent high-resolution studies provide support for this hypothesis (197).

The role of importin-β appears to be to direct the import complex to the nuclear pore. The mechanism by which this occurs is not yet understood in detail, but it is of interest that the nucleoporin repeat domains, GLFG and FXFG, have been shown to interact with importin-β (120, 156). In many cases thus far, the repeats have been isolated and tested for interactions outside the context of the entire protein either by in vitro methods (127, 185, 216) or via the two-hybrid screen (127). Thus, it remains to be determined which of the nucleoporins directly interacts with the transport factor-substrate complex in vivo. Furthermore, at least one yeast nucleoporin, Nup2p (64), and one mammalian nucleoporin, RanBP2/Nup358 (277, 280), contain Ran binding domains (16, 107), a motif found in proteins that interact with the small GTP-binding protein required for nuclear transport, Ran (168, 177).

RanBP2 has been localized to the filaments that extend into the cytoplasm, and it has therefore been proposed that this nucleoporin serves as the initial docking site for the NLS-receptor complex at the periphery of the nuclear envelope in higher eukaryotes (271). One thing to consider with respect to this model is that Nup2p, which is the yeast protein with the greatest homology to RanBP2, is not essential for viability in *S. cerevisiae* (153). It is notable that since the entire yeast genome has been sequenced, there are no other candidates for yeast homologs of RanBP2. This means that, at least in *S. cerevisiae*, this docking step cannot represent an essential step in the transport process. There are several possible explanations for this apparent paradox. First, Nup2p may not be a functional homolog of RanBP2. This can be tested in yeast, where the *NUP2* gene is essential in some genetic backgrounds. Second, it is possible that transport occurs via a different mechanism in yeast than in higher eukaryotes. It seems unlikely, given the extreme conservation observed between transport factors in a variety of species, that the mechanism of translocation of substrates through the nuclear pore is really distinct in different organisms. It is possible, however, that the initial docking step and targeting to the nuclear pore differ. It is possible that higher eukaryotes, which have a higher ratio of cytoplasmic volume to nuclear volume than yeast cells, require a more extended nuclear pore to lure NLS-containing proteins into the nuclear transport machinery. This could explain why the cytoplasmic filaments observed in higher eukaryotes have not yet been identified in yeast (59). Finally, it is possible that binding at RanBP2 represents one of many entrees into the transport pathway. Some genetic experiments suggest that *NUP2* may represent one branch of an import pathway, because it is essential only when *NUP1* is deleted (153). However, since it is not yet clear whether either of these nucleoporins is involved in the initial docking at the pore or even where they are localized in the pore, it would be premature to draw any definite conclusions on the basis of this genetic data. Future studies on the organization and complete structure of the nuclear pore complex will lend insight to the role of these critical interactions in directing transport substrates to the site of translocation.

Translocation into the Nucleus

Once transport substrates are targeted to the nuclear pore, they must undergo translocation through the pore complex. The most important breakthrough in the analysis of the translocation step of nuclear protein import came with the identification of the small GTP-binding protein Ran as a critical cytosolic transport factor (168, 177). This discovery demonstrated that nuclear transport is a cellular process that has reiterated a regulatory mechanism used throughout the cell, a cycle of GTP hydrolysis (29, 32, 39). It also provided a number of paradigms on which to base future studies on the mechanism of nuclear transport. As with many other cellular processes, a number of factors that regulate Ran-mediated GTP hydrolysis have been identified. In fact, the combination of genetic and biochemical approaches has allowed the creation of a list of factors involved in this process (Table 3). It has been relatively straightforward to identify these transport factors and to assign their roles on paper; however, it will be more complicated to weave them into a model that explains all the data that has been gathered thus far and allows the vectorial transport of substrates both into and out of the nucleus (92, 140).

The GTP cycle. (i) Ran. Ran was initially identified as an open reading frame with homology to small G-proteins like Ras (69). Several years later, the protein was purified by a mammalian in vitro protein import assay (179). It was identified as a cytosolic factor required for the import of NLS-containing substrates into the nucleus (168, 177). Highly conserved homologs of Ran have been identified in every organism examined to date (55). In *S. cerevisiae*, there are two nearly identical Ran homologs encoded by *GSP1* and *GSP2* (19, 133). These genes were identified as high-copy suppressors of the *prp20-1* mutant (see the discussion of Prp20p, below). *GSP1* is a highly expressed essential gene, whereas *GSP2* is upregulated only under certain growth conditions and is not essential (19).

Like other G-proteins, Ran cycles between a GDP-bound form and a GTP-bound form (32). In vitro studies demonstrated that nuclear protein import is inhibited by nonhydrolyzable analogs of GTP (168, 177). Several approaches were taken to examine the in vivo effects of perturbing the Ran-mediated GTP cycle. The original in vivo experiments were carried out with *S. cerevisiae* (234), but some have since been duplicated in other systems (37, 63, 165). Overexpression of dominant negative Gsp1p locked in either the GTP-bound or the GDP-bound state is toxic to cells and causes defects in both protein import and poly(A)$^+$ RNA export (141, 234). Recent studies have demonstrated that similar phenotypes are observed in the absence of a functional Gsp1p (274).

Both genetic and biochemical studies have led to the identification of a number of proteins that interact with and regulate Ran (155, 178, 215, 219, 228, 235). Future studies will take advantage of the availability of the crystal structure of Ran (231) to study these interactions at the molecular level.

Since its discovery, Ran has been implicated in a number of cellular processes (reviewed in references 12, 225, and 229). It is not yet clear whether Ran plays multiple cellular roles or whether its central function in nuclear transport leads to pleiotropic effects on other cellular events that occur downstream of nuclear transport (46, 58, 142, 214). There is experimental evidence to support either of these two views, so the controversy is unlikely to be resolved in the immediate future.

(ii) Rna1p/RanGAP1. Like many other cellular G-proteins, most notably Ras (29), the rate of GTP hydrolysis by isolated Ran is negligible (21, 234). Thus, in vivo, a catalyst is required to enhance the rate of this reaction. Proteins that enhance the rate of GTP hydrolysis mediated by G-proteins are called GTPase-activating proteins (GAPs) (104).

The GAP for Ran was originally identified in *S. cerevisiae* as a mutant with pleiotropic defects in macromolecular biosynthesis (108). Studies to further characterize the mutant identified RNA processing and transport defects and demonstrated that the mutant accumulated precursors of tRNA and rRNA (115, 124). Later work demonstrated that the mutant was also defective in the export of poly(A)$^+$ RNA to the cytoplasm (9). These studies suggested that the *RNA1* gene product played a crucial role in many aspects of RNA processing. However, when the gene was cloned (11) and the protein was localized, the localization was found to be primarily cytoplasmic (117). This presented a paradox because a protein involved in many aspects of RNA maturation was expected to be present in the nucleus.

Since the cellular defects attributed to mutant *RNA1* strains were so pleiotropic, the possibility arose that this gene plays some general role in the transport of macromolecules across the nuclear envelope. To examine this possibility, nuclear protein import in an *RNA1* mutant (50), *rna1-1*, that had been historically characterized as an RNA trafficking mutant (9, 115, 116, 124) was examined. Results indicated that the *RNA1* mutant is defective in nuclear protein import as well as RNA processing and transport. This finding also highlighted one of the caveats of in vivo experimentation. Since nuclear transport is a bidirectional process, it is likely that compromising the flow of macromolecular traffic in one direction might also affect the flow in the opposite direction. Thus, any mutation that blocks protein import may also affect the export of RNA and vice versa. This highlights the need for in vitro experimentation to confirm the in vivo observations. In the case of the *RNA1* mutant, in vitro assays were used to demonstrate that Rna1p plays a direct role in protein import to the nucleus (50).

Concurrent with this study, the cellular Ran GAP was purified biochemically (21, 22). This provided the functional connection between the *RNA1* gene and the Ran GAP activity, because the mammalian Ran GAP purified was found to be a homolog of the *S. cerevisiae* Rna1p (15, 21, 22). The present studies support a model in which the Ran GAP, Rna1p, is absolutely required for protein import into the nucleus. It is also possible that the Ran GAP plays a direct role in poly(A)$^+$ RNA export (258), but this is a more difficult question to address since in vitro assays for poly(A)$^+$ RNA export have not yet been developed.

A covalently modified form of the GAP exists in vivo in higher eukaryotes. Two studies have demonstrated that Ran GAP1 from mammalian cells is modified by a small ubiquitin-like molecule (158, 164). Both these studies suggest that the modification alters the interaction between the GAP and the nuclear pore. This modified form of the GAP has also been observed in *Xenopus* (228). While there is not yet any evidence to suggest that the yeast GAP, Rna1p, is modified by a similar ubiquitin-like molecule, the yeast genome does encode a small protein with homology to the modifying moiety. This protein is called Smt3p and was originally identified in a screen for suppressors of a mutated component of the centromere, Mif2p (171).

(iii) Prp20p/RCC1. Another critical regulator of Ran function is the nucleus-localized (193) nucleotide exchange factor for Ran (24). Exchange factors catalyze the release of GDP from the GTP binding protein and consequently the regeneration of the GTP-bound form of the protein (208).

The exchange factor for Ran has been identified in vivo in a number of different screens carried out in several different systems including Chinese hamster ovary (CHO) cells (193), *Drosophila* (82), fission yeast (163, 230), and budding yeast (45, 134, 261). The exchange factor gene was first cloned on the basis of complementation of the temperature sensitivity of the tsBN2 CHO cell line, which undergoes premature chromatin condensation at the nonpermissive temperature (192, 193). The gene was designated RCC1 for regulator of chromatin condensation (57, 193).

In *S. cerevisiae*, the exchange factor was identified in two different screens as a mutant, encoded by *prp20-1* (81, 261) or *mtr1-1* (134), with pre-RNA processing and RNA trafficking defects similar to those described for *rna1-1* (8, 9, 50, 115, 134, 141). The subsequent cloning of the *PRP20* gene led to the realization that Prp20p was homologous to RCC1 (4), and it was later shown that the RCC1 gene could complement a *PRP20* mutant (80), demonstrating that the two proteins are also functional homologs. Another screen in yeast identified a third mutation in *PRP20*, termed *srm1-1* (45), a mutant that restored mating to strains that had had the *STE3* gene, which encodes the pheromone receptor, deleted (248). No direct role for Prp20p in the mating-type pathway has been identified, but it is another cellular pathway that involves a cycle of GTP hydrolysis (39, 114). Since some of the phenotypes attributed to the *PRP20* mutants are so similar to those described for *RNA1* mutants, it is not surprising that subsequent experiments demonstrated that cells lacking a functional Prp20p are also defective in the import of NLS-containing proteins to the nucleus (141, 255).

Although the RCC1 protein was identified as a nucleotide exchange factor for Ran several years ago (24, 25), it was not until the role for Ran in nuclear transport was realized (168, 177) that the in vivo phenotypes of various RCC1 (and *PRP20*) mutants were considered in light of biochemical function. Thus, it is not surprising that mutations in this gene cause pleiotropic defects in nuclear transport, since it regulates the Ran GTP cycle. While the direct contribution of the exchange factor to either protein import or RNA export has been diffi-

cult to address, current models suggest that the regeneration of the GTP-bound form of Ran in the nucleus is required both for the continuation of the protein import cycle and for the export of poly(A)$^+$ RNA (140).

Like Ran, Prp20p and its homologs have been implicated in many cellular processes that may or may not be linked directly to nuclear transport (57). In fact, one possibility is that exchange factors like Prp20p actually do play more than one nuclear role and that the pleiotropic effects attributed to Ran are actually a result of alterations in the functions of these proteins instead. One recent study that demonstrates that Prp20p is a phosphoprotein in vivo (80) raises the question whether the phosphorylation state of the protein is a point of regulation of nuclear transport or whether it modulates some other function.

(iv) NTF2. Another protein that has been implicated in nuclear transport by both in vitro (178, 201) and in vivo (51) experiments is the small Ran binding protein that has been referred to as pp15 (30), p10 (178), and nuclear transport factor 2 (NTF2) (201) in higher eukaryotes and Ntf2p (51) in yeast. This protein was identified biochemically as an essential cytoplasmic component of the transport system that interacted both with Ran (178) and with the nuclear pore protein, p62 (201). The locus was also identified genetically in *S. cerevisiae* as a conditional mutant defective in the import of proteins to the nucleus (51). The function of this protein has been conserved throughout evolution, its localization at the nuclear rim is consistent with a role in nuclear transport, and it interacts both with Ran and with components of the nuclear pore (51, 178, 200). In vivo studies have demonstrated that the gene is essential for viability in *S. cerevisiae* (51, 185). Finally, yeast strains harboring conditional alleles of *NTF2* are defective in protein import but not in poly(A)$^+$ RNA export. These results suggest but do not prove that Ntf2p plays a more direct role in protein import than in RNA export.

Studies of Ntf2p function have been facilitated by the solution of the crystal structure of the homodimeric protein (35, 137). These studies have provided insight into the physical interaction between Ntf2p and Ran. Several lines of in vivo evidence support the notion that the interaction between Ntf2p and the GDP-bound form of Ran (185, 200) is critical to nuclear transport. When wild-type Ntf2p is replaced with a mutant Ntf2p that has altered interactions with Ran/Gsp1p, the resulting strains are inviable (49). Furthermore, conditional *gsp1* mutants are suppressed by the overexpression of Ntf2p (274) but not by the overexpression of any other transport factor. Biochemical experiments have demonstrated that these mutant Gsp1p proteins have a decreased affinity for Ntf2p (274). One interpretation of this data is that when the Ran/Gsp1p-Ntf2p interaction is compromised, increasing the cellular concentration of Ntf2p is sufficient to shift the equilibrium toward formation of the complex. The complex itself may be required for nuclear transport, or, alternatively, it is possible that the interaction with Ntf2p physically stabilizes the Ran mutant protein to make it competent for some critical function.

