

Regulation of Protein Transport to the Nucleus: Central Role of Phosphorylation

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Jans, David A., and Stefan Hübner. Regulation of Protein Transport to the Nucleus: Central Role of Phosphorylation. *Physiol. Rev.* 76: 651–685, 1996.—Nuclear protein transport is integral to eukaryotic cell processes such as differentiation, transformation, and the control of gene expression. Although the targeting role of nuclear localization signals (NLSs) has been known for some time, more recent results indicate that NLS-dependent nuclear protein import is precisely regulated. Phosphorylation appears to be the main mechanism controlling the nuclear transport of a number of proteins, including transcription factors such as NF κ B, c-*rel*, dorsal, and SWI5 from yeast. Cytoplasmic retention factors, intra- and intermolecular NLS masking, and NLS masking by phosphorylation are some of the mechanisms by which phosphorylation specifically regulates nuclear transport. Even nuclear localization of the archetypal NLS-containing simian virus 40 large tumor antigen (T-ag) is regulated, namely by the “CcN motif,” which comprises the T-ag NLS (“N”) determining ultimate subcellular destination, a casein kinase II site (“C”) 13 amino acids NH₂-terminal to the NLS modulating the rate of nuclear import, and a cyclin-dependent kinase site (“c”) adjacent to the NLS regulating the maximal level of nuclear accumulation. The CcN motif appears to be a special form of phosphorylation-regulated NLS (prNLS), where phosphorylation at site(s) close to the NLS specifically regulates NLS function. The regulation of nuclear transport through phosphorylation and prNLSs appears to be common in eukaryotic cells from yeast and plants to higher mammals.

I. INTRODUCTION

Since its identification as early as the 17th century by Leeuwenhoek, the nucleus has proved the object of exhaustive study at all levels and using a variety of techniques. Possession of a nucleus (karyon) by a eukaryotic cell is now known to have far-reaching implications for cellular function, since it essentially means that the genetic information, the DNA, is separated from the site of protein synthesis, the cytoplasm. Separation is effected by a double membrane structure, the nuclear envelope, which is both contiguous with the endoplasmic reticulum (ER) and largely ER-like in terms of lipid and enzyme composition. Because gene transcription and translation take place in separate subcellular compartments, specific transport events must occur: 1) mRNA must make its way from the nucleus into the cytoplasm to be translated into protein, 2) the proteins that are required in the nucleus need to be specifically transported from their site of synthesis in the cytoplasm into the nucleus. Nucleocytoplasmic transport processes in both directions are precisely controlled, whereby the regulation of the subcellular distribution of proteins that regulate gene transcription, RNA processing, or other nuclear events is integral to many cellular processes (147). In a signal transduction context, any growth factor or hormonal signal that affects gene expression must by definition be communicated to the nucleus, which usually involves a protein translocation event. The nuclear localization of many transcription factors (TFs), morphogens, and oncogene products very precisely accompanies changes in the differentiation or metabolic state of eukaryotic cells, indicating that nuclear protein import is a key control point in regulating gene expression and signal transduction (147).

This review seeks to describe what is known about protein transport to the nucleus, dealing with the components of the transport apparatus that have been identified, including those of the nuclear pore complex, nuclear localization sequences, and the proteins that interact with them, and concentrating in particular on the mechanisms regulating signal-dependent nuclear localization. The latter is the means by which particular proteins are conditionally localized in the nucleus, as required, in response to passage through the cell cycle or differentiation-inducing or proliferative signals.

II. THE NUCLEUS

A. Nuclear Pore Complex as a Molecular Sieve

All passive and active transport into and out of the nucleus occurs through the nuclear pore complex (NPC) (8, 124, 337) present in the nuclear envelope. The structure and function of the NPC appears to be conserved in

all eukaryotes, indicating its central role in eukaryote cell function. It is composed of at least 30 distinct protein components in varying stoichiometries and has a near organelle-like mass of $\sim 10^5$ kDa. Between 10^2 and 5×10^7 NPCs are present per nucleus depending on the cellular metabolic and differentiation state. This corresponds to a range of from ~ 3 NPCs/ μm^2 in the nuclear envelope of an inactive oocyte to $\sim 15-20/\mu\text{m}^2$ in that of normal metabolically active differentiated somatic cells. As its name suggests, the NPC has a porelike, molecular sieve function, whereby molecules smaller than 40–45 kDa can apparently diffuse freely into and out of the nucleus. Proteins larger than 45 kDa require a nuclear localization signal (NLS) (118, 155, 156, 177) to be targeted specifically to the nucleus. NLS-dependent protein transport is ATP dependent and temperature dependent (244, 281) and can be inhibited by the lectin wheat germ agglutinin (WGA) (51, 83), which binds to NPC proteins. Antibodies to NPC components block nuclear protein import (12, 50, 77, 106, 258), reinforcing the idea that the NPC is the sole path of protein entry into the nucleus. The NPC pore properties appear to be able to be regulated in response to mitogenic signals (78), probably mediated by the phosphorylation of NPC components (204) or by effects on Ca^{2+} concentrations within the ER and nuclear envelope lumen (see Ref. 107).

B. Nuclear Pore Complex Structure

A number of models of NPC structure have been proposed based largely on electron microscopic (EM) techniques (e.g., Refs. 9, 10). All have to account for the molecular exclusion properties of the NPC as well as its ability to transport much larger molecules (e.g., 970-kDa immunoglobulin M molecules; Ref. 393) in an NLS-dependent fashion. Most models suggest an octagonal rotational symmetry for the 100-nm diameter complex, with two concentric ring structures on the cytoplasmic and nucleoplasmic sides of the nuclear envelope and a central pore of ~ 90 Å in diameter through which active nucleocytoplasmonic transport takes place (9, 10). The eight circular granules that line the cytoplasmic ring have been hypothesized to be the remnants of fibrils compacted during sample preparation (see references in Ref. 8). Figure 1 is a representation of the NPC, showing its relationship to the nuclear lamina and nuclear lattice as well as some of its components.

Electron microscopic data give no hint as to the mechanism by which the central channel opens to allow the passage of molecules up to 40 nm in diameter. Even though molecular chaperone proteins have been implicated in playing a role in nuclear protein transport (141, 316, 387), unfolding of the transport substrate is clearly not the mechanism for this, since very large (280 Å diame-

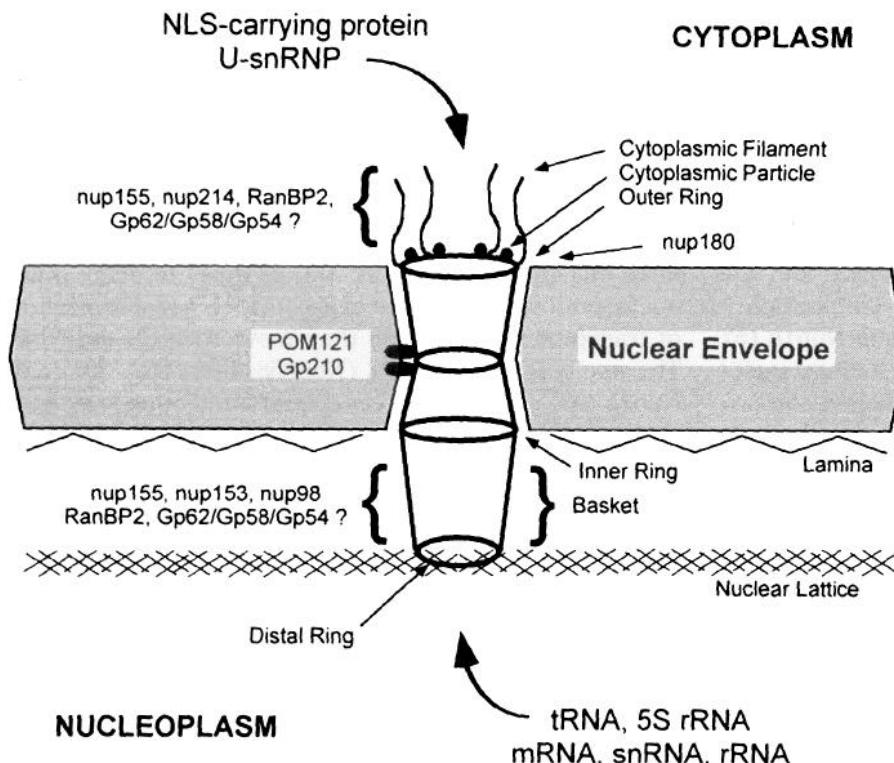


FIG. 1. Nuclear pore complex (NPC) and its relationship to nuclear envelope, nuclear lamina, and nuclear lattice is shown (see text), together with location of selected components of mammalian NPC (see Ref. 74) and direction of transport of various molecules through the NPC. NLS, nuclear localization signal; snRNP, small nuclear ribonucleoprotein particle.

ter) colloidal gold molecules can be transported into the nucleus if covalently labeled with specific NLS-bearing molecules (69, 79). A double iris/camera shutter-like mechanism (9, 124) has been proposed, whereby ATP-dependent stretching and contraction of NPC components in an actin-myosin-like fashion may constitute the mechanistic basis of the opening of the pore during active nuclear transport (10). Indeed, several nuclear envelope-associated ATPases have been identified (22, 23, 309), including a 40- to 46-kDa ATPase that is important for RNA export from the nucleus (309).

An alternative model for transport through the NPC relies in part on the refinement of both sample preparation and EM image averaging techniques. This has elaborated the model of NPC structure to include fibrillar structures attached to the cytoplasmic ring and basketlike structures on the nucleoplasmic side of the pore, which appear to be attached to a smaller ring structure connected to a nuclear lattice of unknown composition (152; see Fig. 1). This has encouraged support for solid phase models of nuclear transport (see Ref. 8), where transport is purported to be directed along a network of filaments mediated by NLS binding proteins (NLSBPs). Movement of transport protein substrates along the filaments may be through molecules analogous to kinesin molecules that are involved in the directed ATP-dependent movement of organelles and vesicles in neuronal cells (e.g., Ref. 313). Immunoelectron microscopic data for the NLSBP Nopp140, which shuttles between nucleus and cytoplasm,

has been interpreted as representing direct evidence for linear tracks of protein movement through the NPC (218).

C. Components of the Nuclear Pore Complex

Quite a few of the multiple protein components constituting the NPC have been characterized at the molecular level (see Ref. 74). These include the nuclear envelope-anchored concanavalin A-binding high-mannose Gp210 protein (106, 378), most of whose mass is located within the nuclear envelope lumen adjacent to the NPC. Its function appears to be to anchor the NPC in the nuclear envelope, with a putative role in fusing the inner and outer nuclear membranes during NPC assembly (106, 378). The 121-kDa WGA binding protein POM121 also appears to be localized in the pore membrane region (119). A further anchoring or attachment role appears to be played by nup180, which is located at the cytoplasmic ring of the NPC and appears to constitute the link between it and the cytoskeleton (376). The signal for specific sorting of Gp210 to the NPC membrane has been shown to be contained within the single transmembrane segment of the molecule (379).

Approximately 5–10% of the NPC mass is made up of a class of peripheral, non-membrane-anchored NPC protein components called nucleoporins. They possess O-linked glycosidic *N*-acetylglucosamine moieties, which are the basis of the WGA-mediated inhibition of nuclear

protein transport, and appear to complex with Gp210. Nuclear pore complexes depleted of WGA-binding proteins are inactive in terms of nuclear protein import (81); as mentioned above, antibodies to nucleoporins reversibly block nuclear protein import (12, 50, 54, 77, 106, 391).

Nucleoporins identified by molecular cloning appear to form two basic classes (see Ref. 74) based on the presence of repeat units (F-X-F-G and G-L-F-G, respectively, where the single letter amino acid code is used and X is any amino acid). The Gp62 protein (35, 333), which is related to the yeast nucleoporins Nsp1p and Nup1p (57, 241), has a distinctive primary structure including a region of heptad hydrophobic repeats similar to coiled-coil proteins such as myosin, which may have functional significance in the context of the models of transport through the NPC. Like Nsp1p and Nup1p (103, 377), Gp62 associates with other nucleoporins and, in particular, forms a stable hetero-oligomeric complex with Gp54 and Gp58 that functions in transport through the NPC (8, 82). A cross-linking approach has demonstrated a WGA-inhibitable complex of Gp62 with mRNA (54), implying that Gp62 also plays a role in mRNA export. This is supported by the fact that antibodies to Gp62 reduce the rate of mRNA export from the nucleus (54). Other nucleoporins purported to have a role in RNA export include nup153 (8, 338); the yeast nucleoporins Nup145p (73), Nup116p (73, 373, 374, 377), and Nup133p (66); and nup159/rat7p (101), both of which also appear to be important in NPC assembly.

The COOH-termini of both Nup145p and Nsp1p are capable of targeting normally cytosolic carrier proteins to the nuclear periphery, implying that they contain specific signals for localization to the NPC (73, 138). In contrast, deletion analysis indicates that the NH₂-terminal of Nup1p contains its NPC targeting signal (28), whilst heptad repeat sequences appear to be responsible for localization to the NPC of the yeast nucleoporin Nic96 (104). The zinc finger-containing nup153 appears to be localized exclusively on the nucleoplasmic side of the NPC, possibly associated with the nucleoplasmic basket structures (8, 338; see Fig. 1).

The distinctive structure of nucleoporins has encouraged speculation that the coiled-coil COOH-terminal domains of Gp62 and Nsp1p (together with their complexing proteins; Refs. 82, 103, 377) may constitute part of the fibrillar structures on the cytoplasmic side of the NPC while the central parts of the molecules may function as hinge domains together with the glycosylated NH₂-termini to "open" and "close" the central pore (see Ref. 8). Recent work from Blobel and co-workers (168, 276) has elaborated the role of the nucleoporins in docking transport substrate complexes, whereby the nucleoporins nup214 (on the cytoplasmic side of the NPC) as well as nup153 and nup98 (on the nucleoplasmic side of the NPC) appear to be directly involved (see sect. IVB2; Refs. 234,

235, 275). Similar results have been obtained for the P-S-F-G, P-A-S-G, and S-A-F-G repeat-containing yeast nucleoporin Nup159 (localized on the cytoplasmic side of the NPC), whose central repeat-containing domain is necessary for the nucleoporin's docking role (168). Radu et al. (276) have similarly shown that the G-L-F-G and F-X-F-G repeat-containing NH₂-terminal of nup98 is necessary for docking transport substrates at the NPC and speculate that these repeats, as well as those in many other nucleoporins, are the sites of association and dissociation of transport substrates as they move through the NPC. The nucleoporins RanBP2 (Nup358; Refs. 380, 391) and Nup2p (63) appear to be capable of interacting with Ran/TC4, which plays a direct role in translocation from the NPC outer surface to the nucleoplasm (see sect. IVB3; Refs. 63, 380, 391), while Gp62 binds a protein that interacts with Ran/TC4 (see sect. IVB3; Ref. 259). Other NPC components proposed to play a direct role in nuclear protein import include Nic96 (103, 104), Gp210 (106, 378, 379), and Nup145p (73). RanBP2 also appears able to bind factors that dock transport substrates at the NPC (see sect. IVB2; Ref. 235). Significantly, nup214 was originally identified as CAN, a putative oncogene implicated in myeloid leukemogenesis (167), stressing the central role of the NPC and its components in cellular processes such as transformation.