Ntf2p also interacts tightly with some repeat-containing nucleoporins (47, 118, 185, 200, 201). This interaction is consistent with the subcellular localization of the protein, but its in vivo consequence is not yet understood. Currently, it is possible to construct a number of models for the role of Ntf2p in nuclear transport. Since it interacts most tightly with the GDP-bound form of Ran (185, 200), it seems likely that it may act as a timing mechanism for the recycling of the protein to the GTP-bound form by the exchange factor. Consistent with this possibility, our attempts to identify any interactions between Prp20p, the exchange factor, and Ntf2p have been unsuccessful (52), even though both proteins interact strongly with the GDP-bound form of Ran. If the recycling reaction requires targeting to the nuclear pore, this might provide logic for the interaction between Ntf2p and the nuclear pore components. A combination of biochemical and genetic approaches will be necessary to refine this model or to develop new models for Ntf2p function.

(v) Yrb1p/RanBP1. A protein that was identified on the basis of its tight interaction with the GTP-bound form of Ran is required to modulate Ran-mediated GTP hydrolysis (23, 54, 235). This protein is encoded in *S. cerevisiae* by the essential gene *YRB1* (195, 235). Yrb1p and its mammalian homolog, RanBP1, fall into a class of proteins that contain a motif referred to as a Ran binding domain (16, 107). Yeast strains harboring conditional alleles of *YRB1* are defective both in the import of proteins to the nucleus and in the export of poly(A)$^+$ RNA (235).

The mechanistic role of Yrb1p/RanBP1 in vivo is not yet clear. Biochemical experiments demonstrate that the protein can function as a GAP activating protein (25, 235). Thus, addition of Yrb1p/RanBP1 to GAP assays enhances the rate of GAP-stimulated Ran-mediated GTP hydrolysis. This observation is consistent with the subcellular localization of the protein to the cytoplasm and the exterior of the nuclear envelope (235), which in yeast is indistinguishable from the localization of the GAP, Rna1p (117, 141, 258). However, in addition to displaying GAP-activating activity, RanBP1 has been shown to stabilize a trimeric complex between RanBP1, importin-β, and Ran (41, 156). Other experiments have demonstrated that a cytoplasmic retention signal in the C-terminal portion of RanBP1 is required for its cytoplasmic localization (218). This latter result is a bit puzzling, as the yeast protein is truncated relative to the human protein and consequently does not contain this domain. Further experimentation is clearly required to reach a complete understanding of the in vivo function of Yrb1p/RanBP1.

Candidate transport factors. (i) Proteins that contain Ran binding domains. As already discussed, several proteins that contain a motif referred to as a Ran binding domain have been identified (16, 107). These include the GAP-activating protein Yrb1p/RanBP1, which interacts tightly with Ran-GTP, as well as the yeast nucleoporin Nup2p (64, 153) and the mammalian nucleoporin RanBP2/Nup358 (277, 280), both of which interact with Ran.

In both budding yeast and fission yeast, another protein containing a Ran binding domain has been identified. The *S. cerevisiae* protein was identified on the basis of sequence homology. The gene encodes a 36-kDa protein that has been referred to as Nup36p (185), although it seems to be localized throughout the nucleus (256) rather than exclusively at the nuclear pore. The protein is not essential for viability and does not interact tightly with Ran (256). A similar protein was identified in fission yeast in a screen for mutants resistant to the Golgi poison, brefeldin A (85). This protein, termed hba1, is essential for viability, is localized to the nucleus, and is phosphorylated in vivo (259). Further experimentation will be required to determine whether these proteins play a role in nuclear transport or whether they interact with GTP binding proteins involved in other cellular processes.

(ii) Other GTPases. There has been some evidence to support a role for a second, as yet unidentified G-protein in nuclear transport (237, 253). In one study, an altered-specificity Ran mutant was used to make this argument (253). However, other researchers, using the same Ran mutant, reached the conclusion that the nucleotide requirement in import was ful-

filled by Ran-mediated hydrolysis of GTP alone (93, 264). Another putative GTPase that interacts genetically with some of the regulators of Ran is Gtr1p. This gene was identified as a suppressor of mutations in *PRP20* (184).

(iii) HSP70. As with many other cellular transport processes, some studies have implicated the chaperone Hsp70p in nuclear protein import (125, 239). Recent work has demonstrated that elevated expression of one of the *HSP70* genes in *S. cerevisiae*, *SSA1*, is sufficient to suppress the temperature-sensitive phenotype of two nuclear transport mutants (240). These results suggest that Hsp70p may facilitate some steps in the nuclear transport process.

Mechanism of translocation. While it is straightforward to list the potential players in translocation, it is much more difficult to propose a model for the actual movement of import substrates through the nuclear pore, a distance of approximately 200 to 300 Å (198). When considering the task that must be accomplished, at least two general mechanisms seem feasible. In one mechanism, the movement through the pore would occur by random nondirected diffusion, but upon reaching some point, an irreversible gate would open and close, committing the substrate to enter the nucleus (detailed in references 92 and 140). The second model would involve a series of associations and dissociations of the substrate with the transport channel as the substrate moved down an energy gradient in a directed stepwise manner (detailed in reference 216). The second model might require repeated rounds of GTP hydrolysis for the repetitive association and dissociation steps. These two models have recently been compared (140).

Release into the Nucleus

Once transport into the nucleus is accomplished, it is essential that the importin-NLS-containing complex dissociate to deliver the substrate to its destination. Since it is not yet possible to distinguish between the two general mechanisms for translocation, the role played by release into the nucleus in nuclear protein import is unclear. The mechanism of translocation of the substrate mentioned above directly affects the contribution of this step to completing transport into the nucleus. If translocation occurs via directed steps down an energy gradient, it is possible that release into the nucleus simply occurs when the final step is reached and no further association points exist. In contrast, if transport occurs by random diffusion, release could rely heavily on a release step that would commit the substrate to be released into the nucleus.

Regardless of the mechanism of translocation, current thinking would require that the two importin subunits dissociate from one another at the nucleoplasmic face of the pore, since importin-α and the transport substrate can be released into the nucleus but importin-β remains associated with the pore (95, 181). One study has demonstrated that when importin-β binds to Ran-GTP, importin-α dissociates from importin-β and releases the importin-α–NLS-containing protein complex into the nucleus (92, 93). Since Ran-GTP is generated in the nucleus by the nucleus-localized exchange factor, this would be a mechanism for ensuring that release does not occur until the substrate has reached its destination.

Recycling of the Transport Factors

One transport step that is rarely considered is the requirement for recycling of the transport factors. At least some of the transport factors actually move through the pore in association with the transport substrate. These factors would include importin-α, which is actually released into the nucleus in concert with the transport substrate (95, 181); importin-β, which seems to remain associated with the pore complex (95); and probably Ran (169). For the flow of substrates into the nucleus to continue, these components require recycling from the nucleus to the cytoplasm. In addition, to maintain the GTPase cycle, the necessary nucleotide-bound state of the G-protein must continually be regenerated.

The way to examine the recycling of transport components is to use an in vivo system that undergoes multiple rounds of transport. For example, the recycling of importin-α in several yeast mutants with defects in nuclear transport has been examined. When the nucleoporin *NUP120* is deleted, importin-α accumulates in the nucleus (6). A similar phenotype is observed when Ran-GDP accumulates in the cell either due to a mutation in the exchange factor or due to overexpression of Ran locked in the GDP-bound form (141). These results suggest that importin-α is indeed recycled through the nuclear pore complex and that it probably requires regeneration of Ran-GTP for this recycling. The localization of importin-α has not been examined in many nucleoporin mutants, and so it remains to be seen whether Nup120p plays a specific role in transit back to the cytoplasm or whether other nucleoporin mutants display a similar phenotype.

Recent evidence has suggested that much of the recycling of transport factors occurs in conjunction with a pathway for export of macromolecules from the nucleus (see the section on U snRNAs, below). It is not yet clear whether the export of the factors, for example importin-α (91), is required to mediate the export of macromolecules or whether these transport components simply hitch a ride on the export substrates as a means of recycling. It certainly seems likely that the regeneration of Ran-GTP may be essential for the export of at least some macromolecules from the nucleus (180, 234). Thus, the replenishing of the pools of import-competent transport factors is inexorably linked to the achievement of vectorial transport of substrates.

Models for Protein Import

Vectorial transport. The question of how vectorial transport is accomplished is critical to our overall understanding of nuclear protein import. One major question that exists with regard to the Ran-mediated cycle of GTP hydrolysis is how transport and GTP hydrolysis are coordinated. It is now clear that GTP is the only nucleotide required for import of proteins to the nucleus (93, 264). There is no apparent requirement for ATP. However, it is not yet clear precisely when the hydrolysis is required or how many rounds of hydrolysis are required for import of a substrate. For the transport system to be efficient, once proteins are imported to the nucleus, they should remain there unless they are specifically targeted for export. As mentioned above, this must be accomplished in a manner that allows for continuous recycling of the G-protein and possibly the transport factors without continuous cycling of the transport substrates.

The subcellular localizations of the GAP and the exchange factor present one possible mechanism for achieving vectorial transport of substrates and recycling of the G-protein. The GAP, Rna1p/RanGAP1, is localized in the cytoplasm, whereas the exchange factor, Prp20p/RCC1, is found in the nucleus. This suggests a simple model in which GTP hydrolysis to generate Ran-GDP is required for import and perhaps regeneration of the GTP-bound form of Ran is required for export (140). Since we do not yet understand the specific requirements for Ran, particularly why the GDP-bound form is required for import, this model requires further refinement. In addition, other observations need to be considered. Although

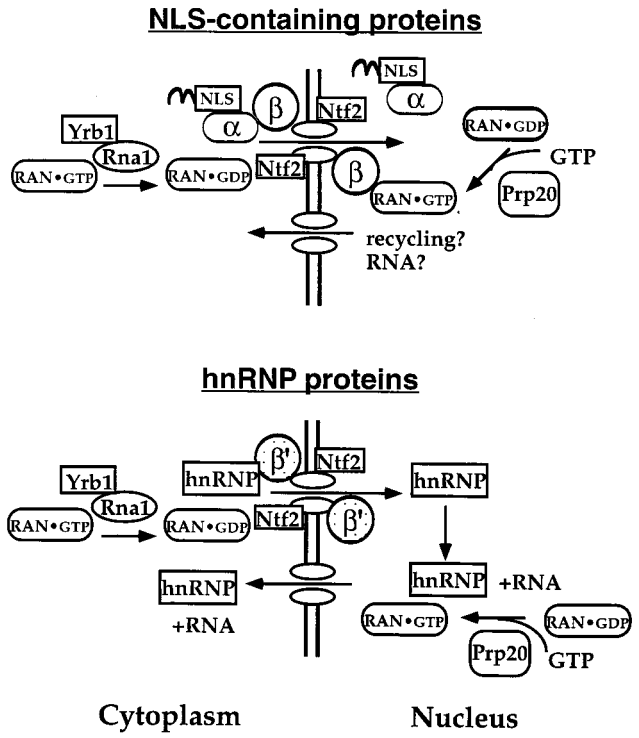

FIG. 1. Models for nuclear protein import. The top model depicts the import of a protein containing a canonical NLS. The bottom model depicts the import of a class of proteins that do not contain a canonical NLS, hnRNPs. It is most likely that translocation of both classes of proteins into the nucleus depends on Ran and proteins that regulate its GTP hydrolysis activity (Rna1p, Yrb1p, Ntf2p, and Prp20p), because mutations in any of the genes cause mislocalization of proteins in either class to the cytoplasm. In contrast, the mechanism of targeting to the nuclear pore appears to be distinct for the two different classes of proteins. Proteins that contain a canonical NLS are targeted to the nuclear pore via an interaction in the cytoplasm with the heterodimeric NLS receptor composed of importin-α and importin-β. In contrast, hnRNPs appear to be targeted to the nuclear rim via an interaction with a distinct importin-β homolog (β′). Details of both mechanisms are given in the text.

the GAP is localized primarily on the cytoplasmic face of the nucleus (117, 141), recent work suggests that it may be able to enter the nucleus (258). Furthermore, although Prp20p has been identified as the yeast homolog of the Ran exchange factor, RCC1, at least two other RCC1 homologs are encoded by the yeast genome (138, 162). It remains to be determined whether these proteins function in nuclear transport or whether they serve as exchange factors for other unrelated G-proteins. Finally, the other transport factors that interact with Ran in a nucleotide-specific manner may contribute to vectorial transport.

NLS-containing proteins. Several recent reviews have presented detailed models for the transport process (92, 140). A simplified model is shown in Fig. 1. NLS-containing proteins interact with the importin-α–importin-β heterodimer in the cytoplasm via direct binding to importin-α. Importin-β then targets the complex to the nuclear pore periphery, where it interacts with repeat-containing nucleoporins. Current thinking dictates that Ran must be in the GDP-bound state to promote import of this complex to the nucleus. Thus, both the GAP (Rna1p) and the GAP-activating protein (Yrb1p) are required to stimulate GTP hydrolysis prior to import. Once Ran-GDP is generated, the importin-NLS-bearing protein complex is translocated into the nucleus through the nuclear pore. It is unclear precisely what role Ran-GDP plays in this translocation. It seems likely that Ran moves into the nucleus (169) in conjunction with the complex, but there is no evidence that it is actually a component of the importin transport complex. Dissociation of the transport complex occurs when the importin complex interacts with Ran in the GTP-bound state (93), which is generated in the nucleus by the exchange factor, Prp20p. It is possible that the small protein Ntf2p plays some role in timing the generation of Ran-GTP, but there is currently no direct evidence to support this hypothesis. At this juncture, importin-α and the NLS-bearing protein are released into the nucleus and importin-β is recycled to the cytoplasm. It is not yet clear exactly where in the nuclear pore this event occurs, but importin-β has been localized to both the cytoplasmic and the nucleoplasmic faces of the nuclear pore (95). Since the steady-state distribution of importin-β is predominantly cytoplasmic and it has not yet been observed to accumulate in the nucleus, it seems that there must be an efficient directional method for returning importin-β to the cytoplasm; however, nothing is known about the details of this process. Importin-α must also be recycled to the cytoplasm, and evidence is mounting that this may occur in conjunction with some components of the RNA transport machinery (91).

Other transport pathways. The transport mechanism presented above describes the import of NLS-containing proteins to the nucleus. It has, however, been known for some time that there are proteins that do not contain canonical NLSs but are nonetheless efficiently targeted to the nucleus. It is not surprising that these proteins are translocated through the nuclear pore in a manner that is Ran dependent but NLS receptor independent (Fig. 1). For example, the yeast heterogeneous RNA binding protein (hnRNP), Npl3p, is an RNA binding protein that shuttles rapidly between the cytoplasm and the nucleus (31, 79, 272). It undergoes rapid cycles of import to and export from the nucleus, yet it does not contain a classical NLS (31). In mutants where Ran-mediated GTP hydrolysis is affected, Npl3p accumulates in the cytoplasm, as do proteins containing standard NLSs (50, 51, 141, 274). In contrast, the localization of Npl3p is unaffected by mutations in either NLS receptor subunit that do affect standard NLS-containing proteins (139).

Homology searches of the yeast genome revealed that there are three importin-β homologs that share approximately 50% sequence similarity with the NLS receptor subunit. One of these genes was previously identified as a multicopy enhancer of protein secretion, *PSE1* (44). The other two genes were uncharacterized open reading frames that have now been termed *KAP104* and *KAP123* (4).

Genetic analysis of *PSE1* and *KAP123* suggests that the proteins they encode may function in the export of mRNA from the nucleus (236). Both of these proteins are localized primarily to the nucleus, and yeast strains with mutations in both *PSE1* and *KAP123* accumulate poly(A)$^+$ RNA in the nucleus.

Recently, direct evidence that supports a role for Kap104p in the transport of some RNA binding proteins has been presented (5, 205). Yeast Kap104p binds directly to two RNA binding proteins termed Nab2p (10) and Hrp1p (Nab4p) (111). Mutations in the *KAP104* gene cause these two proteins, both of which are normally localized primarily to the nucleus (10, 111), to become mislocalized to the cytoplasm (5). A second study has demonstrated that the nuclear localization of the human protein hnRNPA1, which is homologous to Hrp1p (68), is dependent on a human homolog of Kap104p termed transportin (205). These findings suggest that the different importin-β proteins identified in yeast may function in distinct nuclear transport pathways and also imply that import to the

nucleus can occur via pathways that are independent of classical NLSs (Fig. 1).

NUCLEAR EXPORT

Macromolecules exported from the nucleus include proteins and a number of different species of RNA (78, 87, 129). Classical studies of nuclear export have focused on the movement of different species of RNA out of the nucleus. The primary approach to studying RNA export in vivo has been to use in situ hybridization to examine the localization of poly(A)$^+$ RNA in yeast and to search for conditional yeast mutants with aberrant accumulation of poly(A)$^+$ RNA (9, 34, 134). This approach, as well as subsequent genetic screens, has identified components of the general nuclear transport process including nucleoporins (89, 96, 110, 151), regulators of Ran-mediated GTP hydrolysis (134), and other factors that may play specific roles in RNA export (34, 62, 132, 183). A complementary experimental approach has involved microinjection of RNA into *Xenopus* oocytes. This method has been useful in elucidating the different pathways used for export of different RNAs (91). Microinjection studies suggest that different RNAs are exported from the nucleus by independent, saturable pathways (131). These experiments led to the search for export factors that would associate with RNAs and mediate export in a class-specific manner. In fact, a number of recent reviews have focused on the export of macromolecules from the nucleus (78, 87, 129, 150, 175). Thus, this review will only touch upon a few of the most recent findings.

Nuclear Export Signals

Only recently has it been determined that like protein import to the nucleus, protein export from the nucleus is a specific signal-directed event. This realization has come with the identification of specific nuclear export signals (NESs) in the primary amino acid sequence of proteins that are specifically targeted for export from the nucleus (87, 175). Thus far, two different types of NESs have been identified. The first type is a general export signal that has been identified in a variety of proteins, including, thus far, HIV Rev protein (76), the human T-cell leukemia virus type 1 Rex protein (27), protein kinase A inhibitor (266), amphibian transcription factor IIIA (83), and the RNA export mediator, Gle1p/Rss1p (62, 183). It is an approximately 10-amino-acid sequence that is rich in hydrophobic residues, particularly leucine (reviewed in reference 175). The second type is a 38-amino-acid sequence, identified in the C terminus of mammalian hnRNPA1, that serves as a combined signal for both import and export (173, 246). This sequence shares no homology with the leucine-rich NES. The identification of these signals for export from the nucleus has facilitated the search for proteins that may participate in processes, such as RNA trafficking, that may require cycling of factors between the nucleus and the cytoplasm.

RNA Export

The export of RNA from the nucleus is linked to all the processing events that must occur prior to its exit from the nucleus via the nuclear pores (119). A comprehensive review of RNA processing is beyond the scope of this article, but it should be noted that splicing, 3′ polyadenylation, and 5′ capping all affect the export process (71, 106, 148, 159). For more information, the reader is referred to recent articles on RNA splicing (145, 226), polyadenylation (161), and 5′ capping (241).

Several findings led to the conclusion that the substrate for

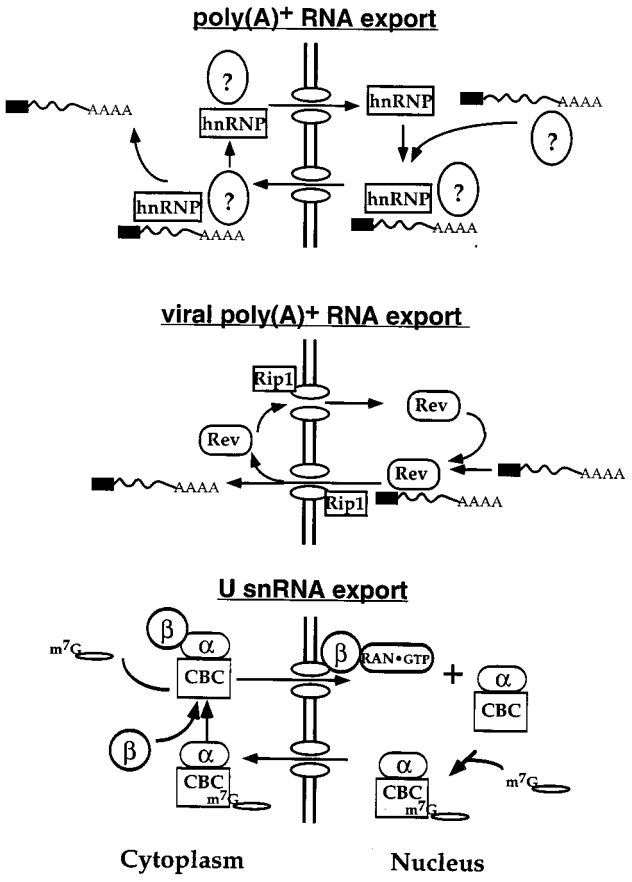

FIG. 2. Models for RNA export. Models for the export of several different classes of RNA are shown. The top model depicts the export of poly(A)$^+$ RNA from the nucleus, a process that appears to be dependent on hnRNPs that shuttle between the cytoplasm and the nucleus (149). The middle model simply identifies the proteins, Rev and Rip, that have been identified thus far that may play a role in the export of viral RNA from the nucleus. The bottom model shows the export of U snRNA from the nucleus (91). Görlich et al. have demonstrated that the CBC, which binds to the cap of U snRNA, interacts with importin-α. They have also demonstrated that this complex is dissociated upon interaction with importin-β. Details of all models are discussed in the text.

RNA export is not the nucleic acid itself but, rather, the associated proteins found in the RNP complex (129). The most notable piece of evidence to support this conclusion is the observation that RNA can be observed moving through the nuclear pore in conjunction with RNA binding proteins (262, 263). This realization led to the reclassification of RNA export as a specialized form of protein export in which the signal for export is found in the associated protein rather than in the RNA itself. Thus, to understand the export of different RNA classes from the nucleus, it is necessary to fully understand protein export and to identify the specificity factors required for each RNA class. Another important question is whether the translocation machinery that is required for movement across the nuclear envelope is the same for all transport substrates. There is evidence that, like protein import, protein export from the nucleus requires Ran-mediated GTP hydrolysis (180). In addition, export of poly(A)$^+$ RNA from the nucleus is compromised in yeast strains with mutations in genes that affect Ran-mediated GTP hydrolysis (9, 134, 234, 235, 274).

It may be necessary to consider different mechanisms of transport for different species of RNAs (summarized in Fig. 2)

and perhaps even for RNAs that fall into the same class. One recent study demonstrated that there is a pathway for the export of heat shock mRNA that is distinct from the standard poly(A)$^+$ export pathway (227). This export pathway may be related to the export mechanism that is exploited by viruses to hijack the host pathways for their own propagation (76).

Candidate Poly(A)$^+$ RNA Export Mediators

hnRNPs. For some time, interest had been growing in a class of proteins termed heterogeneous nuclear RNPs (hnRNPs). hnRNPs are abundant proteins that associate with poly(A)$^+$ RNA in both the nucleus and the cytoplasm (68, 204). In addition, both a yeast hnRNP, Npl3p, and mammalian hnRNPs, hnRNPA1 and hnRNPB, have been shown to shuttle rapidly between the cytoplasm and the nucleus (79, 204). These features make certain hnRNP proteins prime candidates for the role of RNA export mediator. Subsequent experiments that examined both poly(A)$^+$ RNA export and Npl3p export in budding yeast have added support to the hypothesis that such proteins may act as carriers for the export of poly(A)$^+$ RNA from the nucleus. Mutations in the RNA recognition motif of Npl3p block both RNA export and Npl3p export from the nucleus, suggesting that the two exit in concert (149).

Implicit in the model that hnRNPs are mediators of RNA export is the necessity that these proteins must release their RNA "cargo" once the appropriate cellular compartment is reached. Therefore, it seems likely that a mechanism exists to regulate the association and dissociation of the hnRNPs with RNA. One possibility is that some of these functions are regulated by posttranslational modifications. Several hnRNP proteins undergo a novel methylation on arginine residues (86, 113, 243). This modification may contribute to the formation of the appropriate export-competent RNP complex. Since a number of proteins that associate with nucleic acids contain potential sites for methylation, it seems likely that methylation will serve as a general mechanism for regulating the formation of protein-nucleic acid complexes (152). This hypothesis is further supported by the identification of conserved genes encoding arginine methyltransferases in yeast (113) and in higher eukaryotes (2, 112).

Rev proteins and cofactors. Cellular hnRNPs are not the only candidates for mediators of poly(A)$^+$ RNA export. The HIV-1 Rev protein has many of the same characteristics as the hnRNP proteins described above. Rev shuttles between the cytoplasm and the nucleus (172, 217) and promotes the export of unspliced poly(A)$^+$ RNA via the Rev response element (77, 160). In addition, Rev contains a leucine-rich NES that directs its export from the nucleus (76). Several recent studies have set out to identify proteins that interact with Rev along its export pathway in an attempt to identify cellular components of the poly(A)$^+$ RNA transport machinery. These studies have identified a cellular cofactor that is present in both yeast (251) and human (28, 84) cells. These proteins have been called Rips (Rev-interacting proteins). These Rips have homology to nucleoporins (as mentioned in "Components of the nuclear pore complex" above) and thus may play a role in targeting the RNA carrier to the nuclear rim.

A recent study has in fact identified a protein that may be the cellular equivalent of Rev. This protein, Gle1p/Rss1p, was identified in two different screens carried out in *S. cerevisiae*. One screen identified mutants that were synthetically lethal with mutations in the GLFG-containing nucleoporin *NUP100*, *GLE1* (GLFG lethal) (183). The second screen identified high-copy suppressors of yeast strains harboring a mutation in the *NUP159*/*RAT7* gene, *RSS1* (*rat7-1* suppressor) (62). Gle1p/Rss1p is an NES-containing protein that interacts with a number of nucleoporins including yeast Rip1p. Its interaction with Rip1p appears to be mediated by the NES, because point mutations that inactivate the NES also abolish the interaction with Rip1p. Consistent with Gle1p/Rss1p acting as a cellular counterpart of Rev, mutations in this essential gene cause a rapid-onset defect in the export of poly(A)$^+$ RNA from the nucleus (183). Thus, by studying the pathways available for the export of viral poly(A)$^+$ RNA, it may be possible to learn more about the export of cellular poly(A)$^+$ RNA. The identification of the *GLE1*/*RSS1* gene now creates a starting point for new genetic screens that should identify yet more components of this cellular machinery.

A second Rev-interacting protein that may function in some aspect of RNA processing and transport is the eukaryotic initiation factor 5A (eIF-5A) (224). Mutant alleles of the eIF-5A gene were identified in yeast in a screen for conditional mutants that mislocalized nuclear proteins (31, 43).

Another protein that is a potential mediator of poly(A)$^+$ RNA export was identified in a screen for *S. pombe* mutants which accumulate nuclear poly(A)$^+$ RNA (34). This protein, *rae1*, is essential for viability in fission yeast and is localized at the nuclear periphery. The *S. cerevisiae* homolog of *rae1*, Gle2p, was identified in a screen for mutants synthetically lethal in combination with mutations in *NUP100* (182), the same screen in which Gle1p (see above) was identified (183). The *GLE2* gene is not essential for viability, but mutants have defects in the export of poly(A)$^+$ RNA from the nucleus. Gle2p/*rae1* has no homology to hnRNPs or to other proteins that have been implicated in RNA trafficking.