III. SIGNALS FOR NUCLEAR TARGETING

A. Nuclear Localization Signals

Nuclear localization signals (118, 156, 177) are the short peptide sequences that are necessary and sufficient for nuclear localization of their respective proteins (Tables 1 and 2). These sequences have been identified based on two basic criteria: 1) mutation or deletion of the NLS leads to cytoplasmic localization of the protein in question, and 2) the NLS is active in nuclear targeting of a normally cytoplasmic localized carrier protein, either as a peptide covalently coupled to the carrier or when encoded in the same reading frame as a fusion protein.

Nuclear localization sequences are regarded as functioning via recognition/ligand-receptor-like interactions (e.g., with NLSBPs) and not through DNA or histone binding (e.g., Refs. 48, 117, 165, 203, 361). That the NLS is an entry rather than a retention signal has been shown by the fact that NLS-deficient carrier proteins microinjected into the nucleus remain nuclear (203, 306). Photobleaching experiments show that nuclear accumulated NLS-containing proteins are highly laterally mobile (284), implying that binding in the nucleus to chromatin or other structures such as the nucleoskeleton is unlikely to be the mechanism by which NLSs function (65, 124, 337; see however Refs. 174, 306 and references in Ref. 8). Nuclear

TABLE 1. Nuclear localization signals in nuclear proteins

Protein	NLS	Critical Residue	Carrier Proteins Targeted
SV40 large tumor antigen	PKKKRKV ¹³² (38, 155, 156, 178)	K ¹²⁸	PK/BSA/IgG/βG/CSA
Polyoma T	VSRKRP ¹⁹ (38, 282)		PK/CSA (not PK or CSA)
Lamin L ₁	PPKKARED ²⁸⁶ (38, 282)		CSA
Lamin A/C	VRTTKGKRKRIDV ⁴²⁰ (38, 170)	K ⁴¹⁷	
Cofilin	SVTKKRKLE ⁴²² (199)		BSA
Human c-myc	PEEVKKRKAV ³⁶ (2)		PK/HSA/CSA
Ad7 E1a	PAAKRVKLD ³²⁸ (38, 52)		PK
SV40 VP1	RQRRNELKRSF ³⁷⁴ (38, 52)		IgG/GK/CSA
SV40 VP2/3	KRPRP ²⁸⁹ (38, 178, 203)		POL (not PK or CSA)
Human p53	APTKRKGS ⁸ (38, 382)	K ³²⁰	POL/PK/BSA/βG/IgG
NFκB p50	PNKKRK ³²³ (43)	K ³²⁰	PK/βG (not CSA)
NFκB p65	PQPKKKPL ³²³ (38, 53, 311)	K ³⁶⁹ R ³⁷⁰	
Chicken v-rel	QRKRQK ¹⁷² (27, 130)		
Mouse c-abl IV	EEKRKR ²⁸⁶ (394)		
Influenza virus NS1	KSKKQK ²⁹⁵ (95)	K ²⁹² K ²⁹³	βG
Hepatitis virus delta antigen	SALIKKKKKMAP ⁶³¹ (301, 359)		(not βG)
chicken c-ets-1	DRLRR ³⁸ (108)		(not βG)
v-Jun	PKQKKRK ²²¹ (108)		
Ribosomal protein L29	RKLKKKKKKL ⁴⁴ (37)		
Human DNA ligase I	PRKRP ⁸⁹ (37)		
Human hnRNP B1	GKRKNKPK ⁸⁹ (31)		
Human hnRNP A1	KSRKRKL ²⁸³ (41, 340)	R ⁸	IgG
Yeast histone 2B	KTRKHG ¹² (354)	R ²⁵	βG
Monkey v-sis	KHRKHPG ²⁹ (354)	R ¹³⁰ R ¹³¹	βG
Human PDGF-A (longer form) ^c	PKRRTARKQLPKRT ¹³² (226)		CAT
Mouse Mx1	KTLETVPPLERKKREK ¹⁷ (369)		CAT
Prothymosin α	NDFGMYNNQSSNNFGPMKGGNFGGRSSGPY ²⁸⁹ a		
VirD2 protein (octopine, <i>Agrobacterium tumefaciens</i>)	GKKRSKAK ³⁶ (232)	K ³¹	βG
Maize R protein	RTVTIRTVRVRRPPKGKHRK ²⁵⁵ (123) ^b		PK
MyoD	RESGKKRKRKRLKPT ²⁰⁷ (206)	R ⁶¹⁴	PK/CAT/DHFR
Serum response factor	REKKKFLKRR ⁶¹⁵ (400)		
CaM δB ^d	TKKQKT ¹⁰⁷ (209)		βG
Yeast Mata2	EYLSRKKGKLEL ³⁸ (346) ^d		βG
Influenza virus nucleoprotein	GDRRAAAPARP ¹⁰⁹ (317) ^e		βG/GLUC
Hepatitis B virus core protein	MSEKRKRREKL ²⁴⁸ e		
	MISESLRKAIKGKR ⁶¹⁰ e		
	CKRKTNTADRRK ¹¹² (357)		
	CVNEAFETLKRC ¹³⁵ (357)		
	RRGLKR ¹⁰⁰ (278)		
	AKKPDGVKKRKS ³³² (331)	K ³²⁸ R ³²⁹	
	NKIPIKDLLNPQ ¹⁸ (38, 117, 118, 179)		
	VRILESWFAKNIENPYLDT ¹⁵⁹ (117)		
	AAFEDDLRVL ³⁴⁵ (55)		
	SKCLGWLWG ²⁹ (256)		

Critical residue is residue shown by mutation analysis to be essential for nuclear localization signal (NLS) function. Carrier proteins targeted refers to carrier proteins that have been shown to be able to be targeted to nucleus by NLS either as a fusion protein derivative or peptide covalently coupled to the carrier (see Refs. 91, 124, 287). PK, pyruvate kinase; IgG, immunoglobulin; βG, β-galactosidase; CSA, chicken serum albumin; HSA, human serum albumin; GK, galactokinase; POL, polio virus VP1; α₁G, α₁-globin; CAT, chloramphenicol acetyltransferase; DHFR dihydrofolate reductase; GLUC, β-glucuronidase; SV40, simian virus 40; hnRNP, heterogeneous nuclear ribonucleoprotein particle. ^a A homologous sequence is found in human hnRNP A2 (amino acids 289–319) and *Xenopus laevis* hnRNP A1 (amino acids 313–344) (see Ref. 369). ^b Two copies are necessary to target PK to nucleus. The v-sis NLS is also present in platelet-derived growth factor (PDGF)-B related c-sis protooncogene (123, 154, 206). ^c Alternately spliced longer form of PDGF-A (206). ^d Bacterial NLS capable of nuclear targeting in plant and yeast cells. ^e Two of the three R protein NLSs are necessary for nuclear localization (317). ^f Alternatively spliced form (331).

localization sequences are fundamentally different from other peptide signal or targeting sequences in that they are not cleaved during transport. This is because, in contrast to ER and other targeting signals, NLSs are required to function many times, through a number of cell divisions, which in the case of most eukaryotes involves dissolution of the nuclear envelope (8, 124, 337). Breeuwel

and Goldfarb (32) showed that the addition of an NLS to a small protein can cause its transport to the nucleus to be temperature and ATP dependent, properties of NLS-dependent transport. This implies that an NLS confers upon the protein carrying it the specific regulatory properties associated with active rather than passive transport.

As can be seen in Table 1, there is no general consen-

TABLE 2. Bipartite nuclear localization signals in nuclear proteins

Protein	NLS	Carrier Proteins Targeted
Human		
Poly(ADP-ribose) polymerase	KRK GDEVDGVDEVAKKKSKK ²²⁶ (308)	β G
c-fos	KRR IIRRERNKMAAKCRNKKRRL ¹⁶¹ (352)	PK
SRY	KRP MNAFIVWSRDQRRK ⁷⁷ (273)	IgG/ β G
HSF2	KRK VSSSKPEENKIR ¹²² (314)	
	KRK RPLLNNTNGAQKK ²¹⁰ (314)	
Steroid hormone receptors (human)		
Glucocorticoid	RK CQAGMNLEARKT KK ⁴⁹⁵ (33, 267, 268)*	β G
Progesterone	RK CCQAGMVLGGGRKF KK ⁴⁹⁵ (390)*	
Androgen	RKC YEAGMTL GARKL KK ⁴⁴⁸	
Estrogen	RKC YEVGM MMKGGRK D ⁴⁹⁵ (266, 390)*	
Erb-A	RKL AKR KLIEENRE KRR ¹⁹⁶	
Thyroid β	KRL AKR KLIEENRE KRR ¹⁹⁶	
Mouse		
p110 ^{RB1}	KRS AEGGNPPKPLKKL R ⁸⁶⁹ (395)	β G ^b
FGF3	RLLRDAGGRGGVYEHGGAP RRRK ⁷⁶⁴⁷ (161) ^c	β G
Chicken		
Nucleolin	KRK KEMANKS PEAKKK ²⁷³ (307)	
<i>Xenopus</i>		
NO38	KRAAPNAASKVPL KKTR ¹⁵³ (261, 286)	PK/ β G/IgG/CSA
Nucleoplasmin	KRP AATKKAGQAKKKL ¹⁷¹ (38, 64, 65, 178, 286)	β G
N1/N2	RKK RKTEEE PLDKDAKKSKQEP ⁵⁶⁴ (165)	
xnf ^{7d}	KRK IEEPEPEPKKAKV ¹²¹ (192)	PK
Viral		
Herpes ICP-8	RKRAFHGDDPFGE GPPD KK ¹¹⁸⁸ (90)	PK
<i>Saccharomyces cerevisiae</i>		
SWI5	KKYENVVIKRSPR KRGRPRK ⁵⁶⁶ (225)	β G
GCN4	KRARNTEA ARRSRARK ²⁴⁵ (242)	
Plants		
TGA-1A (tobacco)	KKLAQNREA ARKSRLR KK ⁹² (356)	GLUC
TGA-1B (tobacco)	KKKARLVRNRSAQLSR QRKK ³⁶⁴ (356)	GLUC
Opaque-2 (maize)	RRK LEEDLEAFKMTR ¹⁴³ (361)	
	RKR KESNRESARS RSRYRK ²⁴⁷ (362)	GLUC
<i>Agrobacterium tumefaciens</i> ^e		
VirD2 protein (octopine)	KRPRDRHD GELGGR KR R A R ⁴¹³ (136, 346)	β G
VirD2 protein (nopaline)	KRP REDDDGEPSDR KRER ⁴³⁴ (136, 346)	GLUC
VirE2 protein	KLR PEDRYIQTEKYGRREIQ KR 249 (42)	GLUC
	RAIKTKYGS DTEI KLKS ³⁰⁸ (42)	GLUC
Consensus	K/R-K/R-10–12 amino acid spacer-K/R-K/R-K/R (286)	

The two arms of basic residues of the bipartite NLS are shown in bold type. Definitions are as in Table 1. * Steroid hormone receptors contain in addition to the above bipartite NLS conferring constitutive nuclear localization, a second dominant ligand binding-dependent NLS (e.g., wild-type glucocorticoid receptor is cytoplasmic in absence of ligand; Refs. 33, 267, 268), which has also been shown to be present in progesterone (112–114) and estrogen (266) receptors. Ylikomi et al. (390) report that the situation with respect to estrogen and progesterone receptors may be more complicated, whereby 3 NLS-functioning sequences contribute to nuclear localization, in addition to the ligand binding-dependent NLS. ^b Partial nuclear localization (395). ^c Two forms of FGF3 originating from alternative start codons contain the NLS (161). ^d Homologous sequence is found in PwA (19). ^e Bacterial sequences capable of nuclear targeting in plant (and yeast) cells.

sus sequence for NLSs (61, 91, 287). The best characterized is that of the simian virus 40 (SV40) large tumor antigen (T-ag) (PKKKRKV¹³²), whereby a single amino acid substitution of N or T for the critical K¹²⁸ residue of the NLS abolishes its function and results in complete cytoplasmic localization. Although not all NLSs resemble that of T-ag, e.g., those of Mat α 2, influenza virus nucleoprotein, and hepatitis B virus core protein (and see Table 2), a number of the NLSs listed in Table 1 have been identified on the basis of homology to the T-ag NLS and not formally tested using the basic criteria above.

In the absence of a consensus NLS, several secondary structure prediction analyses have been performed (see Refs. 88, 177, 287, 288). Apart from the fact that all known

NLSs are very hydrophilic, generally preceded by a β -turn/random coil region, and highly antigenic surface structures (see Ref. 288), no definitive consensus structural motif has been identified. Secondary structure, however, is known to play a role in the nuclear localization of many NLS-bearing proteins (e.g., Refs. 323, 396), presumably through influencing the accessibility of the NLS (see sect. III D 1).

B. Multiple Nuclear Localization Signals/Bipartite Nuclear Localization Signals

A number of proteins possess two or more NLSs (e.g., polyoma large T antigen, c-myc, N1/N2, influenza virus

NS1, Mata2, and yeast ribosomal protein L29; see Table 1) that are required in concert to achieve "complete" nuclear localization (see also Refs. 236, 238, 240, 311 for p53, influenza virus PBP1 and 2, and adenovirus DNA binding protein, respectively). Multiple copies of an NLS appear to be more efficient than only one copy (65, 69, 88, 179, 288), especially in the case of a "weak" NLS; the maize R protein, for example, requires two of three NLS-functioning sequences to be nuclear (317).