Export of Other RNAs

U snRNAs. Although most studies carried out in yeast have focused on the export of poly(A)$^+$ RNA due to its ease of detection, biochemical studies have identified at least some of the factors involved in the export of another class of RNA, U snRNA (91). One of these factors is a subunit of the NLS receptor, importin-α. This demonstrates the efficiency of the cell. Since importin-α is cotransported into the nucleus with NLS-containing substrates, it must necessarily be recycled to the cytoplasm for another round of transport. Thus, the cell takes advantage of this import-export cycle in both directions. The study by Görlich et al. (91) describes a biochemical approach to the identification of cellular proteins that are complexed with yeast importin-α in vivo. The authors identify a complex containing yeast importin-α and the yeast homologs of the two subunits of the nuclear cap binding complex (CBC) (48, 91). The cap binding complex consists of two subunits encoded by *CBP20* and *CBP80* (also known as *GCR3* in yeast), which have previously been shown to mediate the export of U snRNA (128). This study also demonstrates that the addition of yeast importin-β to the importin-α–CBC complex results in the dissociation of the capped RNA from the complex. Since importin-β is found predominantly in the cytoplasm, this suggests a model in which the substrate is delivered to the appropriate cellular compartment as the result of an importin-β-induced substrate-carrier dissociation.

Thus, in a single round of transport (Fig. 2), the U snRNA would associate with a complex containing the cap binding proteins, Cbp20p and Cbp80p, as well as importin-α in the nucleus. This complex would then be translocated to the cytoplasm, where interaction with importin-β would dissociate the complex and deliver the substrate to the cytoplasm. The importin-α would then be available for another round of nuclear protein import. Presumably the cap binding proteins would

need to be recycled to the nucleus for subsequent rounds of RNA export.

tRNA. Finally, there is at least one class of RNA, tRNA, that seems to be exported from the nucleus in a manner that is fundamentally different from that for the other export substrates. tRNA is able to exit the nucleus in the absence of a functional exchange factor (when RCC1 is lost), when export of other species of RNA is blocked (40). This finding suggests that tRNA exits the nucleus in a manner that is not dependent on the Ran GTPase cycle. One possibility is that tRNA is small enough to diffuse out of the nucleus. However, classical studies suggest that this is not the case, since mutant tRNAs are retained in the nucleus (281, 282). Other transport events that have been reported to occur in the absence of Ran-mediated GTP include the import of U1 snRNP to the nucleus (63) and the export of *SSA4* poly(A)$^+$ RNA following heat shock (227). It remains to be seen whether these non-Ran-dependent transport events are mediated by another G-protein or whether they occur via a completely different mechanism.

The identification of some of the basic components of the RNA transport apparatus now opens the door to numerous genetic studies that should lead to the rapid identification of other genes involved in the process. Studies in yeast have already paved the way for the identification of these factors; therefore, it is logical to believe that ongoing studies will provide a wealth of new information in the coming years.

CONCLUSIONS

It is clear that we are beginning to understand nuclear transport at the mechanistic level. Many of the components of protein import have been identified, although some of their roles are still not fully understood. Export from the nucleus is by nature more complicated than import, as the variety of vehicles that must travel this road may be more diverse than those that take the import highway. However, it is clear that studies that take advantage of all possible scientific methods are beginning to unravel this complex mechanism as well. It is likely that the amount of attention that is currently focused on delineating export mechanisms will result in a rapid expansion of our understanding, as has been the case for protein import over the last 3 years.

With the recent advances in our understanding of nuclear transport, it is intriguing to realize that several of these critical components were actually originally identified, at least one as long as 20 years ago, in genetic screens for budding yeast mutants with defects in nuclear function (19, 108, 261, 278). While these loci were identified many years ago, the subsequent biochemical analysis was necessary to understand their function. This simply emphasizes the fact that it is the combination of all scientific venues that will eventually lead to the complete understanding of the function of the various transport factors in macromolecular trafficking across the nuclear envelope as well as all other complex cellular processes.

ACKNOWLEDGMENTS

We acknowledge the members of the Silver laboratory for reading the manuscript and making valuable suggestions prior to publication. In particular, we thank D. H. Wong, D. M. Koepp, P. Ferrigno, J. K. Hood, M. S. Lee, L. A. Ngyuen, S. Valentini, and J. Lopilato. We also thank our colleagues for providing preprints and communicating results prior to publication.

Work from our laboratory was supported by grants from the National Institutes of Health to P.A.S. A.H.C. was supported by a Biomedical Career Development Award from the Burroughs Wellcome Foundation.

REFERENCES

1. **Aaronson, R. P., and G. Blobel.** 1974. On the attachment of the nuclear pore complex. J. Cell Biol. **62:**746–754.
2. **Abramamovich, C., B. Yakobson, J. Chebath, and M. Revel.** 1997. A protein-arginine methyl transferase binds to the intracytoplasmic domain of the IFNAR1 chain in the type I interferon receptor. EMBO J. **16:**260–266.
3. **Adam, S. A., and L. Gerace.** 1991. Cytosolic proteins that specifically bind nuclear location signals are receptors for nuclear import. Cell **66:**837–847.
4. **Aebi, M., M. W. Clark, U. Vijayraghavan, and J. Abelson.** 1990. A yeast mutant, *PRP20*, altered in mRNA metabolism and maintenance of the nuclear structure, is defective in a gene homologous to the human gene *RCC1* which is involved in the control of chromosome condensation. Mol. Gen. Genet. **224:**72–80.
5. **Aitchinson, J. D., G. Blobel, and M. P. Rout.** 1996. Kap104p: a karyopherin involved in the nuclear transport of messenger RNA binding proteins. Science **274:**624–627.
6. **Aitchinson, J. D., G. Blobel, and M. P. Rout.** 1995. Nup120p: a yeast nucleoporin required for NPC distribution and mRNA transport. J. Cell Biol. **131:**1659–1675.
7. **Aitchinson, J. D., M. P. Rout, M. Marelli, G. Blobel, and R. W. Wozniak.** 1995. Two novel related yeast nucleoporins Nup170p and Nup157p: complementation with the vertebrate homologue Nup155p and functional interactions with the yeast nuclear pore-membrane protein Pom152p. J. Cell Biol. **131:**1133–1148.
8. **Amberg, D. C., M. Fleischmann, I. Stagljar, C. N. Cole, and M. Aebi.** 1993. Nuclear PRP20 protein is required for mRNA export. EMBO J. **12:**233–241.
9. **Amberg, D. C., A. L. Goldstein, and C. N. Cole.** 1992. Isolation and characterization of *RAT1*: an essential gene of *Saccharomyces cerevisiae* required for the efficient nucleocytoplasmic trafficking of mRNA. Genes Dev. **6:**1173–1189.
10. **Anderson, J. T., S. M. Wilson, K. V. Datar, and M. S. Swanson.** 1993. NAB2: a yeast nuclear polyadenylated RNA-binding protein essential for cell viability. Mol. Cell. Biol. **13:**2730–2741.
11. **Atkinson, N. S., R. W. Dunst, and A. K. Hopper.** 1985. Characterization of an essential *Saccharomyces cerevisiae* gene related to RNA processing: cloning of *RNA1* and generation of a new allele with a novel phenotype. Mol. Cell. Biol. **5:**907–915.
12. **Avis, J. M., and P. R. Clarke.** 1996. Ran, a GTPase involved in nuclear processes: its regulators and effectors. J. Cell Sci. **109:**2423–2427.
13. **Azuma, Y., M. M. Tabb, L. Yu, and M. Nomura.** 1995. Isolation of a yeast protein kinase that is activated by the protein encoded by *SRP1* (Srp1p) and phosphorylates Srp1p complexed with nuclear localization signal peptides. Proc. Natl. Acad. Sci. USA **92:**5159–5163.
14. **Bastos, R., N. Panté, and B. Burke.** 1995. Nuclear pore complex proteins. Int. Rev. Cytol. **162B:**257–302.
15. **Becker, J., F. Melchior, V. Gerke, F. R. Bischoff, H. Ponstigl, and A. Wittinghoffer.** 1995. *RNA1* encodes a GTPase-activating protein specific for Gsp1p, the Ran/TC4 homologue of *Saccharomyces cerevisiae*. J. Biol. Chem. **270:**11860–11865.
16. **Beddow, A. L., S. A. Richards, N. R. Orem, and I. G. Macara.** 1995. The Ran/TC4 GTPase-binding domain: identification by expression cloning and characterization of a conserved sequence motif. Proc. Natl. Acad. Sci. USA **92:**3328–3332.
17. **Beg, A. A., S. M. Ruben, R. I. Scheinman, S. Haskill, C. A. Rosen, and A. S. Baldwin.** 1992. I kappa B interacts with the nuclear localization sequences of the subunits of NF-kappa B: a mechanism for cytoplasmic retention. Genes Dev. **6:**1899–1913.
18. **Belanger, K. D., M. A. Kenna, S. Wei, and L. I. Davis.** 1994. Genetic and physical interactions between Srp1p and nuclear pore complex proteins Nup1p and Nup2p. J. Cell Biol. **126:**619–630.
19. **Belhumeur, P., A. Lee, R. Tam, T. DiPaolo, N. Fortin, and M. W. Clark.** 1993. *GSP1* and *GSP2*, genetic suppressors of the *prp20-1* mutant in *Saccharomyces cerevisiae*: GTP-binding proteins involved in the maintenance of nuclear organization. Mol. Cell. Biol. **13:**2152–2161.
20. **Bender, A., and J. R. Pringle.** 1991. Use of a synthetic lethal and multicopy suppressor mutant to identify two new genes involved in morphogenesis in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **11:**1295–1305.
21. **Bischoff, F. R., C. Klebe, J. Kretschmer, A. Wittinghofer, and H. Ponstingl.** 1994. RanGAP1 induces GTPase activity of nuclear Ras-related Ran. Proc. Natl. Acad. Sci. USA **91:**2587–2591.
22. **Bischoff, F. R., H. Krebber, T. Kempf, I. Hermes, and H. Ponstingl.** 1995. Human RanGTPase activating protein RanGap1 is a homologue of yeast Rna1p involved in mRNA processing and transport. Proc. Natl. Acad. Sci. USA **92:**1749–1753.
23. **Bischoff, F. R., H. Krebber, E. Smirnova, W. Dong, and H. Ponstingl.** 1995. Coactivation of RanGTPase and inhibition of GTP dissociation by Ran-GTP binding protein RanBP1. EMBO J. **14:**705–715.
24. **Bischoff, F. R., and H. Ponstingl.** 1991. Catalysis of guanine nucleotide exchange on Ran by the mitotic regulator RCC1. Nature **354:**80–82.
25. **Bischoff, F. R., and H. Ponstingl.** 1991. Mitotic regulator of protein RCC1

is complexed with a nuclear *ras*-related polypeptide. Proc. Natl. Acad. Sci. USA **88:**10830–10834.
26. **Bogerd, A. M., J. A. Hoffman, D. C. Amberg, G. R. Fink, and L. I. Davis.** 1994. *Nup1* mutants exhibit pleiotropic defects in nuclear pore complex function. J. Cell Biol. **127:**319–332.
27. **Bogerd, H. P., R. A. Fridell, R. E. Benson, J. Hua, and B. R. Cullen.** 1996. Protein sequence requirements for function of the human T-cell leukemia virus type 1 Rex nuclear export signal delineated by a novel in vivo randomization selection assay. Mol. Cell. Biol. **16:**4207–4214.
28. **Bogerd, H. P., R. A. Fridell, S. Madore, and B. R. Cullen.** 1995. Identification of a novel cellular cofactor for the Rev/Rex class of regulatory retroviral proteins. Cell **82:**485–494.
29. **Boguski, M. S., and F. McCormick.** 1993. Proteins regulating Ras and its relatives. Nature **366:**643–654.
30. **Bohn, H., R. Johannsen, and W. Kraus.** 1980. New placental protein (PP15) with immunosuppressive properties. Arch. Gynecol. **230:**167–172.
31. **Bossie, M. A., C. DeHoratius, G. Barcelo, and P. Silver.** 1992. A mutant nuclear protein with similarity to RNA binding proteins interferes with nuclear import in yeast. Mol. Biol. Cell **3:**875–893.
32. **Bourne, H. R., D. R. Sanders, and F. McCormick.** 1991. The GTPase superfamily: conserved structure and molecular mechanism. Nature **349:** 117–127.
33. **Briscoe, J., F. Kohlhuber, and M. Müller.** 1996. JAKs and STATs branch out. Trends Cell Biol. **6:**336–340.
34. **Brown, J. A., A. Bharathi, A. Ghosh, W. Whalen, E. Fitzgerald, and R. Dhar.** 1995. A mutation in the *Schizosaccharomyces pombe rae1* gene causes defects in poly(A)$^+$ RNA export and in the cytoskeleton. J. Biol. Chem. **270:**7411–7419.
35. **Bullock, T. L., W. D. Clarkson, H. M. Kent, and M. Stewart.** 1996. The 1.6Å resolution crystal structure of nuclear transport factor 2 (NTF2). J. Mol. Biol. **260:**422–431.
36. **Byrd, D. A., D. J. Sweet, N. Panté, K. N. Konstantinov, T. Guan, A. C. Saphire, P. J. Mitchell, C. S. Cooper, U. Aebi, and L. Gerace.** 1994. Tpr, a large coiled coil protein whose amino terminus is involved in activation of oncogenic kinases, is localized to the cytoplasmic surface of the nuclear pore complex. J. Cell Biol. **127:**1515–1526.
37. **Carey, K. L., S. A. Richards, K. M. Lounsbury, and I. G. Macara.** 1996. Evidence using a green fluorescent protein-glucocorticoid receptor chimera that the RAN/TC4 GTPase mediates an essential function independent of nuclear protein import. J. Cell Biol. **133:**985–996.
38. **Carmo-Fonseca, M., and E. C. Hurt.** 1991. Across the nuclear pores with the help of nucleoporins. Chromosoma **101:**199–205.
39. **Chant, J., and L. Stowers.** 1995. GTPase cascades choreographing cellular behavior: movement, morphogenesis, and more. Cell **81:**1–4.
40. **Cheng, Y., J. E. Dahlberg, and E. Lund.** 1995. Diverse effects of the guanine nucleotide exchange factor RCC1 on RNA transport. Science **267:**1807–1810.
41. **Chi, N. C., E. J. H. Adam, G. D. Visser, and S. A. Adam.** 1996. RanBP1 stabilizes the interaction of Ran with p97 in nuclear protein import. J. Cell Biol. **135:**559–569.
42. **Chi, N. C., J. H. E. Adam, and S. A. Adam.** 1995. Sequence and characterization of cytoplasmic nuclear protein import factor p97. J. Cell Biol. **130:** 265–274.
43. **Chiang, A., A. Kastaniotis, R. Zitomer, and P. A. Silver.** 1993. Unpublished data.
44. **Chow, T. Y.-K., J. J. Ash, D. Dignard, and D. Y. Thomas.** 1992. Screening and identification of a gene, *PSE-1*, that affects protein secretion in *Saccharomyces cerevisiae*. J. Cell. Sci. **101:**709–719.
45. **Clark, K. L., and J. G. F. Sprague.** 1989. Yeast pheromone response pathway: characterization of a suppressor that restores mating to receptor-less mutants. Mol. Cell. Biol. **9:**2682–2694.
46. **Clarke, P. R., C. Klebe, A. Wittinghofer, and E. Karsenti.** 1995. Regulation of Cdc2/cyclin B activation by Ran, a Ras-related GTPase. J. Cell Sci. **108:**1217–1225.
47. **Clarkson, W. D., H. M. Kent, and M. Stewart.** 1996. Separate binding sites on nuclear transport factor 2 (NTF2) for GDP-Ran and the phenylalanine-rich repeat regions on nucleoporins p62 and Nsp1p. J. Mol. Biol. **263:**517–524.
48. **Colot, H. V., F. Stutz, and M. Rosbash.** 1996. The yeast splicing factor Mud13p is a commitment complex component and corresponds to CBP20, the small subunit of the nuclear cap binding complex. Genes Dev. **10:**1699–1708.
49. **Corbett, A. H., W. D. Clarkson, M. Stewart, H. Kent, and P. A. Silver.** 1996. Unpublished data.
50. **Corbett, A. H., D. M. Koepp, M. S. Lee, G. Schlenstedt, A. K. Hopper, and P. A. Silver.** 1995. Rna1p, a Ran/TC4 GTPase activating protein is required for nuclear import. J. Cell Biol. **130:**1017–1026.
51. **Corbett, A. H., and P. A. Silver.** 1996. The *NTF2* gene encodes an essential, highly conserved protein that functions in nuclear transport *in vivo*. J. Biol. Chem. **271:**18477–18484.
52. **Corbett, A. H., and P. A. Silver.** 1996. Unpublished data.
53. **Cortes, P., Z. Ye, and D. Baltimore.** 1994. RAG-1 interacts with the repeated amino acid motif of the human homologue of the yeast protein SRP1. Proc. Natl. Acad. Sci. USA **91:**7633–7637.
54. **Coutavas, E., M. Ren, J. D. Oppenheim, P. D'Eustachio, and M. G. Rush.** 1993. Characterization of proteins that interact with the cell-cycle regulatory protein Ran/TC4. Nature **366:**585–587.
55. **Coutavas, E. E., C. M. Hsieh, M. Ren, G. T. Drivas, M. G. Rush, and P. E'Eustachio.** 1994. Tissue-specific expression of Ran isoforms in the mouse. Mamm. Genome **5:**623–628.
56. **Cuomo, C. A., S. A. Kirch, J. Gyuris, R. Brent, and M. A. Oettinger.** 1994. Rch1, a protein that specifically interacts with the RAG-1 recombination-activating protein. Proc. Natl. Acad. Sci. USA **91:**6156–6160.
57. **Dasso, M.** 1993. RCC1 in the cell cycle: the regulator of chromosome condensation takes on new roles. Trends Biol. Sci. **13:**96–101.
58. **Dasso, M., H. Nishitani, S. Kornbluth, T. Nishimoto, and J. W. Newport.** 1992. RCC1, a regulator of mitosis, is essential for DNA replication. Mol. Cell. Biol. **12:**3337–3345.
59. **Davis, L. I.** 1995. The nuclear pore complex. Annu. Rev. Biochem. **64:**865–896.
60. **Davis, L. I., and G. R. Fink.** 1990. The *NUP1* gene encodes an essential component of the yeast nuclear pore complex. Cell **61:**965–978.
61. **DeHoratius, C., and P. A. Silver.** 1996. Nuclear transport defects and nuclear envelope alterations are associated with mutation of the *Saccharomyces cerevisiae NPL4* gene. Mol. Biol. Cell **7:**1835–1855.
62. **DelPriore, V., C. A. Snay, A. Bahr, and C. N. Cole.** 1996. The product of the *Saccharomyces cerevisiae RSS1* gene, identified as a high-copy suppressor of *the rat7-1* temperature-sensitive allele of the *Rat7*/*NUP159* nucleoporin, is required for efficient mRNA export. Mol. Biol. Cell **7:**1601–1621.
63. **Dickmanns, A., F. R. Bischoff, C. Marshallsay, R. Lührmann, H. Ponstingl, and E. Fanning.** 1996. The thermolability of nuclear protein import in tsBN2 cells is suppressed by microinjected Ran-GTP or Ran-GDP, but not by RanQ69L or RanT24N. J. Cell Sci. **109:**1449–1457.
64. **Dingwall, C., S. Kandels-Lewis, and B. Seraphin.** 1995. A family of Ran binding proteins that includes nucleoporins. Proc. Natl. Acad. Sci. USA **92:**7525–7529.
65. **Dingwall, C., and R. A. Laskey.** 1991. Nuclear targeting sequences—a consensus? Trends Biol. Sci. **16:**178–181.
66. **Doye, V., and E. C. Hurt.** 1995. Genetic approaches to nuclear pore structure and function. Trends Genet. **11:**235–241.
67. **Doye, V., R. Wepf, and E. C. Hurt.** 1994. A novel nuclear pore protein Nup133p with distinct roles in poly(A)$^+$ RNA transport and nuclear pore distribution. EMBO J. **13:**6062–6075.
68. **Dreyfuss, G., M. J. Matunis, S. Pinol-Roma, and C. G. Burd.** 1993. hnRNP proteins and the biogenesis of mRNA. Annu. Rev. Biochem. **62:**289–321.
69. **Drivas, G. T.** 1990. Characterization of four novel ras-like genes expressed in a human teratocarcinoma cell line. Mol. Cell. Biol. **10:**1793–1798.
70. **Dujon, B.** 1996. The yeast genome project: what did we learn? Trends Genet. **12:**263–270.
71. **Eckner, R., W. Ellmeir, and M. L. Birnstiel.** 1991. Mature mRNA 3′ end formation stimulates RNA export from the nucleus. EMBO J. **10:**3513–3522.
72. **Fabre, E., W. C. Boelans, C. Wimmer, I. W. Mattaj, and E. C. Hurt.** 1994. Nup145p is required for nuclear export of mRNA and binds homopolymeric RNA in vitro via a novel conserved motif. Cell **78:**272–289.
73. **Fabre, E., and E. C. Hurt.** 1994. Nuclear transport. Curr. Opin. Cell Biol. **6:**335–342.
74. **Fields, S., and O. Song.** 1989. A novel genetic system to detect protein-protein interactions. Nature **340:**245–246.
75. **Finlay, D. R., E. Meier, P. Bradley, J. Horecka, and D. J. Forbes.** 1991. A complex of nuclear pore proteins required for pore formation. J. Cell Biol. **114:**169–183.
76. **Fischer, U., J. Huber, W. C. Boelens, I. W. Mattaj, and R. Lührmann.** 1995. The HIV-1 Rev activation domain is a nuclear export signal that accesses an export pathway used by specific cellular RNAs. Cell **82:**475–483.
77. **Fischer, U., S. Meyer, M. Teufel, C. Heckel, R. Luhrman, and G. Rautman.** 1994. Evidence that the HIV-1 Rev directly promotes the nuclear export of unspliced RNA. EMBO J. **13:**4105–4112.
78. **Fischer, U., W. M. Michael, R. Lührman, and G. Dreyfuss.** 1996. Signal-mediated nuclear export pathways of protein and RNA. Trends Cell Biol. **6:**290–293.
79. **Flach, J., M. Bossie, J. Vogel, A. H. Corbett, T. Jinks, D. A. Willins, and P. A. Silver.** 1994. A yeast RNA-binding protein shuttles between the nucleus and the cytoplasm. Mol. Cell. Biol. **14:**8399–8407.
80. **Fleischmann, M., M. W. Clark, W. Forrester, M. Wickens, T. Nishimoto, and M. Aebi.** 1991. Analysis of yeast *prp20* mutations and functional complementation by the human homologue RCC1, a protein involved in the control of chromosome condensation. Mol. Gen. Genet. **227:**417–423.
81. **Forrester, W., F. Stutz, M. Rosbash, and M. Wickens.** 1992. Defects in mRNA 3′-end formation, transcription initiation, and mRNA transport associated with the yeast mutation *prp20*: possible coupling of mRNA processing and chromatin structure. Genes Dev. **6:**1914–1926.
82. **Frasch, M.** 1991. The maternally expressed *Drosophila* gene encoding the chromatin-binding protein *BJ1* is a homolog of the vertebrate gene Regu-