Possibly a variant of multiple NLSs are bipartite NLSs (61), which consist of two series of basic residues separated by a 10- to 12-amino acid spacer (286, 308) (Table 2). Although varying the length of this spacer using an S-P-G-G insert had no effect on nuclear targeting efficiency in the case of the nucleoplasmin NLS, a more hydrophobic, bulky Q-P-W-L spacer markedly reduced its targeting efficiency (286). This implies that conformation and/or hydrophobicity may be important, perhaps for corecognition of the two arms of the NLS. Again, few of the bipartite NLS sequences shown in Table 2 have been formally tested using the criteria listed in section IIIA, but have been identified through their homology to the nucleoplasmin NLS (61, 286).

Longer NLS-functioning sequences include those of the yeast TF *gal4* (the NH₂-terminal 74 amino acids; Ref. 323), the human T-cell leukemia virus HTLV-1 Tax transactivator protein (amino acids 2–59; 329), the small nuclear ribonucleoprotein particle (snRNP) U1A protein (amino acids 94–204; Ref. 158), and the stress-induced chaperone HSC70 (208). Probably as a result of secondary structure influencing NLS accessibility, multiple mutation sites/deletions affect the nuclear localization of the latter two proteins. More complex NLSs include that of the nerve growth factor-induced TF NGF1-A (also known as Egr1 or Krox24), whose three C₂H₂-type zinc fingers (amino acids 328–433) within the DNA binding domain are necessary for nuclear localization (213). Homologous sequences are found in the TFs Egr3, Krox20, and NGF1-C, although NLS activity has only been demonstrated in the latter case (see Ref. 213). The influenza virus polymerase PA subunit has two regions responsible for its nuclear localization (amino acids 124–139 and 186–247) (246). Finally, it appears that specific glycosylation signals may function as NLSs under certain circumstances through a distinct pathway that is ATP dependent and temperature dependent and inhibitable by WGA, but not competed by peptide NLS-containing protein carriers (67, 68).

The size of the carrier used to test targeting efficiency of an NLS can be important, as indicated by studies with the *gal4* NLS in which amino acids 1–74 were shown to be necessary to target a β -galactosidase fusion protein (~480 kDa) to the nucleus, whereas only residues 1–29 are needed to target invertase (~120 kDa) (242). Similar results have been reported by Yoneda et al. (393) for T-ag (94 kDa), where the NLS alone (amino acids 126–132)

is sufficient to target bovine serum albumin (BSA; 67 kDa) to the nucleus, but not to target immunoglobulin M (~970 kDa). Larger carrier proteins presumably have more stringent targeting requirements than smaller proteins and probably approximate the *in vivo* situation more realistically.

C. Small Nuclear Ribonucleoprotein Particles

Small nuclear ribonucleoprotein particles are protein-RNA complexes, whereby the RNA component (the snRNA) is first exported into the cytoplasm where it associates with specific proteins before reaccumulation in the nucleus. The spliceosomal snRNAs U1, U2, U4, and U5 share two common structural features: a single-stranded uridine-rich sequence flanked by two hairpin loops called the Sm site and a 2,2,7-trimethylguanosine (m₃G) cap (see Refs. 84, 210, 223). Variants of this include the U3 snRNP, which contains an m₃G cap but no Sm site, and the U6 snRNP, which lacks both the Sm site and m₃G cap (see Refs. 210, 223). Newly transcribed snRNAs are initially capped with a 7-methylguanosine (m⁷G) cap structure that constitutes part of the nuclear export signal (122). Once in the cytoplasm, a set of "Sm proteins" binds to the snRNA to constitute the Sm core domain, and the m⁷G cap structure is hypermethylated to a m₃G cap. Additional snRNP proteins are then added before, during, or after nuclear import.

Although both protein and snRNP nuclear import exhibit saturation kinetics (see Refs. 97, 223), are inhibited by antibodies to NPC components (e.g., Refs. 210, 223), and are ATP dependent and temperature dependent (e.g., Ref. 210), they differ in their sensitivity to WGA (84, 158, 223). Whereas transport of the U1A protein is inhibited by WGA, nuclear import of the U1 snRNP is not (158; see Ref. 210). In contrast to transport of U1–5, U6 snRNP nuclear import can be competed by BSA carrying the T-ag NLS (223). Microinjection studies in *Xenopus* oocytes indicate that the U1, U2, U4, and U5 snRNPs possess a complex bipartite NLS composed of both the Sm-core domain and the m₃G cap (87, 121, 223). Their import can be competed by excess m₃GpppG dinucleotide (87, 223); however, while U1 and U2 snRNAs appear to have an absolute requirement for the m₃G cap, U4 and U5 snRNAs can enter the nucleus as AppG-capped derivatives, albeit with slower import kinetics (84). That snRNP component proteins both contain functional NLSs and are capable of nuclear import independent of snRNA binding has been shown for the U2 snRNP protein U2B (157) and the 70-kDa component of U1 (289, 290). However, in both cases, the NLS functions appear to be linked to the RNA-binding domains of the respective proteins (157, 289, 290).

D. Factors Affecting Nuclear Localization Signal Function

1. Protein context and competing signals

A number of studies have shown that the possession of an NLS may not be sufficient to effect the nuclear localization of a particular protein (see Refs. 91, 287). One factor that plays a role is that of protein context, the position of the NLS within a protein (288). The general consensus is that an NLS has to be on the surface of the protein to be accessible for recognition (231, 288). Moreland et al. (231), for example, showed that the NH₂-terminal NLS (amino acids 1–21) of the yeast ribosomal L3 protein was only functional in targeting a β -galactosidase fusion protein to the nucleus when made accessible via inclusion of a G-P-G-P-G-P-G-P spacer. There are several examples of proteins containing NLSs that are functional only in certain protein contexts. The best characterized is the NF κ B p50/105 protein, whereby the p50 comprises the NH₂-terminal NLS-containing portion of the p105 protein. Whereas the p50 protein is predominantly nuclear, the p105 precursor is exclusively cytoplasmic (27, 130) through intramolecular masking of the NLS (see sect. v). This conclusion is supported by the observation that monoclonal antibodies to the NLS recognize native p50 but fail to recognize p105 unless it has been denatured (130).

A further example of NLS-containing proteins that are cytoplasmic is the oncogenic p160^{gag/v-abl} variant of mouse p150 type IV c-abl (359). Both proteins contain a functional T-ag-like NLS (KKKKMKA⁶³²), but only c-abl is nuclear (301, 359). Nuclear localization of c-abl appears to be mediated in part by c-abl residues 72–126, which are lacking from p160^{gag/v-abl}, since deletion of these residues results in predominantly cytoplasmic localization of c-abl. These residues contain no identifiable NLS, but instead constitute part of the SH3 (*src* homology-3) domain, which is responsible for association with specific protein factors such as the guanosinetriphosphatase-activating protein (GAP)-rho-like 3BP-1. A role for binding to other proteins, in concert with the c-abl-NLS, is implicated in effecting c-abl's nuclear localization (301, 359).

Signals for localization to subcellular compartments other than the nucleus can override NLSs (see Ref. 91). The T-ag NLS inserted into the sequence of the normally plasma membrane-associated polyoma middle T antigen, for example, is not functional in nuclear targeting (288). Comparable effects are seen for a SV40 T-ag frameshift mutant where an altered COOH-terminal results in an additional largely hydrophobic sequence of ~50 amino acids that overrides T-ag NLS-mediated nuclear localization (360). Analogous results have been reported for pyruvate kinase fusion proteins carrying short sequences of the c-fos gene, including the bipartite NLS and "cytoplasmic

localization" determinants (352) and for the snRNP-U1A protein (158). The hepatitis delta antigen is a further example whereby two NLSs (RKLKKKIKKL⁴⁴ and PRKR⁸⁹) are separated by a hydrophobic domain (amino acids 50–65) that impairs the function of one of the NLSs (37).

The question of dominance of signals for targeting to different subcellular compartments, and in particular of the competition between NLSs and mitochondrial localization and secretory signals, has been addressed in several studies (see Refs. 91, 161). It appears that the most NH₂-terminal signal dominates (161, 300). The yeast TRM1 protein, required in both nucleus and mitochondria for tRNA modification, interestingly appears to carry functional signals for both mitochondrial (NH₂-terminal) and nuclear (amino acids 70–213) localization, as shown by the targeting of β -galactosidase fusion proteins (189). In analogous fashion, the fibroblast growth factor class ligand FGF3 possesses a secretory signal in addition to a weak bipartite NLS (161; see Table 2).

2. Piggy back and cell type-specific effects

A number of examples exist of proteins greater than 45 kDa in molecular mass which, although lacking a functional NLS, are predominantly nuclear, whereby the mechanism appears to be via association with NLS-bearing proteins and cotransport into the nucleus ("piggy back"). The nonhistone nuclear high-mobility group protein HMG1 is able to cotransport a specifically reacting 170-kDa monoclonal antibody to the nucleus (353). Nuclear localization of NLS-defective p53 observed in T-ag-expressing COS cells (311) is presumed to be through specific complexation with T-ag and cotransport into the nucleus. Similar observations have been made for the adenovirus DNA polymerase, which is nuclear localized through association with the preterminal protein pTP1 (NLS RLPVRRRRRVP³⁷³) (398) and for the two subunits of the mammalian pancreas-specific TF PTF1, which require a third 75-kDa glycoprotein for nuclear localization (330). Nuclear localization of NLS-deleted c-fos fusion protein variants is similarly thought to be through cotransport with c-fos's NLS-containing AP-1 transcription complex partner c-jun (352). Cytoplasmically localized mutant steroid hormone receptors can be transported to the nucleus by the specifically interacting 90-kDa heat shock protein (HSP90) if the latter contains the NLS of nucleoplasmin (159); expression of wild-type progesterone receptor can lead to nuclear localization of HSP90, which is normally cytoplasmic (159). Predominant localization of the NLS-deficient cyclin-dependent kinase (cdk) cdc2 in the nucleus is believed to be through complex formation with cyclin B, which possesses three putative NLSs: PKKRHA⁶¹, SKKRRQP¹¹⁷, and PKKLKKD¹⁶⁰ (29, 255). The ability of cdc2 and other cdks to form complexes with many other nuclear factors, including p107^{Rb}, cyclin A, and E2F (e.g.,

Ref. 15), provides a number of possibilities both for their nuclear transport and for the transport of proteins and TFs that interact with them. The reverse process, that of retention of an NLS-bearing protein in the cytoplasm, has been demonstrated in the case of p53 anchored in the cytoplasm either through association with NLS-defective T-ag (45) or with nonnuclear localized mutant p53 in inactive oligomers (212).

Cell-type specific differences in terms of NLS effectiveness have been observed for T-ag (88), adenovirus DNA binding protein (236), and p53 (311). Adenosine 3',5'-cyclic monophosphate (cAMP)-dependent protein kinase (PKA) regulation of nuclear localization of rNFIL-6 (rat nuclear factor induced by interleukin-6) also appears to be cell type specific, since it localizes to the nucleus upon the elevation of intracellular cAMP levels in rat PC-12 pheochromocytoma cells, but does not do so in HeLa cells (see Ref. 222). Cell type variation has also been observed for U1 snRNPs, whereby the m₇G cap is required for U1 snRNP transport in oocytes, but not in somatic cells, where it only accelerates nuclear uptake (85). The reason for such cell type variability may relate in part to interactions with other proteins such as those mentioned above and to specific mechanisms of regulation of nuclear protein transport (see sect. v).

3. Alternative splicing

A simple strategy to regulate the nuclear import of a particular protein appears to be alternatively spliced mRNAs that either contain or lack an exon encoding an NLS. One example is the multifunctional calmodulin (CaM)-dependent protein kinase (CaMPK) kinase δ_B variant, which is nuclear in contrast to the δ_A and δ_C forms. Alternative splicing introduces an NLS (see Table 1), normally present in the sequences of the predominantly nuclear α_B and γ_A CaMPK isoforms, into the δ_B primary sequence (331). Alternative splicing mechanisms also appear to control subcellular localization of the CaMPK α -isoform (21, 331). The p85^{s6k} (s6 kinase) similarly shows nuclear localization in contrast to the alternatively spliced variant p70^{s6k}, which lacks the putative NH₂-terminal NLS (RRRRR⁷) (280). Alternative splicing and/or alternative translation start sites appear to be a common means of varying the NLS-mediated subcellular localization of a number of polypeptide hormone ligands such as those of the platelet-derived and fibroblast growth factor classes (see Refs. 123, 146, 154).

E. Signals for Localization to Subnuclear Compartments

1. Nucleolar localization signals

There is some evidence for signal-mediated targeting to subnuclear compartments such as the nucleolus. Nucle-

olar localization signals (NOSs) appear to be related to NLSs in that they also comprise short sequences of essentially basic amino acids, although this may relate to the fact that entry into the nucleus is initially required before a subnuclear compartment is encountered. An example of this is the *Xenopus* nucleolar protein NO38, which has distinct NLS (see Table 2) and NOS (present in the COOH-terminal 108 amino acids) sequences (261); chicken nucleolin is similar (307). Dang and Lee (53) have proposed a consensus NOS sequence, which comprises regions homologous to both the T-ag and polyoma T-ag NLSs (see Table 1), separated by a glutamine residue. Examples of these include the NOSs of HSP70 (FKRKHKKDISQNKRAVRR²⁸⁷), of the human immunodeficiency virus Tat protein (GRKKRRQRRRAP⁵⁹) (53), and of the HTLV-1 (p27x) Rex protein (MPKTRRRP-RRSQRKRPPPT²⁰) (188, 328). However, although such NOSs appear to be able to target heterologous proteins such as pyruvate kinase (53) and β -galactosidase (188) to the nucleolus, these appear not to be the sole type of functional NOS, with quite different sequences being responsible for the nucleolar localization of a number of other proteins.

Because no membrane structure surrounds the nucleolus, it seems likely that binding in the nucleolus is the major mechanism of localization. This contention is supported by results for the nucleolar-localized rRNA gene TF mouse upstream binding factor (mUBF; Ref. 205), which possesses an NLS (amino acids 449–480, including the KKAK⁴⁵⁶ sequence) capable of targeting β -galactosidase to the nucleus, but no identifiable NOS (205). Nucleolar localization requires sequences other than the NLS, including the first of six HMG boxes (~80–90 amino acid sequences homologous to repeat sequences of members of the HMG class of nuclear nonhistone proteins) and the acidic COOH-terminal region (205). It is suggested that the mUBF NLS targets mUBF to the nucleus, followed by sequestration in the nucleolus through binding to other proteins at the rRNA promoter mediated by the mUBF HMG boxes and acidic tail (205). Casein kinase II-specific phosphorylation within the mUBF COOH-terminal activates promoter binding possibly through increasing the affinity of these interactions (364). Nucleolar localization of both chicken nucleolin, which is dependent on both RNA-binding motifs and a G-R-rich COOH-terminal domain (307), and of Nsr1 (386), which also contains RNA recognition sequences, has also been concluded to be due to binding to specific nucleolar components.