lator of Chromosome Condensation, RCC1. EMBO J. **10:**1225–1236.
83. **Fridell, R. A., U. Fischer, R. Luhrman, B. E. Meyers, J. L. Meinkoth, M. H. Malim, and B. R. Cullen.** 1996. Amphibian transcription factor IIIA proteins contain a sequence elements functionally equivalent to the nuclear export signal of human immunodeficiency virus type 1 Rev. Proc. Natl. Acad. Sci. USA **93:**2936–2940.
84. **Fritz, C. C., M. L. Zapp, and M. R. Green.** 1995. A human nucleoporin-like protein that specifically interacts with HIV Rev. Nature **376:**530–533.
85. **Fujiwara, T., K. Oda, S. Yokota, A. Takatsuki, and Y. Ikehara.** 1988. Brefeldin A causes disassembly of the Golgi complex and accumulation of secretory proteins in the endoplasmic reticulum. J. Biol. Chem. **263:**18545–18552.
86. **Gary, J. D., W.-J. Lin, M. C. Yang, H. R. Herschman, and S. Clarke.** 1996. The predominant protein-arginine methyltransferase from *Saccharomyces cerevisiae*. J. Biol. Chem. **271:**12585–12594.
87. **Gerace, L.** 1995. Nuclear export signals and the fast track to the cytoplasm. Cell **82:**341–344.
88. **Goldberg, M. W., and T. D. Allen.** 1995. Structural and functional organization of the nuclear pore. Curr. Opin. Biol. **7:**301–309.
89. **Goldstein, A. L., C. A. Snay, C. V. Heath, and C. N. Cole.** 1996. Pleiotropic nuclear defects associated with a conditional allele of the novel nucleoporin Rat9p/Nup85p. Mol. Biol. Cell **7:**917–934.
90. **Görlich, D., P. Henklein, R. A. Laskey, and E. Hartmann.** 1996. A 41 amino acid motif in importin-α confers binding to importin-β and hence transit into the nucleus. EMBO J. **15:**1810–1817.
91. **Görlich, D., R. Kraft, S. Kostka, F. Vogel, E. Hartmann, R. A. Laskey, I. W. Mattaj, and E. Izaurralde.** 1996. Importin provides a link between nuclear protein import and U snRNA export. Cell **87:**21–32.
92. **Görlich, D., and I. W. Mattaj.** 1996. Nucleocytoplasmic transport. Science **271:**1513–1518.
93. **Görlich, D., N. Panté, U. Kutay, U. Aebi, and F. R. Bischoff.** 1996. Identification of different roles for RanGDP and RanGTP in nuclear protein import. EMBO J. **15:**5584–5594.
94. **Görlich, D., S. Prehn, R. A. Laskey, and E. Hartmann.** 1994. Isolation of a protein that is essential for the first step of nuclear protein import. Cell **79:**767–778.
95. **Görlich, D., F. Vogel, A. D. Mills, E. Hartmann, and R. A. Laskey.** 1995. Distinct functions for the two importin subunits in nuclear protein import. Nature **377:**246–248.
96. **Gorsch, L. C., T. C. Dockendorff, and C. N. Cole.** 1995. A conditional allele of the novel repeat-containing yeast nucleoporin *RAT7*/*NUP159* causes rapid cessation of mRNA export and reversible clustering of nuclear pore complexes. J. Cell Biol. **129:**939–955.
97. **Grandi, P., V. Doye, and E. C. Hurt.** 1993. Purification of NSP1 reveals complex formation with 'GLFG' nucleoporins and a novel nuclear pore protein NIC96. EMBO J. **12:**3061–3071.
98. **Grandi, P., S. Emig, C. Weise, F. Hucho, T. Pohl, and E. C. Hurt.** 1995. A novel nuclear pore protein Nup82p which specifically binds to a fraction of Nsp1p. J. Cell Biol. **130:**1263–1273.
99. **Grandi, P., N. Schlaich, H. Tekotte, and E. C. Hurt.** 1995. Functional interaction of Nic96p with a core nucleoporin complex consisting of Nsp1p, Nup49p and a novel protein Nup57p. EMBO J. **14:**76–87.
100. **Greber, U. F., A. Senior, and L. Gerace.** 1990. A major glycoprotein of the nuclear pore complex is a membrane-spanning polypeptide with a large lumenal domain and a small cytoplasmic tail. EMBO J. **9:**1495–1502.
101. **Grote, M., U. Kubitscheck, R. Reichelt, and R. Peters.** 1995. Mapping of nucleoporins to the center of the nuclear pore complex by post-embedding immunogold electron microscopy. J. Cell Sci. **108:**2963–2972.
102. **Guan, T., S. Muller, G. Klier, N. Panté, J. M. Blevitt, M. Haner, B. Paschal, U. Aebi, and L. Gerace.** 1995. Structural analysis of the p62 complex, an assembly of O-linked glycoproteins that localizes near the central gated channel of the nuclear pore complex. Mol. Biol. Cell **6:**1591–1603.
103. **Gyuris, J., E. Golemis, H. Chertkov, and R. Brent.** 1993. Cdi1, a human G1 and S phase protein phosphatase that associates with Cdk2. Cell **75:**791–803.
104. **Hall, A.** 1990. Ras and GAP—who's controlling whom? Cell **61:**921–923.
105. **Hallberg, E., R. W. Wozniak, and G. Blobel.** 1993. An integral membrane protein of the pore membrane domain of the nuclear envelope contains a nucleoporin-like region. J. Cell Biol. **122:**513–521.
106. **Hamm, J., and I. W. Mattaj.** 1990. Monomethylated cap structures facilitate RNA export from the nucleus. Cell **63:**109–118.
107. **Hartmann, E., and D. Görlich.** 1995. A Ran-binding motif in nuclear pore proteins. Trends Cell Biol. **5:**192–193.
108. **Hartwell, L.** 1967. Macromolecular synthesis in temperature-sensitive mutants of yeast. J. Bacteriol. **93:**1662–1670.
109. **He, X., N. G. W. N. Hayashi, Y. Azuma, T. E. Patterson, F. R. Bischoff, H. Ponstigl, T. Nishimoto, and S. Sazer.** Unpublished data.
110. **Heath, C. V., C. S. Copeland, D. C. Amberg, V. DelPriore, M. Snyder, and C. N. Cole.** 1995. Nuclear pore complex clustering and nuclear accumulation of poly(A)$^+$ RNA associated with mutation of the *Saccharomyces cerevisiae RAT2*/*NUP120* gene. J. Cell Biol. **131:**1677–1697.
111. **Henry, M., C. Z. Borland, M. Bossie, and P. A. Silver.** 1996. Potential RNA binding proteins in *Saccharomyces cerevisiae* identified as suppressors of temperature-sensitive mutations in *NPL3*. Genetics **142:**103–115.
112. **Henry, M. F., H. S. Scott, M. D. Lalioti, C. Rossier, P. A. Silver, and S. Antonarakis.** Molecular cloning, genetic, and biochemical characterization of two putative human arginine methyltransferases (HRMT1L1 and HRMT1L2) which map to chromosomes 21q22.3 and 19q, respectively. Submitted for publication.
113. **Henry, M. F., and P. A. Silver.** 1996. A novel methyltransferase (Hmt1p) modifies poly(A)$^+$ RNA binding proteins. Mol. Cell. Biol. **16:**3668–3678.
114. **Herskowitz, I.** 1995. MAP kinase pathways in yeast: for mating and more. Cell **80:**187–197.
115. **Hopper, A. K., F. Banks, and V. Evangelidis.** 1978. A yeast mutant which accumulates precursor tRNAs. Cell **19:**211–219.
116. **Hopper, A. K., L. D. Schultz, and R. A. Shapiro.** 1980. Processing of intervening sequences: a new yeast mutant which fails to excise intervening sequences from precursor tRNAs. Cell **19:**741–751.
117. **Hopper, A. K., H. M. Traglia, and R. W. Dunst.** 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:**309–321.
118. **Hu, T., T. Guan, and L. Gerace.** 1996. Molecular and functional characterization of the p62 complex, an assembly of nuclear pore complex glycoproteins. J. Cell Biol. **134:**589–601.
119. **Huang, S., T. J. Deerinck, M. H. Ellisman, and D. L. Spector.** 1994. *In vivo* analysis of the stability and transport of nuclear poly(A)$^+$ RNA. J. Cell Biol. **126:**877–899.
120. **Hurt, E. C.** 1996. Importins/karyopherins meet nucleoporins. Cell **84:**509–515.
121. **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:**4323–4334.
122. **Hurt, E. C., A. McDowall, and T. Schimmang.** 1988. Nucleolar and nuclear envelope proteins of the yeast *Saccharomyces cerevisiae*. Eur. J. Cell Biol. **46:**554–563.
123. **Hurwitz, M. E., and G. Blobel.** 1995. *NUP82* is an essential yeast nucleoporin required for poly(A)$^+$ RNA export. J. Cell Biol. **130:**1275–1281.
124. **Hutchinson, H. T., L. H. Hartwell, and C. S. McLaughlin.** 1969. Temperature-sensitive yeast mutant defective in ribonucleic acid production. J. Bacteriol. **99:**807–814.
125. **Imamoto, N., Y. Matsuoka, T. Kurihara, K. Kohno, M. Miyagi, F. Sakiyama, Y. Okada, S. Tsunasawa, and Y. Yoneda.** 1992. Antibodies against 70-kD heat shock cognate protein inhibit mediated nuclear import of karyophilic proteins. J. Cell Biol. **119:**1047–1061.
126. **Imamoto, N., T. Shimamoto, T. Takao, T. Tachibana, S. Kose, M. Matsubae, T. Sekimoto, Y. Shimonshi, and Y. Yoneda.** 1995. *In vivo* evidence for involvement of 58 kDa component of nuclear pore-targeting complex in nuclear protein import. EMBO J. **14:**3617–3626.
127. **Iovine, M. K., J. L. Watkins, and S. R. Wente.** 1995. The GLFG repetitive region of the nucleoporin Nup116p interacts with Kap95p, an essential yeast nuclear import factor. J. Cell Biol. **131:**1699–1713.
128. **Izaurralde, E., J. Lewis, C. Gamberi, A. Jarmalowski, C. McGuigan, and I. W. Mattaj.** 1995. A cap-binding protein complex mediating U snRNA export. Nature **376:**709–712.
129. **Izaurralde, E., and I. W. Mattaj.** 1995. RNA export. Cell **81:**153–159.
130. **Jans, D. A.** 1995. The regulation of protein transport to the nucleus by phosphorylation. Biochem. J. **311:**705–716.
131. **Jarmolowski, A., W. C. Boelens, E. Izaurralde, and I. W. Mattaj.** 1994. Nuclear export of different classes of RNA is mediated by specific factors. J. Cell Biol. **124:**627–635.
132. **Kadowaki, T., S. Chen, M. Hitomi, E. Jacobs, C. Kumagai, S. Liang, R. Schneiter, D. Singleton, J. Wisniewska, and A. M. Tartakoff.** 1994. Isolation and characterization of *Saccharomyces cerevisiae* mRNA transport-defective (*mtr*) mutants. J. Cell Biol. **126:**649–659.
133. **Kadowaki, T., D. Goldfarb, L. M. Spitz, A. M. Tartakoff, and M. Ohno.** 1993. Regulation of RNA processing and transport by a nuclear guanine nucleotide release protein and members of the Ras superfamily. EMBO J. **12:**2929–2937.
134. **Kadowaki, T., Y. Zhao, and A. Tartakoff.** 1992. A conditional yeast mutant deficient in mRNA transport from nucleus to cytoplasm. Proc. Natl. Acad. Sci. USA **89:**2312–2316.
135. **Kalderon, D., B. L. Roberts, W. D. Richardson, and A. E. Smith.** 1984. A short amino acid sequence able to specify nuclear location. Cell **39:**499–509.
136. **Kenna, M. A., J. G. Petranka, J. L. Reilly, and L. I. Davis.** 1996. Yeast N1e3p/Nup170p is required for normal stoichiometry of FG nucleoporins within the nuclear pore complex. Mol. Cell. Biol. **16:**2025–2036.
137. **Kent, H. M., W. D. Clarkson, T. L. Bullock, and M. Stewart.** 1996. Crystallization and preliminary X-ray diffraction analysis of nuclear transport factor 2. J. Struct. Biol. **116:**325–328.
138. **Kirkpatrick, D., and F. Solomon.** 1994. Overexpression of the yeast homologs of the mammalian checkpoint gene RCC1 suppresses the class of α-tubulin mutations that arrest with excess microtubules. Genetics **137:**381–392.
139. **Koepp, D. M., P. Ferrigno, and P. A. Silver.** 1996. Unpublished data.