2. Signals for targeting to other subnuclear compartments

The existence of other discrete subnuclear compartments has been proposed (see Ref. 337), which include those defined on the basis of "specific" subnuclear local-

ization of proteins involved in RNA splicing ("nuclear speckles"; Ref. 188) and snRNP-protein components ("foci"; Ref. 36). The functionally interchangeable R-S-rich regions of the nuclear speckle-localizing *Drosophila melanogaster* RNA processing regulators *Tra* and *Su(w^a)* can target β -galactosidase to nuclear speckles (188), implying that localization may be sequence specific. Such sequences appear to be unique to RNA processing regulators such as SC35 and SF2/ASF (see Ref. 36).

IV. CELLULAR NUCLEAR PROTEIN IMPORT MACHINERY

A. Systems to Measure Nuclear Protein Import

1. In vivo systems

Most studies concerned with the identification of NLSs have used transfection to introduce protein-expressing plasmid constructs into eukaryotic cells, followed by cell fixation 24–48 h later, and qualitative scoring for subcellular localization using indirect immunofluorescence. Such an approach provides no information with respect to the rate of nuclear import, and the high levels of protein expression in the cells analyzed are often not physiologically relevant, especially in the case of TFs, which are normally present in low amounts.

Some of these drawbacks can be avoided by examining the rate of transport of carrier proteins containing covalently attached multiple NLS peptides microinjected into single living mammalian cells, in conjunction with subcellular fractionation methods or immunofluorescence (32, 38, 97, 178, 393). In quantitative experiments using radiolabeled protein, Goldfarb et al. (97) showed that nuclear protein uptake is saturable. The carrier protein constructs used in this and other studies, however, have generally been far from physiological due to very high NLS-to-protein molecular weight ratios, which have been shown to be an important factor in several studies (e.g., Refs. 242, 393).

Confocal laser-scanning microscopy (CLSM) has recently come to the forefront as a quantitative means of estimating fluorescence, enabling the measurement of relative protein concentrations in different subcellular compartments (see references in Ref. 145). We have successfully combined the technique of microinjection of fluorescently labeled fusion proteins and quantitative CLSM to analyze nuclear import kinetics in single living mammalian cells and to resolve the transport process temporally (149–151, 283, 365). Kinetic analysis has confirmed the NLS dependence of nuclear protein transport (284), as well as showing that both the rate and maximal extent of NLS-dependent nuclear protein import can be precisely

regulated according to proliferative signals and the stage of the cell cycle (see sect. V).

2. In vitro systems

The establishment of in vitro or reconstituted nuclear import assay systems has greatly contributed to the understanding of nuclear transport at the molecular level (4, 5, 7, 83, 221, 230, 243, 244, 300, 310, 316, 336, 387). One approach has been to isolate nuclei from various sources, including those reconstituted in vitro from bacteriophage λ DNA (see Ref. 243) and reconstitute active NLS-dependent nuclear protein import through the addition of cytosolic extract from *Xenopus* eggs (83, 243, 244). Another has been to use adherent cells treated with the detergent digitonin, which selectively permeabilizes the cell membrane but leaves the nuclear envelope intact (4, 5, 7, 221, 230). We have established an in vitro system based on rat hepatoma cells that have been mechanically perforated (149, 365), using a modification of a procedure first used to investigate intracellular vesicle trafficking (326), and CLSM, enabling the individual steps of nuclear transport to be resolved kinetically (149). Nuclear protein import in vitro is dependent on ATP, temperature (83, 149, 243), and exogenously added cytosol (5, 7, 149) and can be inhibited by both WGA and the alkylating agent *N*-ethyl-maleimide (NEM) (5, 7, 244, 245). Reconstitution of U1 and U2 snRNP nuclear import in vitro also indicates a dependence on ATP, temperature, and cytosolic factors, whereby, consistent with in vivo observations (see sect. III D 2), there is an m₃G cap requirement when *Xenopus* oocyte-derived cytosol, in contrast to that derived from somatic cells, is used (210).

Figure 2 summarizes current knowledge concerning the events involved in NLS-dependent nuclear protein import and the specific factors mediating them. Nuclear localization signal-dependent nuclear transport in vitro involves two basic stages (227, 244, 281): those of ATP-independent "docking" or binding at the nuclear envelope/NPC and of ATP-dependent active transport into the nucleus. Both are dependent on cytosolic factors; however, the cytosolic factors required for the two steps are distinct (227, and see below). The approach of purifying factors from cytosolic extract capable of reconstituting active NLS-dependent protein import in vitro has enabled the identification and characterization at the molecular level of some of the protein factors necessary for the various steps of nuclear protein import (see sect. IV B 3).

B. Cellular Components of the Nuclear Protein Import Apparatus

1. Nuclear localization signal binding proteins

Initial attempts to characterize the cellular factors involved in nuclear protein import used the NLS as a

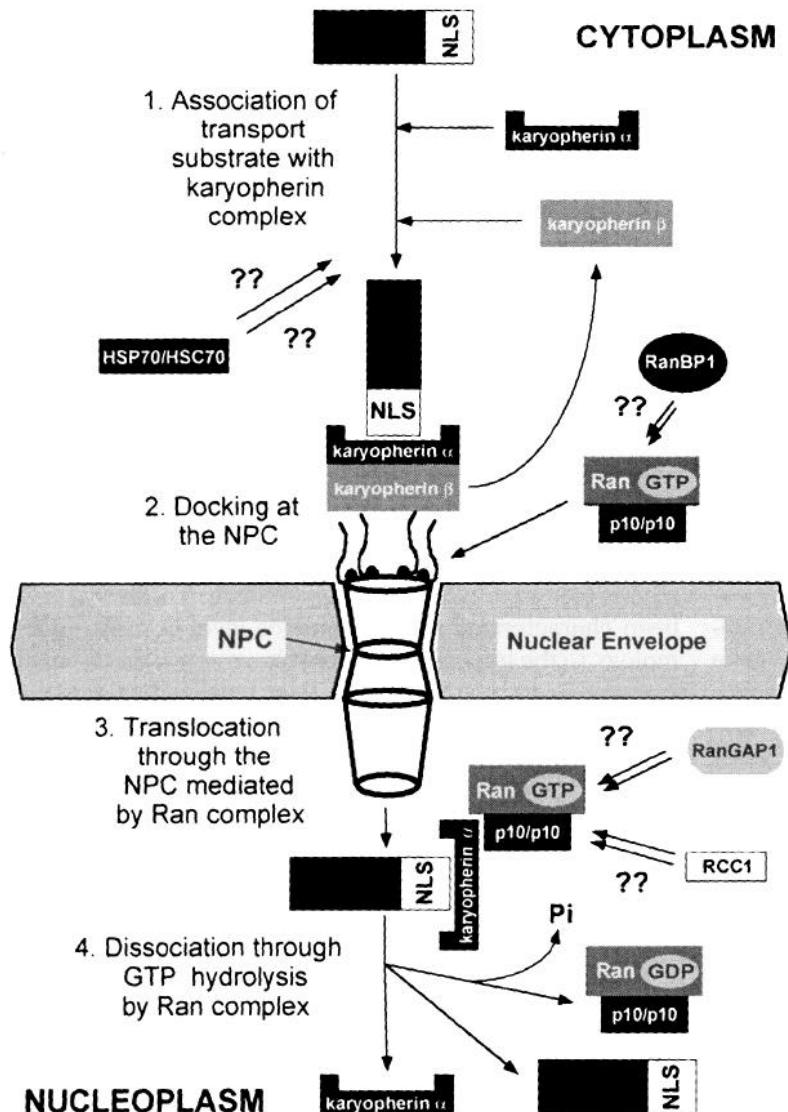


FIG. 2. Nuclear import machinery. Basic steps of nuclear protein import (essentially as proposed by Moore and Blobel; Ref. 227) are shown: 1) association with NLS-recognizing karyopherin α/β (importin 60/90) protein complex, 2) docking at NPC mediated by karyopherin β , 3) translocation through NPC mediated by association of Ran/TC4(GTP-bound)-p10 dimer complex (see Ref. 227), and 4) dissociation within the nucleus effected by GTP hydrolysis by Ran/TC4. It should be pointed out that there is no direct evidence for association or direct interaction of p10 with Ran/TC4 (see Ref. 229). Although not shown specifically in figure, association on part of both p10 (through nucleoporin Gp62; Ref. 259) and Ran/TC4 [mediated by nucleoporins RanBP2 (233, 380, 391) and Nup2 (63)] with NPC has been demonstrated. RanBP2 also appears to be capable of binding karyopherin β (Ref. 235; also not shown), probably thereby mediating interaction between docking factor and Ran/TC4-p10 dimer complexes. Although RCC1 and RanGAP1 probably play a role in nuclear regulation of Ran/TC4 GTPase activity and RanBP1 also interacts with Ran/TC4, the precise role of molecular chaperone HSP/HSC70 is unclear; it may be involved in the initial association step of transport substrate with karyopherin α (see sect. IVB4).

probe to isolate NLS-specific receptors or NLSBPs. Quite a number of these have now been identified from various sources based on cross-linking and/or ligand-blot experiments using labeled NLS peptides such as those of T-ag, histone 2B, and nucleoplasmin (see Refs. 6, 20, 141, 142, 182–184, 190, 191, 217, 218, 324, 335, 336, 383–385, 392). Perhaps surprisingly, two of the NLSBPs characterized at the molecular level, yeast p67 Nsr1 (183) and rat Nopp140 (218), appear to be nucleolarly localized. Nsr1 possesses two RNA recognition motifs and has been shown to be involved in ribosome biogenesis and pre-rRNA processing (184). The fact that Nopp140, isolated on the basis of affinity for the T-ag NLS, is largely nucleolarly localized (218) is hard to rationalize in the light of the fact that T-ag is normally excluded from the nucleolus, putting into question the physiological relevance of the binding interaction between the two. Despite this, a cytoplasm-nucleolus shuttling NLS-receptor role has been proposed for the

protein, supported by targeting of antibodies to Nopp140 to the nucleolus, apparently by a piggy-back mechanism (218). That hormonal signals may modulate nuclear protein transport through potentially regulating NLSBP activities is implied by the fact that phosphorylation may be essential for NLS binding activity by both Nsr1 and Nopp140 (183, 218), as well as for the NLSBP NBP70 from yeast (335, 336). Antibodies to the latter inhibit binding at the NPC (336), as well as nuclear import in reconstitution experiments (4), underlining its potentially important role in nuclear transport.

2. Factors mediating docking at the nuclear pore complex

Approaches to purify factors from *Xenopus* egg cytosol able to support NLS-dependent nuclear protein import *in vitro* defined two fractions (A and B), separated on the

basis of anion exchange chromatography, which together can reconstitute nuclear protein import in digitonin-permeabilized cells (227). *Fraction A* is necessary for docking at the NPC, while *fraction B* is required for translocation into the nucleus (227, 230). The role of two *fraction A* proteins in the docking process has now been definitively demonstrated (4, 98), namely, that of importin 60/karyopherin α (54/56 kDa), first identified by Adam and Gerace (5), and importin 90/karyopherin β (97 kDa), which form a heterodimeric complex (71, 98). Cloning of cDNA for the former from *Xenopus* (99) showed it to be largely homologous to a previously characterized yeast gene SRP-1 (NPI-1) (388, 389), a suppressor of an RNA polymerase mutation, and its human homologue Rch1 (46, 49). More recent cloning of human SRP-1 α (58 kDa, also called karyopherin $\alpha 1$; Ref. 370), mouse importin (140), and pendulin (encoded by the *Drosophila* overgrown hematopoietic organs-31 or *oho31* gene; see Refs. 173, 347) has revealed further members of the protein family. All possess a series of eight hydrophobic 42-amino acid repeats initially defined for the *Drosophila* segment polarity gene product armadillo (262). A family of karyopherin β proteins may also exist; thus far, cDNAs for *Xenopus* (importin 90–90 kDa) (98), bovine (40), rat (275), and yeast (importin 90H; Refs. 71, 98) homologues have been isolated. All appear to possess one armadillo repeat sequence, together with three less well-conserved sequences (40, 98).

The NLS-binding role of the importin 60/karyopherin α class of proteins is underlined by the fact that the proteins initially used to isolate SRP-1 and Rch1 using the yeast two-hybrid system have been NLS-containing proteins (46, 49, 254). Initial structural analyses indicate that four of the eight 42-amino acid repeats are necessary for binding activity on the part of Rch1, which recognizes the NLS-containing NH₂-terminal 288 amino acids of its interacting protein partner, the recombination activating protein Rag1 (46). Amino acid substitutions abolishing the nuclear targeting activity of a bipartite NLS-containing Cap-binding protein CBP80 eliminated binding of the latter to SRP-1 α , which also appears to be able to bind nucleoplasmmin (370).

The karyopherin α/β -heterodimer mediates docking of transport substrates at the NPC (71, 100) through the α -subunit's NLS binding activity and the β -subunit's affinity for specific nucleoporins such as nup214, nup153, nup98, and nup159 at both the cytoplasmic and nucleoplasmic side of the NPC (168, 234, 275; see Fig. 2). Although karyopherin α appears to enter the nucleus during transport, karyopherin β does not (100, 234). Human karyopherin $\alpha 2$ (Rch1) can function in docking at the nuclear envelope in concert with *Xenopus* importin 90, indicating the multigene family nature of these proteins (98).

An intriguing observation is that one of the importin

60/karyopherin α family members, namely pendulin, appears to be both developmentally regulated in terms of expression during *Drosophila* embryogenesis and exhibits cell cycle-dependent nuclear localization (173, 347; see Table 3). The implication of this is that different importin 60/karyopherin α forms may play roles in transporting particular proteins to the nucleus at different stages of development and/or the cell cycle, meaning that regulation of nuclear protein import may also be exerted at this level. Also of physiological significance is the fact that the gene encoding pendulin, *oho31*, is a tumor suppressor (347), underlining the central role of protein transport to the nucleus in growth regulation and deregulation.

In the light of the undisputed central role of the karyopherin/importin family proteins in mediating the docking of NLS-carrying transport substrates at the NPC as outlined here, the role in nuclear transport of the NLSBPs identified by NLS-ligand blot and other techniques (see sect. IVB1) is largely unclear. Those that have been characterized at the sequence level possess no homology to the importin/karyopherin proteins, and it seems reasonable to speculate that they may in fact be cytoplasmic retention or other protein factors (see sect. V) which regulate nuclear import before the steps of NPC docking and translocation, rather than specific NLSBP/docking proteins.

3. Factors mediating translocation through the nuclear pore complex

Although there are a number of points of contention (e.g., Refs. 98, 166, 221), the translocation step of nuclear import appears to be dependent on the 25-kDa monomeric GTP-binding protein Ran/TC4 (*fraction B1*) and its "associated" protein p10 (*fraction B2*) (229). The latter is essentially identical to the previously identified placental protein pp15 (also known as NTF2), which appears to be specifically recognized by the nucleoporin Gp62 (259). The requirement for the latter for active nuclear import does not appear to be absolute (see Refs. 98, 100) but may depend on the stage of the cell cycle; nuclei from quiescent HeLa cells apparently require pp15 for nuclear accumulation (229), whereas those from actively growing cells do not (98, 100). Whereas Ran/TC4 is predominantly nuclear, digitonin-permeabilized cells appear to be deficient of nuclear Ran/TC4 (but not of karyopherin α) (233). Exogenously added fluorescent Ran/TC4 interestingly appears to access the nucleoplasm but localizes predominantly to the nuclear envelope, indicating that there are specific Ran/TC4 binding sites at the NPC (see Ref. 233). This conclusion is supported by recent cloning of the Ran binding protein RanBP2 (391), which is a nucleoporin, and the demonstration that the yeast nucleoporin Nup2p can also interact with Ran/TC4 (63).

Apparently due to the dependence of active transport

on Ran/TC4, the nonhydrolyzable GTP analogue guanosine 5'-O-(3-thiotriphosphate) appears to inhibit nuclear import (221); however, the effect appears to depend on the cytosol used (4) and may not be physiologically relevant (see Refs. 166, 221). It has also been suggested that GTP and not ATP is necessary for active nuclear protein import (228, 229, 233; see however Ref. 4). Although association of p10 with Ran/TC4 has not been formally demonstrated, the active form of Ran/TC4 has been proposed to be a ~60-kDa complex consisting of the GTP-bound form of Ran/TC4 (305) and two molecules of p10 (229). Mutations that stabilize the GTP-bound form result in defects in both nuclear protein localization and RNA export (305). Modulators of Ran/TC4 function that regulate its GTPase activity include the Ran GTPase activating protein RanGAP1 (a 100/120-kDa dimer of 65-kDa subunits; Ref. 26) and RCC1, whose yeast homologue (PRP20) catalyzes GDP-GTP exchange on the yeast homologues of Ran/TC4 (GSP1 and GSP2). A further potential modulator of Ran/TC4 function appears to be the 24-kDa RanBP1 protein, which lacks RanGAP activity (47). Moore and Blobel (228) have proposed that the GTP-bound Ran/TC4-p10 dimer complex may associate with the karyopherin-NLS-carrying protein complex to release it from the docking complex at the NPC. Successive docking/undocking interactions with the F-X-F-G and G-L-F-G repeat sequences of nucleoporins such as nup98 are proposed to be the molecular basis of subsequent translocation through the NPC (276) while GTP hydrolysis by Ran/TC4 ultimately effects release of the transport substrate from the karyopherin/Ran/TC4-p10 dimer complex on the nucleoplasmic side of the NPC (see Fig. 2). The protein RanBP2 would appear to play a potentially central role in NPC function during nuclear protein import through being an interaction site for both Ran/TC4 (380, 391) and karyopherin β (see Ref. 235). Other nucleoporins such as Gp62, which appears to recognize pp15 specifically (259), and Nup2p, which can interact with Ran/TC4 (63), probably act in concert with RanBP2 to mediate the transition from docking at the NPC to translocation into the nucleus.

4. Molecular chaperones

In a novel approach to identify NLSBPs, Yoneda et al. (392) raised antibodies to an "anti-T-ag-NLS peptide" (D-D-D-E-D) and showed that they both bound specifically to nuclear envelopes and could block the nuclear uptake of nucleoplasmin or T-ag-NLS-BSA conjugates. The antibodies also appeared to recognize proteins binding to NLSs other than that of T-ag (142). Later experiments showed that the antibodies were specific for the stress protein/molecular chaperone HSP70 and its cytoplasmic correlate HSC70 and that other antibodies to HSC/HSP70 could also block T-ag-NLS-BSA nuclear uptake (141). Independent experiments have since shown that HSC/

HSP70 is necessary for NLS-dependent nuclear transport in reconstituted systems (316), whereas HSC/HSP70 activity in terms of in vitro nuclear protein import could be substituted by bacterially expressed HSP70 or HSC70 but not by the mitochondrial chaperone cpn60 (316). HSC/HSP70 appears to be able to bind NLSs directly (141) and hence may function as an NLSBP, but its exact role in nuclear transport would appear to be unclear at this stage, since it does not appear to be required for the nuclear import of all NLS-carrying proteins, e.g., nuclear import of the bipartite NLS-containing GR does not require HSC/HSP70 (387). It is conceivable that HSC/HSP70's role may be indirect, perhaps functioning in its molecular chaperone capacity to stabilize factors such as karyopherins during association with transport substrates, rather than through a direct NLSBP role.

A genetic approach has succeeded in implicating a role in nuclear protein transport for another molecular chaperone, the ER chaperone SEC63 (NPL1; Ref. 300), which, like HSP70, exhibits homology to the *Escherichia coli* heat shock protein Dnaj. The exact role of this protein in nuclear transport can only be speculated upon since SEC63 appears to be primarily necessary for the transport of proteins into the ER where it is exclusively localized. Of significance in this regard, however, may be the fact that the NPC component Gp210 has a consensus ER-localization signal (313), implying that some NPC proteins may be assembled via the ER.

5. Other components

Other proteins believed to be involved in the nuclear transport process include the NEM-sensitive factors NIF-1 and NIF-2, identified via reconstitution of nuclear transport activity in vitro (245). These appear not to be NLSBPs (see Ref. 337) but are necessary for transport substrate binding to the NPC of isolated rat nuclei (245). Their relationship to the factors described above has not yet been established, but it seems likely that they are related to forms of karyopherin β /importin 90. Adam and Adam (4) have demonstrated NEM inactivation of karyopherin β activity (40) but only a limited effect of NEM treatment on the 54/56-kDa NLS binding subunit (karyopherin α), thus providing the mechanistic basis of NEM sensitivity of the karyopherin-mediated docking step of nuclear transport (5, 244). In addition, there is evidence that GTP-binding proteins other than Ran/TC4 may play a role, whereby a pertussis toxin-sensitive GTP-binding protein is implicated in nuclear protein transport by digitonin-permeabilized rat liver nuclei (341).

Genetic approaches (e.g., Ref. 300) have identified a number of other genes involved in nuclear transport (see Ref. 385). One example is npl3, which appears to have a role in both protein import and rRNA maturation (89). A temperature-sensitive *Saccharomyces cerevisiae* npl3

TABLE 3. Examples of regulated nuclear protein transport

Protein	Stimulus/Kinase/Phosphatase	Effect on Nuclear Transport (Sequences Involved)
<i>Signal transduction related</i>		
T-ag	CKII ^a	CKII site increases rate of nuclear import by ~40-fold (150, 278, 283) (S ¹¹¹ S ¹¹² DDE-10 amino acid spacer- <i>PKKKRKV</i>)
NF κ B	IL-1 α ^b /TNF- α PKC/PKA	Phosphorylation (of I κ B and/or NF κ B subunits) results in unmasking of NF κ B p50/p65 NLSs and NF κ B nuclear localization (18, 93, 130, 185, 319, 345, 394) ^c (human NF κ B p50/p105 RRKS ²³⁶ DLETSE-16 amino acid spacer- <i>QRKRRQK</i> ; human NF κ B p65 RRPS ²⁶⁵ DRELSE-16 amino acid spacer- <i>EEKRKR</i> ; I κ B α putative PKA/PKC site GRPS ²⁶⁶ TR)
c-rel	PKA	PKA site enhances nuclear localization, whereby mutation of S ²²⁶ to alanine abolishes nuclear localization (237) (chicken c-rel: RRPS ²⁶⁶ -22 amino acid spacer- <i>KAKRQR</i>)
Nucleoplasmin	CKII	CKII phosphorylation increases rate of nuclear import (355) (<i>KRPAATKKAGQAKKKL</i> -5 amino acid spacer-S ¹⁷⁷ S ¹⁷⁸ EED)
GR	GH PP2A?	Hormone binding by GR releases it from a cytoplasmic complex with HSP90 and results in its nuclear localization (267). Nuclear retention of GR is impaired during G2, concomitant with a change in GR phosphorylation. Inhibition of PP2A similarly leads to inefficient nuclear retention of GH-occupied GR, as does transformation by v-mos oncogenic kinase, implying that site-specific (cell cycle-dependent) dephosphorylation of GR is involved in GH-dependent nuclear translocation (59, 137, 274) [A constitutive NLS <i>RKCLQAGMNLEARKTKK</i> (amino acids 479–495 of the rat sequence) is repressed by a second ligand binding-dependent NLS function, which includes amino acids 600–626 and 696–777] (33)
Lamin B ₂ GrH receptor	PKC GrH	PKC mediated phosphorylation inhibits nuclear import (131) (RS ⁴¹⁰ G ⁴¹¹ <i>RGKRRRIE</i>) Ligand-dependent nuclear translocation/"anchorage" of GrH receptor is accompanied by tyrosine phosphorylation (198) (Receptor residues 294–454 are essential for nuclear anchorage)
rNFIL-6	cAMP PKA?	Elevation of intracellular cAMP stimulates phosphorylation of rNFIL-6 and its translocation to nucleus (22) (<i>KPS</i> ¹⁰¹ <i>KKPS</i> ¹⁰⁵ ???)
c-fos	Serum PKA	Phosphorylation of c-fos reverses its binding to a putative inhibitor protein and retention in cytoplasm (11, 292, 351) (<i>KRRIRRIRNKMAAKCRNRRRL</i> -200 amino acid spacer—RKGS ³⁶² SS ³⁶⁴) ^d
ISGF-3	Interferon- α/β Tyk2/JAK1 (?)	Interferon-induced cytosolic tyrosine phosphorylation of ISGF-3 subunits p113 and p91/84 is required to effect their association and translocation to the nucleus (186, 304, 321) ^e (Phosphorylated site is Tyr ⁷⁰¹ in p91/84) (321)
GAF	Interferon- γ allergic reactions/growth factors JAK1/JAK2 (?)	Interferon-induced cytosolic tyrosine phosphorylation of ISGF-3 α subunit p91 (STAT-91) is required to effect its nuclear translocation (153, 294, 320, 322) ^f (Phosphorylated site is Tyr ⁷⁰¹ in p91) (321)
STAT-3	EGF/IL-6/LPS	Stimulation induces tyrosine phosphorylation and nuclear translocation of STAT-3 (p89) (296, 399)
STAT-5	PRL/EGF JAK2	Stimulation induces tyrosine phosphorylation and nuclear translocation of STAT-5 (p92) (295)
NF-AT _{p6c}	Ca ²⁺ /FK506/cyclosporin PKC (?)/PP2B	Ca ²⁺ -dependent activation of calcineurin (PP2B) results in dephosphorylation of cytoplasmic NF-AT and its nuclear localization (234, 293). Through different mechanisms, immunosuppressants FK506/cyclosporin inhibit phosphatase and thereby induce nuclear translocation of NF-AT ⁸ (Putative NLS/PKC sites: NF-AT _c KPNS ⁶⁶¹ -29 amino acid spacer- <i>GKRKRS</i> ⁶⁶⁶ Q)
SREBP-1	Oxysterols (cholesterol intake)	Sterols inhibit proteolysis of membrane-associated 125-kDa SREBP-1 precursor to a smaller soluble NLS-containing NH ₂ -terminal fragment which translocates to nucleus; phosphorylation of SREBP-1 is implicated in this process (367) (Putative NLS in amino acids 1–410)
Glucokinase RP PKA C subunit	High glucose cAMP	RP is predominantly nuclear; it is translocated to nucleus in response to high glucose (348) PKA C subunit translocates from cytoplasm to nucleus upon dissociation from PKA holoenzyme complex subsequent to cAMP binding by the regulatory (R-) subunit, ^h which plays a cytoplasmic anchor role in the absence of cAMP (219, 247, 260) ⁱ (Putative NLS: porcine C _β -subunit <i>AKRVKGRT</i> ¹⁹³ ; see Ref. 76)
p42 ^{mapk} /p44 ^{erk1}	Serum/EGF/GrH p45 ^{mapk} / PKC/p56 ^{ck}	Mitogenic stimulation results in phosphorylation, activation, and translocation to nucleus of p42/p44 MAPKs (39, 72, 111, 175) (Phosphorylation sites include Thr ¹⁸³ and Tyr ¹⁸⁵ in mouse p42 ^{mapk} ; p44 ^{erk1} has a Tyr ¹⁸⁵ but no Thr ¹⁸³ equivalent; see Ref. 72)
p90 ^{rsk}	Growth factors MAPK	Activated MAPK phosphorylates and activates RSK (rsk-gene encoded) kinases, inducing their nuclear translocation (39)
PKC- α	Phorbol ester α -Thrombin	Phorbol ester binding by the PKC 82-kD α -isoform effects conformational changes which relieve intramolecular NLS masking and result in its localization to nuclear envelope (70, 144, 180, 181, 325) (Putative NLSs: ¹⁶⁸ <i>KRGRIYLK</i> and ⁵⁷⁵ <i>KHPGKRLG</i> ; see Refs. 70, 144) ^j
PKC- β	Bryostatin I	Nuclear translocation of PKC- β can be induced by bryostatin I (134, 135)
<i>Cell cycle dependent/developmental</i>		
T-ag	cdk/cdc2	cdc2-mediated phosphorylation reduces maximal level of nuclear accumulation by ~70% through increasing affinity of binding to a putative cytoplasmic retention factor (149) (ST ²⁴² <i>PKKKRKV</i>)
SWI5	cdk/CDC28	Phosphorylation-mediated nuclear exclusion through NLS masking; mutation of <i>CDC28</i> -serines to alanine results in constitutive nuclear localization (151, 225) (S ⁵²² PSK-109 amino acid spacer- <i>KKYENVVIKRS</i> ⁶⁴⁶ <i>PRKGRGRPKDGTSSVSSS</i> ⁶⁶⁴ PIK)
c-myc	Fertilization Ca ²⁺ ?/PKC?	c-myc is stable and cytoplasmic in mature <i>Xenopus</i> oocytes but rapidly translocated to nucleus upon fertilization, concomitant with both an increase in phosphorylation and a reduction in cellular half-life (115, 162) (<i>PAAKRVLKD</i> ³²⁶)
v-jun	Serum cdk ?/p44 ^{erk1} ?	Cell cycle-dependent determination of rate of nuclear localization conferred by S ²⁴⁸ (faster during G2, when dephosphorylated) (ASKS ²⁴⁸ <i>RKRKL</i>) (41, 340) ^k
PTF1	??	Nuclear entry of the p64 and p48 subunits at early stages of pancreatic development (<i>day 15</i> in mouse embryo) through association with a glycosylated third protein p75 (265, 330)
Dorsal	Toll PKA/Ca ²⁺ ? Pelle kinase	Relocalization from cytoplasm to nucleus during development in a ventral-to-dorsal gradient, involving components of toll signaling pathway, including cytoplasmic retention factor cactus, ^l and "releasing factors" pelle kinase and the pelle activator tube. Phosphorylation of dorsal by PKA and/or pelle leads to release from cactus and nuclear localization, whereby activated toll receptor, pelle, and increased cytoplasmic Ca ²⁺ are required (94, 102, 110, 171, 250, 315, 375) (RRPS ³¹² -22 amino acid spacer- <i>RRKQRQR</i> ; cactus putative PKA/PKC site MNKS ⁴⁴ RMQ)
Lodestar Wee 1 kinase MyoD	CKII? ?? PKA	Nuclear exclusion until prophase (DESS ⁴⁰⁶ DS ⁴⁰⁸ DS ⁵⁰⁰ <i>EDDKNKKR</i>) (96) Wee 1 is nuclear in interphase cells and cytoplasmic in prophase (14) Nuclear localization only in vicinity of sites of mesoderm induction in developing embryo. PKA activation is necessary for nuclear localization, with COOH-terminal of MyoD responsible for its cytoplasmic retention (297, 358) ^m (<i>CKRKTTNADRRKATT</i> ¹¹⁶ MR and <i>CVNEAFETLKRC</i> ¹³⁶) (357)