140. **Koepp, D. M., and P. A. Silver.** 1996. A GTPase controlling nuclear trafficking: running the right way or walking randomly. Cell **87:**1–4.
141. **Koepp, D. M., D. H. Wong, A. H. Corbett, and P. A. Silver.** 1996. Dynamic localization of the nuclear import receptor and its interactions with transport factors. J. Cell Biol. **133:**1163–1176.
142. **Kornbluth, S., M. Dasso, and J. Newport.** 1994. Evidence for a dual role for TC4 protein in regulating nuclear structure and cell cycle progression. J. Cell Biol. **125:**705–719.
143. **Kraemer, D., R. W. Wozniak, G. Blobel, and A. Radu.** 1994. The human CAN protein, a putative oncogene product associated with myeloid leukemogenesis, is a nuclear pore complex protein that faces the cytoplasm. Proc. Natl. Acad. Sci. USA **91:**1519–1523.
144. **Kraemer, D. M., C. Strambio-de-Castillia, G. Blobel, and M. P. Rout.** 1995. The essential yeast nucleoporin *NUP159* is located on the cytoplasmic side of the nuclear pore complex and serves in karyopherin-mediated binding of transport substrate. J. Biol. Chem. **270:**19017–19021.
145. **Kramer, A.** 1996. The structure and function of proteins involved in mammalian pre-mRNA splicing. Annu. Rev. Biochem. **65:**367–409.
146. **Küssel, P., and M. Frasch.** 1995. Yeast Srp1, a nuclear protein related to *Drosophila* and mouse pendulin, is required for normal migration, division, and integrity of nuclei during mitosis. Mol. Gen. Genet. **248:**351–363.
147. **Lanford, R. E., and J. S. Butel.** 1984. Construction and characterization of an SV40 mutant defective in nuclear transport of T antigen. Cell **37:**801–813.
148. **Lawrence, J. B., A. W. Cochrane, C. V. Johnson, A. Perkins, and C. A. Rosen.** 1991. The HIV-1 Rev protein: a model system for coupled RNA transport and translation. New Biol. **1991:**1220–1232.
149. **Lee, M. S., M. Henry, and P. A. Silver.** 1996. A protein that shuttles between the nucleus and the cytoplasm is an important mediator of RNA export. Genes Dev. **10:**1233–1246.
150. **Lee, M. S., and P. A. Silver.** 1997. RNA movement between the nucleus and the cytoplasm. Curr. Opin. Genet. Dev., **7:**212–219.
151. **Li, O., C. V. Heath, D. C. Amberg, T. C. Dockendorff, C. S. Copeland, M. Snyder, and C. N. Cole.** 1995. Mutation or deletion of the *Saccharomyces cerevisiae RAT3/NUP133* gene causes temperature-dependent nuclear accumulation of poly(A)$^+$ RNA and constitutive clustering of nuclear pore complexes. Mol. Biol. Cell **6:**401–417.
152. **Liu, Q., and G. Dreyfuss.** 1995. In vivo and in vitro arginine methylation of RNA-binding proteins. Mol. Cell. Biol. **15:**2800–2808.
153. **Loeb, J. D. J., L. I. Davis, and G. R. Fink.** 1993. *NUP2*, a novel yeast nucleoporin, has functional overlap with other proteins of the nuclear pore complex. Mol. Biol. Cell **4:**209–222.
154. **Loeb, J. D. L., G. Schlenstedt, D. Pellman, D. Kornitzer, P. A. Silver, and G. Fink.** 1995. A nuclear import receptor homologue required for mitosis in yeast. Proc. Natl. Acad. Sci. USA **92:**7647–7651.
155. **Lounsbury, K. M., A. L. Beddow, and I. G. Macara.** 1994. A family of proteins that stabilize the Ran/TC4 GTPase in its GTP-bound conformation. J. Biol. Chem. **269:**11285–11290.
156. **Lounsbury, K. M., S. A. Richards, R. R. Perlungher, and I. G. Macara.** 1996. Ran binding domains promote the interaction of Ran with p97/beta-karyopherin, linking the docking and translocation steps of nuclear import. J. Biol. Chem. **271:**2357–2360.
157. **Macaulay, C., and D. J. Forbes.** 1996. Assembly of the nuclear pore: biochemically distinct steps revealed with NEM, GTP gamma S, and BAPTA. J. Cell Biol. **132:**5–20.
158. **Mahajan, R., C. Delphin, T. Guan, L. Gerace, and F. Melchior.** 1997. A small ubiquitin-related polypeptide involved in targeting RanGAP1 to nuclear pore complex protein RanBP2. Cell **88:**97–107.
159. **Malim, M. H., and B. R. Cullen.** 1993. Rev and the fate of pre-mRNA in the nucleus: implications for the regulation of RNA processing in eukaryotes. Mol. Cell. Biol. **13:**6180–6189.
160. **Malim, M. H., J. Hauber, S. Y. Le, J. V. Maizel, and B. R. Cullen.** 1989. The HIV-1 Rev trans-activator acts through a structured target sequence to activate nuclear export of unspliced viral mRNA. Nature **338:**254–257.
161. **Manley, J. L.** 1995. A complex protein assembly catalyzes polyadenylation of mRNA precursors. Curr. Opin. Genet. Dev. **5:**222–228.
162. **Mannhaupt, G., R. Stucka, S. Ehnle, I. Vetter, and H. Feldmann.** 1994. Analysis of a 70 kb region on the right arm of yeast chromosome II. Yeast **10:**1363–1381.
163. **Matsumoto, T., and D. Beach.** 1991. The *spi1* GTPase interacts with RCC1 in cell cycle dependency. Cold Spring Harbor Symp. Quant. Biol. **56:**385–398.
164. **Matunis, M. J., E. Coutavas, and G. Blobel.** 1996. A novel ubiquitin-like modification modulates the partitioning of the Ran-GTPase activating protein RanGAP1 between the cytosol and the nuclear pore complex. J. Cell Biol. **135:**1457–1470.
165. **Matynia, A., K. Dimitrov, U. Mueller, X. He, and S. Sazer.** 1996. Perturbations in the *spi1* GTPase cycle of *Schizosaccharomyces pombe* through its GTPase-activating protein and guanine nucleotide exchange factor components result in similar phenotypic consequences. Mol. Cell. Biol. **16:**6352–6362.
166. **Maul, G.** 1977. The nuclear and cytoplasmic pore complex: structure, dynamics, distribution, and evolution. Int. Rev. Cytol. Suppl. **6:**75–186.
167. **Meier, E., B. R. Miller, and D. J. Forbes.** 1995. Nuclear pore complex assembly studied with a biochemical assay for annulate lamellae formation. J. Cell Biol. **129:**1459–1472.
168. **Melchior, F., B. Paschal, J. Evans, and L. Gerace.** 1993. Inhibition of nuclear protein import by nonhydrolyzable analogues of GTP and identification of the small GTPase Ran/TC4 as an essential transport factor. J. Cell Biol. **123:**1649–1659.
169. **Melchior, F., D. J. Sweet, and L. Gerace.** 1995. Analysis of Ran/TC4 function in nuclear protein import. Methods Enzymol. **257:**279–291.
170. **Melchior, F., K. Weber, and V. Gerke.** 1993. A functional homologue of the *RNA1* gene product in *Schizosaccharomyces pombe*: purification, biochemical characterization, and identification of leucine-rich repeat motif. Mol. Biol. Cell **4:**569–581.
171. **Meluh, P. B., and D. Koshland.** 1995. Evidence that the *MIF2* gene *Saccharomyces cerevisiae* encodes a centromere protein with homology to the mammalian centromere protein CENP-C. Mol. Biol. Cell **6:**793–807.
172. **Meyer, B. E., and M. H. Malim.** 1994. The HIV-1 Rev trans-activator shuttles between the nucleus and the cytoplasm. Genes Dev. **8:**1538–1547.
173. **Michael, W. M., M. Choi, and G. Dreyfus.** 1995. A nuclear export signal in hnRNPA1: a signal-mediated, temperature-dependent nuclear protein export pathway. Cell **83:**415–422.
174. **Moll, T., G. Tebb, U. Surana, H. Robitsch, and K. Nasmyth.** 1991. The role of phosphorylation and the CDC28 protein kinase in cell cycle-regulated nuclear import of the *S. cerevisiae* transcription factor Swi5. Cell **66:**743–758.
175. **Moore, M. S.** 1996. Protein translocation: nuclear export—out of the dark. Curr. Biol. **6:**137–140.
176. **Moore, M. S., and G. Blobel.** 1994. A G protein involved in nucleocytoplasmic transport: the role of Ran. Trends Biochem. Sci. **19:**211–216.
177. **Moore, M. S., and G. Blobel.** 1993. The GTP-binding protein Ran/TC4 is required for protein import into the nucleus. Nature **365:**661–663.
178. **Moore, M. S., and G. Blobel.** 1994. Purification of a Ran-interacting protein that is required for protein import into the nucleus. Proc. Natl. Acad. Sci. USA **91:**10212–10216.
179. **Moore, M. S., and G. Blobel.** 1992. The two steps of nuclear import, targeting to the nuclear envelope and translocation through the nuclear pore, require different cytosolic factors. Cell **69:**939–950.
180. **Moroianu, J., and G. Blobel.** 1995. Protein export from the nucleus requires the GTPase Ran and GTP hydrolysis. Proc. Natl. Acad. Sci. USA **92:**4318–4322.
181. **Moroianu, J., G. Blobel, and A. Radu.** 1995. Previously identified protein of uncertain function is karyopherin α and together with karyopherin β docks import substrate at nuclear pore complexes. Proc. Natl. Acad. Sci. USA **92:**2008–2011.
182. **Murphy, R., J. L. Watkins, and S. R. Wente.** 1996. *GLE2*, a *Saccharaomyces cerevisiae* homologue of the *Schizosaccharomyces pombe* export factor *RAE1*, is required for nuclear pore complex structure and function. Mol. Biol. Cell **7:**1921–1937.
183. **Murphy, R., and S. R. Wente.** 1996. An RNA-export mediator with an essential nuclear export signal. Nature **383:**357–360.
184. **Nakashima, N., N. Hayashi, E. Noguchi, and T. Nishimoto.** 1996. Putative GTPase Gtr1p genetically interacts with the RanGTPase cycle in Saccharomyces cerevisiae. J. Cell Sci. **109:**2311–2318.
185. **Nehrbass, U., and G. Blobel.** 1996. Role of the nuclear transport factor p10 in nuclear import. Science **272:**120–122.
186. **Nehrbass, U., E. Fabre, S. Dihlmann, W. Herth, and E. C. Hurt.** 1993. Analysis of nucleo-cytoplasmic transport in a thermosensitive mutant of nuclear pore protein NSP1. Eur. J. Cell Biol. **62:**1–12.
187. **Nehrbass, U., H. Kern, A. Mutvei, H. Horstmann, B. Marshallsay, and E. C. Hurt.** 1990. NSP1: A yeast nuclear envelope protein localized at the nuclear pores exerts its essential function by its carboxy-terminal domain. Cell **61:**979–989.
188. **Nehrbass, U., M. P. Rout, S. Maguire, G. Blobel, and R. W. Wozniak.** 1996. The yeast nucleoporin Nup188p interacts genetically and physically with the core structures of the nuclear pore complex. J. Cell Biol. **133:**1153–1162.
189. **Nelson, M., and P. A. Silver.** 1989. Context affects nuclear protein localization in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **9:**384–389.
190. **Newmeyer, D. D., D. R. Finlay, and D. J. Forbes.** 1986. *In vitro* transport of a fluorescent nuclear protein and exclusion of non-nuclear proteins. J. Cell Biol. **103:**2091–2102.
191. **Newmeyer, D. D., and D. J. Forbes.** 1988. Nuclear import can be separated into distinct steps in vitro: nuclear pore binding and translocation. Cell **52:**641–653.
192. **Nishimoto, T., E. Eilen, and C. Basilico.** 1978. Premature chromosome condensation in a tsDNA$^-$ mutant of BHK cells. Cell **15:**475–483.
193. **Ohtsubo, M., R. Kai, N. Furuno, T. Sekiguchi, H. Havashida, K. Kuma, T. Miyata, S. Fukushige, T. Murotu, K. Matsubara, and T. Nishimoto.** 1987. Isolation and characterization of the active cDNA of the human cell cycle gene (RCC1) involved in the regulation of onset of chromosome condensation. Genes Dev. **1:**585–593.
194. **O'Neil, R. E., and P. Palese.** 1995. NPI-1, the human homolog of SRP-1,

interacts with influenza virus nucleoprotein. Virology **206:**116–125.
195. **Ouspenski, I. I., U. W. Mueller, A. Matynia, S. Sazer, S. J. Elledge, and B. R. Brinkley.** 1995. Ran-binding protein-1 is an essential component of the Ran/RCC1 molecular switch system in budding yeast. J. Biol. Chem. **270:**1975–1978.
196. **Panté, N., and U. Aebi.** 1995. Exploring nuclear pore complex structure and function in molecular detail. J. Cell Sci. Suppl. **19:**1–11.
197. **Panté, N., and U. Aebi.** 1996. Sequential binding of import ligands to distinct nucleopore regions during their nuclear import. Science **273:**1729–1732.
198. **Panté, N., and U. Aebi.** 1996. Toward the molecular dissection of protein import into nuclei. Curr. Opin. Cell Biol. **8:**397–406.
199. **Panté, N., R. Bastos, I. McMorrow, B. Burke, and U. Aebi.** 1994. Interactions and three-dimensional localization of a group of nuclear pore complex proteins. J. Cell Biol. **126:**603–617.
200. **Paschal, B. M., C. Delphin, and L. Gerace.** 1996. Nucleotide-specific interaction of Ran/TC4 with nuclear transport factors NTF2 and p97. Proc. Natl. Acad. Sci. USA **93:**7679–7683.
201. **Paschal, B. M., and L. Gerace.** 1995. Identification of NTF2, a cytosolic factor for nuclear import that interacts with nuclear pore complex protein p62. J. Cell Biol. **129:**925–937.
202. **Peifer, M., S. Berg, and A. B. Reynolds.** 1994. A repeating amino acid motif shared by proteins with diverse cellular roles. Cell **76:**789–791.
203. **Pemberton, L. F., M. P. Rout, and G. Blobel.** 1995. Disruption of the nucleoporin gene *NUP133* results in clustering of nuclear pore complexes. Proc. Natl. Acad. Sci. USA **92:**1187–1191.
204. **Piñol-Roma, S., and G. Dreyfuss.** 1993. hnRNP proteins: localization and transport between the nucleus and the cytoplasm. Trends Cell Biol. **3:**151–155.
205. **Pollard, V. W., W. M. Michael, S. Nakielny, M. C. Siomi, F. Wang, and G. Dreyfuss.** 1996. A novel receptor-mediated nuclear protein import pathway. Cell **86:**985–994.
206. **Powers, M. A., and D. J. Forbes.** 1994. Cytosolic factors in nuclear transport: what's importin? Cell **79:**931–934.
207. **Powers, M. A., C. Macauly, F. R. Masiarz, and D. J. Forbes.** 1995. Reconstituted nuclei depleted of a vertebrate GLFG nuclear pore protein, p97, import but are defective in nuclear growth and replication. J. Cell Biol. **128:**721–736.
208. **Quilliam, L. A., R. Khosravi-Far, S. Y. Huff, and C. J. Der.** 1995. Guanine nucleotide exchange factors: activators of the Ras superfamily of proteins. Bioessays **17:**395–404.
209. **Radu, A., G. Blobel, and M. S. Moore.** 1995. Identification of a protein complex that is required for nuclear protein import and mediates docking of import substrate to distinct nucleoporins. Proc. Natl. Acad. Sci. USA **92:**1769–1773.
210. **Radu, A., G. Blobel, and R. Wozniak.** 1994. Nup107 is a novel nuclear pore complex protein that contains a leucine zipper. J. Biol. Chem. **269:**17600–17605.
211. **Radu, A., G. Blobel, and R. W. Wozniak.** 1993. Nup155 is a novel nuclear pore complex protein that contains neither repetitive sequence motifs nor reacts with WGA. J. Cell Biol. **121:**1–9.
212. **Radu, A., M. S. Moore, and G. Blobel.** 1995. The peptide repeat domain of nucleoporin Nup98 functions as a docking site in transport across the nuclear pore complex. Cell **81:**215–222.
213. **Rao, A.** 1994. NF-ATp: a transcription factor required for the co-ordinate induction of several cytokine genes. Immunol. Today **15:**274–281.
214. **Ren, M., E. Coutavas, P. D'Eustachio, and M. G. Rush.** 1994. Effects of mutant Ran/TC4 proteins on cell cycle progression. Mol. Cell. Biol. **14:**4216–4224.
215. **Ren, M., A. Villamarin, A. Shih, E. Coutavas, M. S. Moore, M. LoCurico, V. Clarke, J. D. Oppenheim, P. D'Eustachio, and M. G. Rush.** 1995. Separate domains of the Ran GTPase interact with different factors to regulate nuclear import and RNA processing. Mol. Cell. Biol. **15:**2117–2124.
216. **Rexach, M., and G. Blobel.** 1995. Protein import into the nuclei: association and dissociation reactions involving transport substrate, transport factors, and nucleoporins. Cell **83:**683–692.
217. **Richard, N., S. Iacampo, and A. Cochrane.** 1994. HIV-1 Rev is capable of shuttling between the nucleus and the cytoplasm. Virology **204:**123–131.
218. **Richards, S. A., K. M. Lounsbury, K. L. Carey, and I. G. Macara.** 1996. A nuclear export signal is essential for the cytosolic localization of the Ran binding protein, RanBP1. J. Cell Biol. **134:**1157–1168.
219. **Richards, S. A., K. M. Lounsbury, and I. G. Macara.** 1995. The C terminus of the nuclear Ran/TC4 GTPase stabilizes the GDP-bound state and mediates interactions with RCC1, Ran-GAP, and HTF9A/RanBP1. J. Biol. Chem. **270:**14405–14411.
220. **Rihs, H. P., D. A. Jans, H. Fan, and R. Peters.** 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:**633–639.
221. **Robbins, J., S. M. Dilworth, R. A. Laskey, and C. Dingwall.** 1991. Two interdependent basic domains in nucleoplasmin nuclear targeting sequence: identification of a class of bipartite nuclear targeting sequence. Cell **64:**615–623.
222. **Rout, M. P., and G. Blobel.** 1993. Isolation of the yeast nuclear pore complex. J. Cell Biol. **123:**771–783.
223. **Rout, M. P., and S. R. Wente.** 1994. Pores for thought: nuclear pore complex proteins. Trends Cell Biol. **4:**357–365.
224. **Ruhl, M., M. Himmelspach, G. M. Bahr, F. Hammerschmid, H. Jaksche, B. Wolff, H. Aschauer, G. K. Farrington, H. Probst, D. Bevec, and J. Hauber.** 1993. Eukaryotic initiation factor 5A is a cellular target of the human immunodeficiency virus type I Rev activation domain mediating *trans*-activation. J. Cell Biol. **123:**1309–1320.
225. **Rush, M. G., G. Drivas, and P. D'Eustachio.** 1996. The small nuclear GTPase Ran: how much does it run? Bioessays **18:**103–112.
226. **Rymond, B. C., and M. Rosbash.** 1992. Yeast pre-mRNA splicing, p. 143–192. *In* E. W. Jones, J. R. Pringle, and J. R. Broach (ed.), The molecular and cellular biology of the yeast *Saccharomyces cerevisiae*: gene expression, vol. II. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
227. **Saavedra, C., K.-S. Tung, D. C. Amberg, A. K. Hopper, and C. N. Cole.** 1996. Regulation of mRNA export in response to stress in *Saccharomyces cerevisiae*. Genes Dev. **10:**1608–1620.
228. **Saitoh, H., C. A. Cooke, W. H. Burgess, W. C. Earnshaw, and M. Dasso.** 1996. Direct and indirect association of the small GTPase Ran with nuclear pore proteins and soluble transport factors: studies in *Xenopus laevis* egg extracts. Mol. Biol. Cell **7:**1319–1334.
229. **Sazer, S.** 1996. The search for the primary function of the Ran GTPase continues. Trends Cell Biol. **6:**81–85.
230. **Sazer, S., and P. Nurse.** 1994. A fission yeast RCC1-related protein is required for the mitosis to interphase transition. EMBO J. **13:**606–615.
231. **Scheffzek, K., C. Klebe, K. Fritz-Wolf, W. Kabsch, and A. Wittinghofer.** 1995. Crystal structure of the nuclear Ras-related protein Ran in its GDP-bound form. Nature **374:**378–381.
232. **Schlaich, N. L., and E. C. Hurt.** 1995. Analysis of nucleocytoplasmic transport and nuclear envelope structure in yeast disrupted for the gene encoding the nuclear pore protein Nup1p. Eur. J. Cell Biol. **67:**8–14.
233. **Schlenstedt, G., E. Hurt, V. Doye, and P. A. Silver.** 1993. Reconstitution of nuclear protein transport with semi-intact yeast cells. J. Cell Biol. **123:**785–798.
234. **Schlenstedt, G., C. Saavedra, J. D. J. Loeb, C. N. Cole, and P. A. Silver.** 1995. The GTP-bound form of the yeast Ran/TC4 homologue blocks nuclear protein import and appearance of poly $(A)^+$ RNA in the cytoplasm. Proc. Natl. Acad. Sci. USA **92:**225–229.
235. **Schlenstedt, G., D. H. Wong, D. M. Koepp, and P. A. Silver.** 1995. Mutants in a yeast Ran binding protein are defective in nuclear transport. EMBO J. **14:**5367–5378.
236. **Seedorf, M., and P. A. Silver.** Importin/karyopherin protein family members required for mRNA export from the nucleus. Submitted for publication.
237. **Seydel, U., and L. Gerace.** 1991. A 28,000-Da GDP/GTP-binding protein specific to the nuclear envelope. J. Biol. Chem. **266:**7602–7608.
238. **Sharma, K., E. Fabre, H. Tekotte, E. C. Hurt, and D. Tollervy.** 1996. Yeast nucleoporin mutants are defective in pre-tRNA splicing. Mol. Cell. Biol. **16:**294–301.
239. **Shi, Y., and J. O. Thomas.** 1992. The transport of proteins into the nucleus requires the 70-kilodalton heat shock protein or its cytosolic cognate. Mol. Cell. Biol. **12:**2186–2192.
240. **Shulga, N., P. Roberts, Z. Gu, L. Spitz, M. M. Tabb, M. Nomura, and D. S. Goldfarb.** 1996. *In vivo* nuclear transport kinetics in *Saccharomyces cerevisiae*: a role for heat shock protein 70 during targeting and translocation. J. Cell Biol. **135:**329–339.
241. **Shuman, S.** 1995. Capping enzyme in eukaryotic mRNA synthesis. Prog. Nucleic Acid Res. Mol. Biol. **50:**101–129.
242. **Sidorova, J. M., G. E. Mikesell, and L. L. Breeden.** 1995. Cell cycle-regulated phosphorylation of Swi6 controls its nuclear localization. Mol. Biol. Cell **6:**1641–1658.
243. **Siebel, C. W., and C. Guthrie.** 1996. The essential yeast RNA binding protein Npl3p is methylated. Proc. Natl. Acad. Sci. USA **93:**13641–13646.
244. **Simos, G., H. Tekotte, H. Grosjean, A. Segref, K. Sharma, D. Tollervey, and E. C. Hurt.** 1996. Nuclear pore proteins are involved in the biogenesis of functional tRNA. EMBO J. **15:**2270–2284.
245. **Siniossoglou, S., C. Wimmer, M. Rieger, V. Doye, H. Tekotte, C. Weise, S. Emig, A. Segref, and E. C. Hurt.** 1996. A novel complex of nucleoporins, which includes Sec13p and a Sec13p homolog, is essential for normal nuclear pores. Cell **84:**265–275.
246. **Siomi, H., and G. Dreyfuss.** 1995. A nuclear localization domain in the hnRNP A1 protein. J. Cell Biol. **129:**551–560.
247. **Soderqvist, H., and E. Hallberg.** 1994. The large C-terminal region of the integral pore membrane protein, POM121, is facing the nuclear pore complex. Eur. J. Cell Biol. **64:**186–191.
248. **Sprague, G. F., R. Jensen, and I. Herskowitz.** 1983. Control of yeast cell type by the mating type locus: positive regulation of the α-specific *STE3* gene by the MATα1 product. Cell **32:**409–415.
249. **Stochaj, U., M. Osborne, T. Kurihara, and P. Silver.** 1991. A yeast protein that binds nuclear localization signals: purification, localization, and antibody inhibition of binding activity. J. Cell Biol. **113:**1243–1254.
250. **Stochaj, U., and P. A. Silver.** 1992. A conserved phosphoprotein that spe-