TABLE 3—Continued

Protein	Stimulus/Kinase/Phosphatase	Effect on Nuclear Transport (Sequences Involved)
<i>Cell cycle dependent/developmental—Continued</i>		
xnf7	cdk/cdc2 (?)	Nuclear entry at the midblastula transition stage of embryonic development associated with dephosphorylation at 4 sites (192, 193, 279). A cytoplasmic retention domain within xnf7 has been defined, which is functional even in the presence of an additional NLS (193) (KT ¹⁰⁹ PQKRKIEEPEPPEPKAKV-87 amino acid spacer-CT ²⁰⁸ PVT ²¹¹ PVEKKT ²¹⁸ RP; cytoplasmic retention domain EEYYAHYVGLNRRQNEWVDKSR ²⁷⁹)
PwA33	cdk ??	Nuclear entry at the midblastula transition stage of embryonic development (19) (Putative NLS: KRKLIEDGDDQKKRKV ¹⁴⁸ ; putative cytoplasmic retention domain EEFVHYVGLNRRQNEWVDKSR ¹⁰⁰)
Cyclin B1 (B-type cyclins)	cdk ??	Nuclear entry at beginning of mitosis. Cytoplasmic retention during G ₂ is via cyclin B1 residues 109 to 154 (numbering according to human sequence; also conserved in cyclin B2). ¹ Phosphorylation within this region is implicated in release from cytoplasmic retention at onset of mitosis (269, 270)
p110 ^{RB1}	cdk/cdc2	Cell cycle-dependent hyperphosphorylation reduces nuclear association ("nuclear tethering") (343, 344) (Putative CcN motif: human T ⁶⁰¹ AADMYL ⁶⁰⁶ PVRS ⁶¹² PKKRTSTTR, where T ⁶⁰¹ is a putative CKII site and serines 608 and 612 cdk sites) ²
p53	cdk ??	Nuclear entry at G ₁ /S phase transition; nuclear exclusion during G ₁ (299, 312) (CcN motif: human SS ³¹² PQPKKKK) ³ (38, 311)
HMG1/2	??	HMG1/2 is predominantly nuclear in undifferentiated cells but largely cytoplasmic (and more highly expressed) in differentiated cells (176)
E1	cdk/cdc2 ?	E1, which exists in multiple multimeric forms, is cytoskeletal in G ₀ , cytoplasmic and nuclear in G ₁ , cytoskeletal and perinuclear in S phase, and nuclear in G ₂ . It is phosphorylated by cdc2 at G ₂ /M transition (109) (Putative NLS: SS ¹ PLSKKKRVS) (239)
Polo	??	Polo kinase is predominantly cytoplasmic during interphase, enters nucleus in prophase, and exits in telophase (197) (Putative NLS/phosphorylation sites: T ⁹¹ FEDS ⁹⁶ Q-9 amino acid spacer-KKRS ¹⁰⁹ -5 amino acid spacer-KRKKS ¹¹⁹ TT ¹²¹ EEF; see Ref. 197)
Swallow	??	Swallow is cytoplasmic during oogenesis in <i>Drosophila</i> but becomes nuclear during mitotic divisions in early embryogenesis, entering nucleus at beginning of mitosis and exiting at end of mitosis (128)
Pendulin ^m	cdk/cdc2	Pendulin is exclusively cytoplasmic during interphase in <i>Drosophila</i> embryos and translocates to nucleus in G ₂ and M phase (173, 347) (Putative NLS/cdc2 sites: S ²² RMRR-7 amino acid spacer-RKSKK ⁹⁹ -9 amino acid spacer-S ⁹⁶ PLK-5 amino acid spacer-S ⁹⁶ PVQ) (173) ⁴
Adenovirus 5 Ela protein	??	Nuclear targeting up to early neurula stage of <i>Xenopus</i> embryonic development through a "developmentally regulated" NLS (drNLS) (332) (drNLS: amino acids 142–182: FV-20 amino acid spacer-MCSLCYMRTCGMF, distinct from constitutive COOH-terminal NLS: KRP ²⁸⁵ RP)
<i>Stress/heat shock</i>		
HSP70	Heat shock/stress	Heat shock induces nuclear and nucleolar translocation of normally cytoplasmic HSP70 (171, 349) (NLS: FKRKHKKDISQNKR ²⁰⁷ R) ⁵ (53)
Cofilin	Heat shock/stress CaMKP	Heat shock induces unmasking of cofilin NLS via dephosphorylation at a CaMKP site resulting in its nuclear localization (248, 253) (RKSS ²⁴ TPEEKKRKA) (2)
HSF2	Heat shock	Heat shock induces nuclear localization of HSF2 through unmasking of 2 NLSs near NH ₂ -terminal of HSF2, which are normally masked by heptad repeat hydrophobic coiled-coil structures in NH ₂ - and COOH-terminals of molecule (314) (KRKVSSSKPPEENKIR ¹² and KRKRPLINTNGAQKK ²¹⁹)

Single letter amino acid code is used. NLSs are in italics, and phosphorylated residues are numbered according to their position in the respective primary amino acid sequence. IL-1 α , interleukin-1 α ; TNF- α , tumor necrosis factor- α ; PP2A, protein phosphatase type 2A; GH, growth hormone; Tyk2, JAK1/JAK2, nonreceptor tyrosine kinases; LPS, lipopolysaccharide; PRL, prolactin; RP, regulatory protein; MAPK, mitogen-activated protein kinase; MAPKK, MAPK kinase; PP2B, protein phosphatase type 2B (calcineurin); PTF1, pancreas-specific TFI; p110^{RB1}, product of "retinoblastoma-susceptibility factor" tumor-suppressor gene RB1; HMG, high mobility group nuclear protein; E1, ubiquitin activating enzyme 1; CaMKP, multifunctional calmodulin-dependent protein kinase. Other definitions are as in Table 1.

^a Regulation of T-ag nuclear localization by casein kinase (CKII) is included under signal transduction-related examples, since CKII is integrally involved in response to various mitogens (3, 129, 132, 200, 220, 263, 264, 271, 363). There may, however, be sufficient basal CKII activity to make rapid T-ag nuclear transport constitutive in absence of mitogenic stimulation.

^b The fact that IL-1 α is a physiological stimulus for NF κ B activation/nuclear localization is interesting in context of NF κ B, rel, and dorsal homologues, since toll receptor in dorsal pathway has homology to IL-1 receptor. Common nuclear signaling mechanisms are clearly implicated.

^c Degradation of I κ B or of p105 COOH-terminal is mechanism of NLS unmasking. Ankyrin repeats 6 and 7 of p105/I κ B γ are responsible for masking of p50-NLS. Although COOH-terminal 67 amino acids of I κ B α are responsible for NLS masking of p65, NH₂-terminal, including 2 consensus CKII phosphorylation sites (DS⁹²GLDS⁹⁶MKD), also present in I κ B γ and pp40^{rel}, may play a regulatory role (349). HTLV-1-encoded Tax protein can also induce NF κ B nuclear translocation through destabilizing the I κ B/NF κ B complex (133, 339).

^d cAMP-dependent protein kinase (PKA) site of c-fos is absent from v-fos, which is constitutively nuclear.

^e Treatments blocking tyrosine phosphorylation prevent nuclear translocation of ISGF-3 and GAF (116, 139, 186). Tyrosine phosphorylation clearly precedes subunit association and nuclear translocation (56, 116, 139, 186, 321, 322), with no nonphosphorylated p91 able to be detected in the nucleus (54); DNA binding in case of ISGF-3 appears to be mediated mainly by p48 subunit (ISGF-3 γ), which is found in both nucleus and cytoplasm and binds DNA constitutively (see Refs. 304, 312).

^f GrH and erythropoietin stimulated factors GHSF and EPOSF, respectively, which may have p91 component in common with ISGF-3 and GAF, are also induced to translocate to nucleus upon tyrosine phosphorylation (80).

^g NF-ATp/c ("p" denotes preexisting, "c", cytoplasmic; both of which are essentially identical; see Ref. 249) has a rel similarity region related to those of NF κ B and dorsal, (249), including the NLS (shown).

^h The PKA RII subunit is itself anchored to a "A-kinase anchor protein" AKAP79, which interestingly also binds calcineurin (PP2B) through a distinct binding site (44).

ⁱ PKA C subunit nuclear translocation is not dependent on catalytic activity, since it is not blocked by N-ethylmaleimide treatment (which destroys catalytic activity) or PKA-specific peptide inhibitor PK-I 5–24 (76) and is not impaired in PKA mutants possessing reduced C-subunit activity (260). The 75-amino acid PKI inhibitor protein inhibits nuclear translocation, possibly through masking putative NLS (not masked by PKI 5–24 peptide; see Ref. 76) in similar fashion to R subunit (see Ref. 75).

^j Deletion of either regulatory or kinase domains of PKC- α leads to constitutive nuclear localization, probably through NLS unmasking (70).

^k Constitutively nuclear c-jun possesses Cys²⁴⁸, which is implicated in redox-mediated regulation of c-jun's DNA binding activity (1). Phosphorylation does not affect v-jun's DNA binding capabilities (13), implying that Ser²⁴⁸ does not enhance nuclear localization through affecting DNA binding. Importantly, sequence shown is capable of conferring cell cycle-dependent nuclear localization on an IgG carrier (41, 340).

^l COOH-terminal (LQISNL¹⁷⁸SIST¹⁷⁹) of dorsal is implicated in cytoplasmic retention, since its deletion results in nuclear localization of dorsal gene product (250, 298). Proteolysis of dorsal or cactus may also be involved.

^m PKA specific inhibitors inhibit nuclear localization of MyoD, but this, according to mutagenic analysis, may not be mediated by phosphorylation sites near NLSs (see Ref. 358). Cytoplasmic retention of MyoD cannot be overridden by inclusion of T-ag NLS (297).

ⁿ Cyclin B1 residues 109–160 can induce cytoplasmic location of normally nuclear human cyclin A; cytoplasmic retention of cyclin B1, however, can be overridden by nucleoplasmic NLS (270).

^o Although not sufficient for complete nuclear localization, a "bipartite" NLS purported to participate in nuclear localization of mouse RB has been identified (see Tables 2 and 4; Ref. 395).

^p Two additional NLSs have been identified in the p53 COOH-terminal (311; see Table 1).

^q Pendulin is encoded by *Drosophila* overgrown hematopoietic organ-31 (*oho31*) gene (173, 347).

^r Mouse pendulin and SRP1, yeast SRP1, and *Xenopus* importin 60 retain homologous bipartite NLSs, although only mouse pendulin retains cdk site (see Ref. 173).

^s Homologous sequences have been identified in rat (amino acids 594–623; AV-17 amino acid spacer-MXILXYSXMF⁶¹²), mouse, and human GlucH binding domains of GlucR (see Ref. 314), known to include a part of ligand binding-dependent NLS (33, 267).

mutant accumulates RNA in the nucleus (327). The primary sequence of the protein encoded by npl3, which has the capacity to shuttle between nucleus and cytoplasm (89), includes RNA recognition motifs. The exact role in nuclear protein import of this and other gene products identified by similar approaches is unclear, but it seems reasonable to speculate that they may not be directly involved in the transport process itself, but perhaps in biosynthesis and/or regulation of the components described above, together with the NPC.

V. REGULATED (CONDITIONAL) NUCLEAR LOCALIZATION

Like other proteins, TFs regulating gene expression in the nucleus are synthesized in the cytoplasm and therefore subject to the mechanisms regulating nuclear protein import. While some proteins appear to be constitutively targeted to the nucleus such as histones, others are only conditionally targeted to the nucleus, often being preferentially in the cytoplasm (see Ref. 147). Advantages of cytoplasmic localization for a TF include the potential to control its nuclear activity through regulating its nuclear uptake and its direct accessibility to cytoplasmic signal-transducing systems. Transcription factors able to undergo inducible nuclear import include the glucocorticoid receptor (GR; Ref. 267), the α -interferon-regulated factor ISGF-3 (304), the nuclear *v-jun* oncogenic counterpart of the AP-1 transcription complex member *c-jun* (41, 340), the yeast TF SWI5 (225), the *D. melanogaster* morphogen dorsal (291, 334), and the nuclear factors NF κ B (93, 319) and NF-AT (nuclear factor of activated T cells; Ref. 196). Table 3 lists selected examples of proteins whose nuclear import is known to be regulated.

Pathways of signal transduction from extracellular signal to nucleus leading to ultimate regulation of gene expression (see Ref. 146) include that of hormone-stimulated response to elevated cAMP levels. This results in translocation of the PKA catalytic (C) subunit to the nucleus (146, 247, 260), where it phosphorylates and thereby activates nuclear TFs such as the cAMP response element binding protein. In a similar fashion, mitogen-activated protein kinases move to the nucleus in response to cellular stimulation by mitogens, while the protein kinase C α -isoform (PKC- α) translocates to the nuclear envelope upon phorbol ester or other stimulation (70, 144, 180, 181, 325, 394). In the latter case, deletion of the regulatory domain of PKC- α appears to mimic phorbol ester binding-induced conformational changes, which unmask the NLS (amino acids 310–346) (70, 144). The PKC β -isoform also appears to be capable of nuclear translocation induced by bryostatin I (130, 134, 135).

The mechanisms regulating NLS-dependent nuclear transport can be regarded as constituting a step of nuclear

transport additional to and before those of docking at the NPC and nuclear translocation dealt with in sect. IV (see Ref. 148). Although all proteins presumably undergo essentially the same recognition interactions with respect to the latter as outlined in Figure 2, the mechanisms regulating the nuclear uptake of proteins, in contrast, are highly specific to the particular protein in question.

A. Cytoplasmic Retention

One mechanism of regulating nuclear protein import is that of cytoplasmic retention, whereby a cytoplasmically localized "anchor" protein or retention factor specifically binds an NLS-containing protein and prevents it from migrating to the nucleus. Cytoplasmic retention has been described for *c-fos* (292) and also for the GR, where HSP90 complexes with and retains the GR in the cytoplasm in the absence of GH (267). Hormone binding by the GR dissociates the complex to allow NLS-dependent nuclear translocation of the receptor (267), more recent results suggesting that cell cycle-dependent dephosphorylation of the GR may also be involved (see Refs. 137, 147). Cytoplasmic retention has also been described for the B-type cyclins, which translocate to the nucleus as cyclin B-cdk complexes at the onset of mitosis (270). Cyclin B1 residues 109–160 can induce cytoplasmic location of the normally nuclear human cyclin A, indicating that these residues are responsible for B1 cytoplasmic retention; cytoplasmic retention of cyclin B1 can be overridden by the addition of the nucleoplasmic NLS. Phosphorylation has been implicated in regulating the release of cyclin B1 from cytoplasmic retention (270).

Further examples of proteins exhibiting regulation of nuclear import by cytoplasmic retention are the *Xenopus* proteins xnf7 (*Xenopus* nuclear factor 7) and PwA33, both of which are cytoplasmic until dephosphorylation at the midblastula transition stage of embryonic development when they become nuclear (19, 190, 193, 279). In conjunction with four phosphorylation sites, a 22-amino acid cytoplasmic retention domain within xnf7 (also present in PwA33; see Table 3) has been shown to be responsible for overriding the xnf7 NLS (193). This cytoplasmic retention is dominant even in the presence of an additional NLS (193), indicating that the cytoplasmic retention of xnf7, unlike that of NF κ B (see sect. vB), does not function through direct masking of the NLS.

Cytoplasmic retention also appears to be the mechanistic basis of the largely cytoplasmic localization of the developmentally regulated helix-loop-helix myogenic factor MyoD. MyoD is nuclear only in the region of mesoderm induction (297), whereby PKA activation is necessary (358). The COOH-terminal of MyoD is responsible for its cytoplasmic retention, which cannot be overridden by inclusion of the T-ag NLS (297).

As alluded to in the previous section, the PKA C-subunit moves to the nucleus upon dissociation from an inactive PKA holoenzyme complex with the PKA regulatory (R) subunit subsequent to cAMP binding by the R subunit upon hormonal stimulation (247, 260). The PKA R subunit can be regarded as playing a cytoplasmic anchor role similar to that of HSP90 and the rel/dorsal family I κ B proteins (see sect. vB), since it functions to retain the C subunit in the cytoplasm in the vicinity of the perinuclear Golgi in the case of the type II R subunit and PKA holoenzyme (260) in the absence of cAMP-mediated stimulation. Interestingly, the RII subunit itself appears to be specifically anchored to an "A-kinase anchor protein" AKAP79 (44).

Proteolysis regulates nuclear translocation of the sterol regulatory element-binding protein SREBP-1, a member of the basic-helix-loop-helix-leucine zipper TF family. In analogous fashion to NF κ B p50 (see sect. vB), SREBP-1 is synthesized as a precursor (125 kDa) which is anchored to the nuclear envelope and ER through the COOH-terminal portion of the molecule (367). Proteolytic cleavage to a smaller (68 kDa) soluble NLS-containing NH₂-terminal fragment by a calpain-like protease results in its nuclear translocation (367). Oxysterols inhibit the proteolytic cleavage, whereby phosphorylation of SREBP-1 is implicated in this process (see Ref. 367).

Kinetic analysis both *in vivo* and *in vitro* indicates that phosphorylation by *cdc2* at Thr-124 adjacent to the NLS inhibits nuclear transport of T-ag fusion proteins, drastically reducing the level of maximal nuclear accumulation (149). Cytoplasmic anchoring appears to be the basis of this inhibition, since maximal inhibition of transport of tetrameric T-ag- β -galactosidase fusion proteins (containing 4 copies of each of the NLS and the *cdc2* site) is effected by a stoichiometry of phosphorylation of only one at the *cdc2* site (149), indicating that one phosphorylated *cdc2* site is sufficient to retain the protein in the cytoplasm even in the presence of three non-*cdc2* phosphorylated sites. Phosphorylation presumably increases the affinity of the specific interaction between T-ag and the putative cytoplasmic retention factor. The inhibitory effect of *cdc2* phosphorylation of Thr-124 can be overcome by increasing the concentration of cytosolic T-ag-fusion protein (Jans, unpublished data), implying that there may be a finite titratable level of this cellular factor.

B. Cytoplasmic Retention Factors of the Rel/Dorsal Family

The protooncogene *c-rel*, the *Drosophila* morphogen dorsal and NF κ B make up a family of TFs which share a 300-amino acid region, the *rel* homology domain required for DNA binding, nuclear localization, and dimerization. Nuclear transport of these TFs appears to be specifically regulated by a family of sequence-related inhibitor proteins or cytoplasmic retention factors.

The active (nuclear) form of NF κ B, which regulates expression of the kappa light chain, is composed of the p50 and p65 (relA) protein components. A cytoplasmic anchor function has been described for the NF κ B-binding inhibitor protein I κ B in retaining the NLS-carrying NF κ B p65 subunit in an inactive complex in the cytoplasm. Phorbol ester or other treatment induces release of p65 and its migration to the nucleus (93, 319). Experiments largely performed *in vitro* imply that this can also be effected by phosphorylation either of I κ B by PKC or of p65 by PKA (98; see Ref. 250). The mechanism of cytoplasmic retention in the case of the I κ Bs appears to be through NLS masking (see sect. vC). Release from retention is effected by proteolytic degradation of I κ B, which is triggered by I κ B phosphorylation, and results in unmasking of the NF κ B p65-NLS. Inhibitor studies implicate the ubiquitin/proteasome pathway in this process (194, 224, 257, 350). There are two forms of I κ B, α and β , both of which appear to be able to bind *c-rel* as well as NF κ B p65 (345). Through their degradation in response to different stimuli, only I κ B α is responsive to phorbol esters and tumor necrosis factor (TNF)- α , whereas both I κ B forms respond to interleukin-1 and lipopolysaccharide; I κ B α and β appear to mediate transient and persistent NF κ B activation, respectively (345). Specific stimulatory signals thus regulate NF κ B activation through their ability to modulate different cytoplasmic anchor proteins, and thereby the kinetics of nuclear localization of the TF.

A role similar to that of I κ B has been proposed for the sequence-related molecule cactus, which negatively regulates nuclear localization of the *D. melanogaster* morphogen dorsal (291, 334). Phosphorylation of dorsal has been shown to effect its release from cactus and nuclear translocation (375), dorsal being constitutively nuclear in the absence of cactus (291, 334). Overexpression of dorsal in the presence of cactus can overcome cytoplasmic retention, implying that it is titratable (see Ref. 250). Other I κ B family members include I κ B γ , a discrete gene product identical to the 70-kDa COOH-terminal of the NF κ B p50 precursor p105 which may be specific to pre-B cells (143, 195, 345), and the protooncogene *bcl-3* (126, 252, 381), both of which can bind the mature NF κ B p50 subunit *in vitro* and may function to retain it in the cytoplasm *in vivo*. The pp40^{rel} avian homologue of I κ B α is implicated in retaining *c-rel* in the cytoplasm (58).

All of the I κ B/cactus family members contain five to seven ankyrin repeats, structural elements involved in protein-protein interactions; I κ B α has five, I κ B β and cactus six, and *bcl-3* and I κ B γ seven repeats. Some of these repeats appear to be directly involved in cytoplasmic retention, since deletion of ankyrin repeat 7 together with part of repeat 6 inactivates *bcl-3* binding of NF κ B p50 (381). Removal of the COOH-terminal 67 amino acids of I κ B α also eliminates binding to p65 (see Ref. 349), while the ankyrin repeats of cactus have been shown to be re-

sponsible for binding dorsal (160). Significantly, most of the I κ B family members contain a consensus PKA/PKC phosphorylation site (see Table 3) within ankyrin repeat 6, meaning that it may well be the target site of PKC phosphorylation upon phorbol ester or other stimulation. The site is absent from I κ B β , which may constitute the mechanistic basis of its activation kinetics differing from those of I κ B α (345). A regulatory role in NF κ B activation has recently been proposed for the NH₂-terminal of I κ B α , which contains two phosphorylation sites (349); these sites are also present in I κ B γ but are absent from I κ B β , cactus, and *bcl-3*. Cactus has been shown to bind to dorsal amino acids 168–350, which includes the NLS and a consensus site for PKA (see below and Table 3; Ref. 160).

Although the physiological relevance is unclear, the HTLV-1 protein Tax appears to be able to stimulate NF κ B translocation to the nucleus through destabilization of the cytoplasmic I κ B α /NF κ B complex, which does not result in I κ B α proteolysis (339). Tax has also been shown to be capable of binding to the ankyrin repeats of I κ B γ , thereby dissociating complexes with NF κ B, and has been found in stable cytoplasmic complexes with I κ B γ (133).

Observations that I κ B α can be nuclear if overexpressed (394), and that *bcl-3* appears to be predominantly nuclear, and able to piggy back NLS-deficient NF κ B p50 into the nucleus, as well as compete for NF κ B p50 in the presence of I κ B α and localize it to the nucleus (397), have encouraged speculations that cytoplasmic retention factors may in fact fulfill some sort of chaperoning function (251, 397). Even though these observations relate specifically to nonphysiological conditions of overexpressed cDNAs and hence should not be overinterpreted, it is possible that the role of cytoplasmic retention factors is more complex than their name suggests, particularly with respect to their subcellular location.

C. Nuclear Localization Signal Masking

A number of proteins possessing apparently functional NLSs are predominantly cytoplasmic due to the inaccessibility or masking of their NLSs. This may be effected through interaction with another protein (e.g., a factor binding to the NLS itself) or conformational effects whereby the NLS is masked by other parts of the molecule (147). Phosphorylation is an efficient and potentially rapidly responsive means of modulating NLS accessibility (147).

1. Intra- and intermolecular masking

It has more recently been established that masking of the NLSs of both of the NF κ B components is the mechanism by which they are retained in the cytoplasm (18, 130, 394). The COOH-terminal of the p105 precursor of NF κ B p50 (or the I κ B γ molecule; see sect. vB) appears to retain

the NF κ B p50 subunit in the cytoplasm through intramolecular masking of its NLS. Antibodies specific to the NLS recognize p50 but not p105, implying that the NLS is inaccessible in the larger precursor (130). The mechanism of unmasking of the NLS appears to be through proteolysis of the p105 COOH-terminal (27, 285), which may be proteasome mediated (257, 350).

Intermolecular masking of its NLS by I κ B is the mechanism of NF κ B p65's cytoplasmic retention (394), whereby deletion or mutation of the p65 NLS eliminates binding to I κ B (18). As mentioned above, NLS unmasking appears to be brought about through proteasome-mediated I κ B degradation (17) induced by PKC phosphorylation (probably by the ζ -isotype, which is known to be activated in response to TNF- α stimulation). Protein kinase A phosphorylation of p65 (93; see Ref. 250) can also effect the dissociation of p65 from I κ B. Nuclear localization of the NF κ B TF, summarized in Figure 3, is thus dually regulated by specific intra- and intermolecular masking of the NLSs of the two NF κ B subunits (see Ref. 147). Signal transduction-triggered phosphorylation regulates the masking events precisely to enable rapid response in terms of nuclear translocation and gene induction.

Intramolecular NLS masking appears to be the means of regulation of heat shock-induced nuclear localization of HSF2, which is normally cytoplasmic. Heat shock treatment appears to result in unmasking of two NLSs near the NH₂-terminal of HSF2 which are normally masked by NH₂- and COOH-terminal heptad repeat hydrophobic coiled-coil structures (314). Point mutations in either of two of the three NH₂-terminal or in the COOH-terminal repeat effect constitutive nuclear localization of HSF2 (314).

2. Nuclear localization signal masking by phosphorylation

Nuclear localization signal masking can also be directly effected by phosphorylation close to an NLS, which masks or inactivates it through charge or conformational effects (147). This is exemplified by cell cycle-dependent nuclear exclusion of the *S. cerevisiae* TF SWI5, which is involved in mating type switching, SWI5 nuclear exclusion being effected by phosphorylation by the cdk *CDC28* (225). Three *CDC28* sites, one of which is within the spacer of the SWI5 bipartite NLS (see Table 3), inhibit nuclear localization by inactivating or masking the SWI5-NLS (225). At anaphase, *CDC28* activity falls and SWI5 is dephosphorylated to effect its nuclear entry; removal by mutation of the *CDC28* sites results in constitutive nuclear localization (151, 225).