cifically binds nuclear localization sequences is involved in nuclear import. J. Cell Biol. **117:**473–482.
251. **Stutz, F., M. Neville, and M. Roshbash.** 1995. Identification of a novel nuclear pore-associated protein as a functional target of the HIV-1 Rev protein in yeast. Cell **82:**495–506.
252. **Sukegawa, J., and G. Blobel.** 1993. A nuclear pore complex protein that contains zinc finger motifs, binds DNA, and faces the nucleoplasm. Cell **72:**29–38.
253. **Sweet, D. J., and L. Gerace.** 1996. A GTPase distinct from Ran is involved in nuclear protein import. J. Cell Biol. **133:**971–983.
254. **Sweet, D. J., and L. Gerace.** 1995. Taking from the cytoplasm and giving to the pore: soluble transport factors in nuclear protein import. Trends Cell Biol. **5:**444–447.
255. **Tachibana, T., N. Imamoto, H. Seino, T. Nishimoto, and Y. Yoneda.** 1994. Loss of RCC1 leads to the suppression of nuclear protein import in living cells. J. Biol. Chem. **269:**24542–24545.
256. **Taura, T., and P. A. Silver.** Yrb2p is a nuclear protein that interacts with Prp20p, a yeast Rcc1 homologue, with and mediates nuclear functions distinct from nuclear transport. Submitted for publication.
257. **Torok, I., D. Strand, R. Schmitt, G. Tick, T. Torok, I. Kiss, and B. M. Melcher.** 1995. The overgrown hematopoietic organs-31 tumor suppressor gene of Drosophila encodes an importin-like protein accumulating in the nucleus at the onset of mitosis. J. Cell Biol. **129:**1473–1489.
258. **Traglia, H. M., J. P. O'Conner, K.-S. Tung, S. Dallabrida, W.-S. Shen, and A. K. Hopper.** 1996. Nucleus-associated pools of Rna1p, the *Saccharomyces cerevisiae* Ran/TC4 GTPase activating protein involved in nucleus/cytoplasm transit. Proc. Natl. Acad. Sci. USA **93:**7667–7672.
259. **Turi, T. G., U. W. Mueller, S. Sazer, and J. K. Rose.** 1996. Characterization of a nuclear protein conferring brefeldin A resistance in *Schizosaccharomyces pombe*. J. Biol. Chem. **271:**9166–9171.
260. **vanDeursen, J., J. Boer, L. Kasper, and G. Grosved.** 1996. G_2 arrest and impaired nucleocytoplasmic transport in mouse embryos lacking the protooncogene *CAN*/*Nup214*. EMBO J. **15:**5574–5583.
261. **Vijayraghavan, U., M. Company, and J. Abelson.** 1989. Isolation and characterization of pre-mRNA splicing mutants in *Saccharomyces cerevisiae*. Genes Dev. **3:**1206–1216.
262. **Visa, N., A. T. Alzhanova-Ericsson, X. Sun, E. Kiseleva, B. Bjorkroth, T. Wurtz, and B. Daneholt.** 1996. A pre-mRNA-binding protein accompanies the RNA from the gene through the nuclear pores and into polysomes. Cell **84:**253–264.
263. **Visa, N., E. Izzaurralde, J. Ferreira, B. Daneholt, and I. W. Mattaj.** 1996. A nuclear cap-binding complex binds Balbani ring pre-mRNA cotranscriptionally and accompanies the ribonucleoprotein particle during nuclear export. J. Cell Biol. **133:**5–14.
264. **Weis, K., C. Dingwall, and A. I. Lamond.** 1996. Characterization of the nuclear protein import mechanism using Ran mutants with altered nucleotide binding specificities. EMBO J. **15:**7120–7128.
265. **Weis, K., U. Ryder, and A. I. Lamond.** 1996. The conserved amino-terminal domain of hSRP1 alpha is essential for nuclear protein import. EMBO J. **15:**1818–1825.
266. **Wen, W., J. L. Meinkoth, R. Y. Tsien, and S. S. Taylor.** 1995. Identification of a signal for rapid export of proteins from the nucleus. Cell **82:**463–473.
267. **Wente, S. R., and G. Blobel.** 1994. *NUP145* encodes a novel yeast glycine-leucine-phenylalanine-glycine (GLFG) nucleoporin required for nuclear envelope structure. J. Cell Biol. **125:**955–969.
268. **Wente, S. R., and G. Blobel.** 1993. A temperature-sensitive *NUP116* null mutant forms a nuclear envelope seal over the yeast nuclear pore complex thereby blocking nucleocytoplasmic traffic. J. Cell Biol. **123:**275–284.
269. **Wente, S. R., M. P. Rout, and G. Blobel.** 1992. A new family of yeast nuclear pore complex proteins. J. Cell Biol. **119:**705–723.
270. **Wilken, N., U. Kossner, J. L. Senécal, U. Scheer, and M. C. Dabauvalle.** 1993. Nup180, a novel nuclear pore complex protein localizing to the cytoplasmic ring and associated fibrils. J. Cell Biol. **123:**1345–1354.
271. **Wilken, N., J. L. Senecal, U. Scheer, and M. C. Daubavalle.** 1995. Localization of the Ran-GTP binding protein RanBP2 at the cytoplasmic side of the nuclear pore complex. Eur. J. Cell Sci. **68:**211–219.
272. **Wilson, S. M., K. V. Datar, M. R. Paddy, J. R. Swedlow, and M. Swanson.** 1994. Characterization of nuclear polyadenylated RNA-binding proteins in *Saccharomyces cerevisiae*. J. Cell Biol. **127:**1173–1184.
273. **Wimmer, C., V. Doye, P. Grandi, U. Nehrbass, and E. C. Hurt.** 1992. A new subclass of nucleoporins that functionally interact with nuclear pore protein NSP1. EMBO J. **11:**5051–5061.
274. **Wong, D. H., A. H. Corbett, H. M. Kent, M. Stewart, and P. A. Silver.** Interaction between the small GTPase Ran/Gsp1p and Ntf2p is required for nuclear transport. Mol. Cell. Biol., in press.
275. **Wozniak, R. W., E. Bartnik, and G. Blobel.** 1989. Primary structure analysis of an integral membrane glycoprotein of the nuclear pore. J. Cell Biol. **108:**2083–2092.
276. **Wozniak, R. W., G. Blobel, and M. P. Rout.** 1994. POM152 is an integral protein of the pore membrane domain of the yeast nuclear envelope. J. Cell Biol. **125:**31–42.
277. **Wu, J., J. Matunis, D. Kraemer, G. Blobel, and E. Coutavas.** 1995. Nup358, a cytoplasmically exposed nucleoporin with peptide repeats, Ran-GTP binding sites, zinc fingers, a cyclophilin A homologous domain, and a leucine-rich region. J. Biol. Chem. **270:**14209–14213.
278. **Yano, R., M. Oakes, M. M. Tabb, and M. Nomura.** 1992. Cloning and characterization of SRP1, a suppressor of temperature-sensitive RNA polymerase I mutations in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **12:**5640–5651.
279. **Yano, R., M. L. Oakes, M. M. Tabb, and M. Nomura.** 1994. Yeast Srp1p has homology to armadillo/plakoglobin/β-catenin and participates in apparently multiple nuclear functions including the maintenance of the nucleolar structure. Proc. Natl. Acad. Sci. USA **91:**688.
280. **Yokoyama, N., N. Hayashi, T. Seki, N. Panté, T. Ohba, K. Nishii, K. Kuma, T. Hayashida, T. Miyata, U. Aebi, M. Fukui, and T. Nishimoto.** 1995. A giant nucleopore protein that binds Ran/TC4. Nature **376:**184–188.
281. **Zasloff, M., M. Rosenberg, and T. Santos.** 1982. Impaired nuclear transport of a human variant tRNAiMet. Nature **300:**81–84.
282. **Zasloff, M., T. Santos, P. Romeo, and M. Rosenberg.** 1982. Transcription and precursor processing of normal and mutant human tRNAiMet genes in a homologous cell-free system. J. Biol. Chem. **257:**7857–7863.