Nuclear localization signal-dependent nuclear transport of lamin B2 is inhibited by phosphorylation at two PKC sites NH₂-terminally adjacent to the NLS (see Table

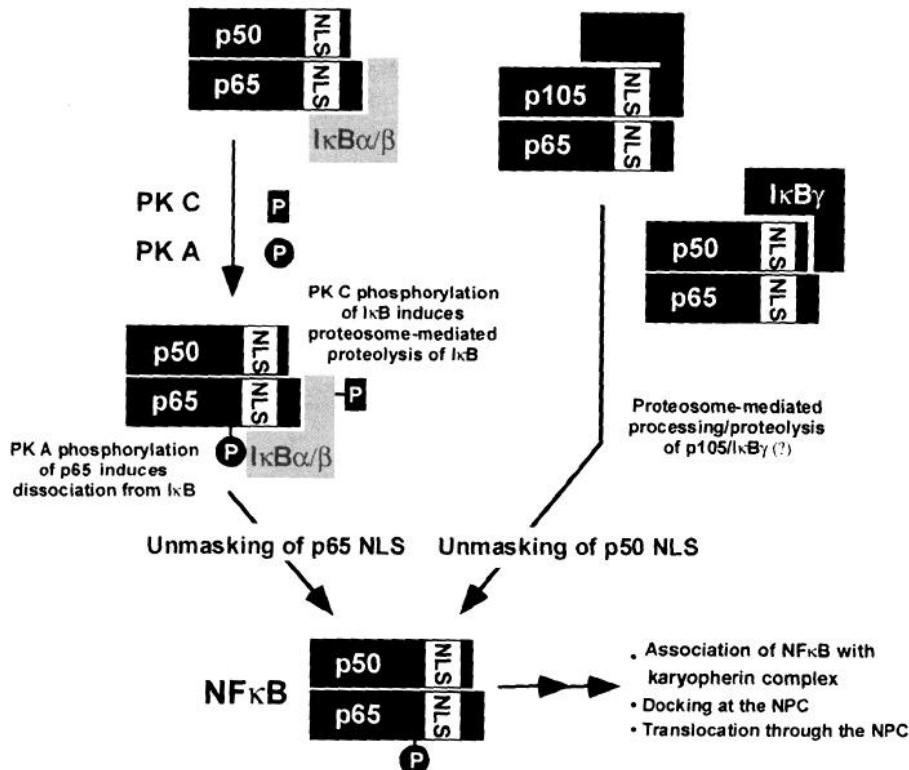


FIG. 3. Regulation of NFκB nuclear transport by phosphorylation and intra-intermolecular masking. NFκB p65 is retained in cytoplasm by IκB α or β , which binds directly to p65-NLS (18). Unmasking of p65-NLS and p65 translocation to the nucleus can be effected by either phosphorylation of IκB by protein kinase C (PKC; probably PKC- ζ), which induces proteosome-mediated proteolysis of IκB (17, 60) and/or phosphorylation of p65 by cAMP-dependent protein kinase (PKA) (see Ref. 250), which presumably dissociates the complex with IκB. Proteosome-mediated proteolysis of NFκB p105 subunit to the mature NFκB p50 form is means by which NLS, masked by COOH-terminal portion of the molecule in unprocessed p105 (130) or by the distinct gene product IκB γ (probably only present in pre-B cells; Ref. 345), is unmasked in p50 (27, 130, 285). Precise details of PKA and PKC regulation of p65 cytoplasmic retention by IκB are contentious (e.g., see Ref. 349), but consensus of experimental evidence supports the simplistic view shown.

3) both *in vivo* and *in vitro* in response to phorbol ester stimulation (131). Negative charge through phosphorylation close to the NLS is presumed to inactivate NLS function. In analogous fashion, nuclear translocation of the actin binding protein cofilin upon heat shock treatment is accompanied by dephosphorylation at a consensus CaMPK site adjacent to the cofilin-NLS (see Table 3; Refs. 2, 253). Phosphorylation at this site is proposed to mask the function of the NLS (253) in similar fashion to CDC28-phosphorylation-mediated inactivation of the SWI5-NLS (225).

Nuclear localization signal-dependent nuclear translocation of the nuclear oncogene *v-jun* (in contrast to that of its cellular protooncogene counterpart *c-jun*) has been shown to be dependent on the stage of the cell cycle (41, 340). Interestingly, *v-jun* possesses a Cys to Ser mutation at position 248, adjacent to its NLS (see Table 3), implying that cell cycle-dependent phosphorylation at this site may mask NLS function in similar fashion to SWI5, cofilin, and lamin B2 above.

D. Phosphorylation Enhancing Nuclear Localization Signal-Dependent Nuclear Transport

The casein kinase II (CKII) site at serine 112 close to the T-ag NLS has been shown both *in vivo* and *in vitro*

to regulate the kinetics of T-ag nuclear transport. The site increases the rate of transport so that maximal accumulation within the nucleus occurs within 15–20 min, compared with the 10 h taken when CKII phosphorylation is prevented through deletion or mutation of the CKII site (150, 283, 284). Aspartic acid at position 112 can simulate phosphorylation at the CKII site in terms of accelerating the rate of nuclear import (150), implying that negative charge at the CKII site is mechanistically important in this process. The phosphorylated CKII site presumably represents a signal in addition to, or recognized in concert with, the NLS, which is specifically recognized by the cellular nuclear transport apparatus to effect accelerated transport (150). Preliminary evidence from *in vitro* studies (D. A. Jans and P. Jans, unpublished data) implies that phosphorylation at the CKII site may increase the rate of docking at the nuclear envelope/NPC. Because this step is almost certainly karyopherin mediated (see Fig. 2), it can be speculated that phosphorylation at the CKII site may directly modulate the affinity of association of T-ag with karyopherin α/β , thereby facilitating/accelerating T-ag docking at the NPC and subsequent translocation into the nucleus (see Ref. 150).

The *Xenopus* nuclear phosphoprotein nucleoplasmmin has also recently been reported to exhibit CKII enhancement of the rate of nuclear import in microinjected *Xenopus* oocytes (355), whereby dephosphorylated nucleoplasmmin is accumulated at a much slower rate than untreated protein. Phosphorylation at CKII sites is respon-

sible for the enhanced transport, since the phosphorylating activity can be both inhibited by heparin and can use GTP as efficiently as ATP as a phosphate donor, characteristics distinctive of CKII (271). In addition, the specific CKII inhibitor 5,6-dichloro-1- β -ribofuranosyl-benzimidazol reduces the rate of nucleoplasmin nuclear import (355). Although the relevant phosphorylation sites have not been confirmed to be the CKII site consensus serines 177 and 178 (355), it seems reasonable to postulate that these sites near the nucleoplasmin NLS (see Table 3) mediate this regulatory effect, thus resembling the regulation of T-ag nuclear import by the CKII site.

Protein kinase A is directly implicated in enhancing nuclear localization of the *c-rel* protooncogene (95, 237). In comparable fashion to the CKII site and T-ag, alanine at the PKA site (Ser²⁶⁶) 22 amino acids NH₂-terminal to the NLS (see Table 3) abolishes *c-rel* nuclear localization, whereas aspartic acid at the site simulates PKA phosphorylation in inducing nuclear translocation (95). Linker insertion analysis indicates that spacing between the PKA site and NLS (22 amino acids) is of prime importance in regulating *c-rel* nuclear localization (237), implying that the two sequence elements may be recognized in concert, as has been speculated for the T-ag NLS and the CKII site (150).

Significantly, this PKA site, together with the NLS, is conserved in all members of the *rel/dorsal* family within the *rel* homology domain, which has been shown to be the region of dorsal to which cactus binds (160). Cotransfection experiments in Schneider cells clearly implicate PKA and this conserved site (Ser³¹²; see Table 3) in regulating dorsal nuclear localization in response to the activated toll receptor pathway (250). Coexpression of the cDNAs for dorsal and the PKA C subunit enhances dorsal nuclear localization, which can be blocked by PKA-specific peptide inhibitors (250). Similar to the results for *c-rel*, mutation of Ser³¹² to glutamine significantly reduces toll-mediated nuclear localization, whereas aspartic acid in place of Ser³¹² can partially induce nuclear transport, indicating that negative charge at the site is mechanistically important (250). Phosphorylation by PKA of *c-rel*, dorsal, and perhaps also of the NF κ B subunits thus appears to enhance their nuclear transport, possibly through modulating their interactions with cytoplasmic retention factors. Further examples of putative PKA enhancement of nuclear localization (see Table 3) include rNFIL-6, which translocates to the nucleus upon the elevation of intracellular cAMP levels in rat PC-12 pheochromocytoma cells (222), *c-fos* (292, 351), and the myogenic factor MyoD (358).

E. Developmentally Regulated Nuclear Localization Signals

A "developmentally regulated" NLS-functioning sequence (drNLS) has been reported for a 45-amino acid

region of the adenovirus E1a protein (see Table 3; Ref. 332), which is distinct from the constitutive E1a NLS shown in Table 1. This sequence is functional in targeting a carrier protein to the nucleus up to the early neurula stage of *Xenopus* embryonic development, when it is inactivated in a hierarchical fashion among the embryonic germ layers (332). Significantly, a sequence homologous to the drNLS of E1a has been identified in the rat (see Table 3) as well as mouse and human GR hormone binding domain known to contain the ligand binding-dependent NLS (267).

F. Phosphorylation-Regulated Nuclear Localization Signals

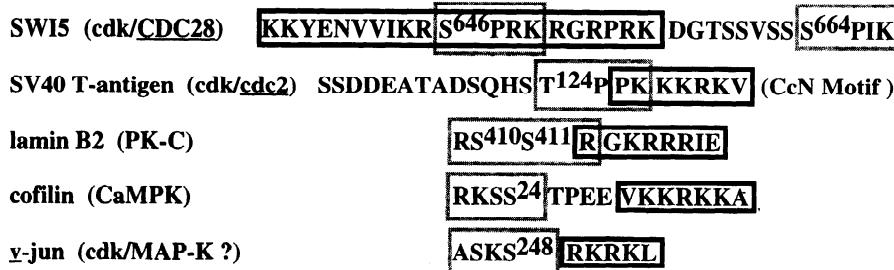
1. Definition of phosphorylation-regulated nuclear localization signals

From a number of examples listed above, there appears to be a consensus of phosphorylation sites close to NLSs modifying NLS activity. The phosphorylation sites, together with the NLS, constitute phosphorylation-mediated regulatory modules for nuclear protein localization, which can be called phosphorylation-regulated NLSs (prNLSs) (148). They can be grouped into those in which phosphorylation either enhances or inhibits NLS-dependent nuclear transport (see Fig. 4).

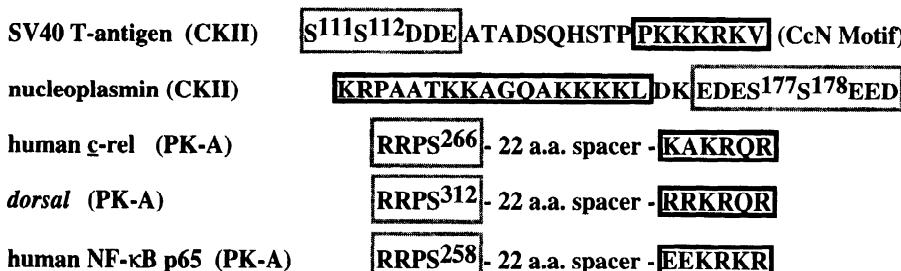
As discussed in sect. vC, NLS-dependent nuclear transport of lamin B2 is inhibited by PKC phosphorylation at sites NH₂-terminally adjacent to the NLS (see Table 3) (131), whereas nuclear localization of the actin binding protein cofilin is similarly negatively regulated by a CaMPK site NH₂-terminal to the NLS (see Table 3; Refs. 2, 248, 253). Nuclear import of both SWI5 (151, 225) and *v-jun* (41, 340) is inhibited by cell cycle-dependent phosphorylation within and/or close to the NLSs of the respective proteins. Cell cycle-dependent nuclear localization conferred by the prNLSs of both SWI5 and *v-jun* appears to be able to be transferred to carrier proteins (41, 151). All of these proteins can be regarded as containing prNLSs, regulatory modules for nuclear import consisting of an inhibitory phosphorylation site and an NLS (Fig. 4).

As seen in section vD, other phosphorylation sites are known to enhance NLS-dependent nuclear protein import. For example, CKII increases the nuclear import of T-ag through phosphorylation 13 amino acids NH₂-terminal to the NLS (150, 283). Similarly, although it has not been formally demonstrated that the consensus CKII sites COOH-terminally adjacent to the NLS are the sites affecting nucleoplasmin nuclear import, CKII phosphorylation has been shown to increase the rate of import of nucleoplasmin into the nucleus (355). In similar fashion, the PKA site 22 amino acids NH₂-terminal to the NLS of both the *c-rel* protooncogene (237) and dorsal (250) has been shown to enhance nuclear localization. The PKA site, to-

Phosphorylation inhibiting NLS function:



Phosphorylation enhancing NLS function:



gether with the NLS, is conserved in NF κ B p65, which has been shown to exhibit PKA-mediated enhancement of nuclear localization (see Ref. 250), implying that it may function analogously to the identical sites in *c-rel* and *dorsal*. All of these proteins can be regarded as containing prNLSs, modules for regulated nuclear import, consisting of an NLS flanked by a phosphorylation site which enhances or activates its function (Fig. 4).

As indicated by the above examples, as well as by those in Table 3, many different kinases regulate nuclear import through prNLSs. This means that a variety of hormonal or other signals can affect the inhibition or enhancement of nuclear localization of particular proteins through regulation of the activity of the kinases phosphorylating within the respective prNLSs. Phosphorylation-regulated NLSs thus constitute a highly specific mechanism of controlling nuclear protein import precisely according to the stage of the cell cycle or to the signal transduction, metabolic, proliferative, or differentiation state of the cell.

2. The CcN motif, a phosphorylation-regulated nuclear localization signal regulated by dual phosphorylation

As implied in section vF1, T-ag appears to possess a specialized sort of prNLS, comprising both enhancing and inhibitory phosphorylation sites (see Fig. 4). The CKII site increases the rate of NLS-dependent nuclear import, whereas phosphorylation at the *cdc2* site adjacent to the

FIG. 4. Phosphorylation-regulated NLSs (prNLSs) where phosphorylation sites close to NLS regulate nuclear protein import. Single letter amino acid code is used, with phosphorylated residues numbered and phosphorylation sites (kinases named in brackets) and NLSs (bold) boxed. It should be noted that enhancement of nucleoplasmin nuclear import by casein kinase II (CKII) and of NF κ B p65 by PKA has not been formally shown to be due to phosphorylation sites shown. The prNLSs of simian virus 40 (SV40) large tumor antigen (T-ag; CcN motif), SWI5 and *v-jun* are completely sufficient to confer phosphorylation-regulated nuclear localization on a heterologous protein (41, 149, 150, 151, 225, 283, 340).

NLS inhibits transport (149), markedly reducing the level of maximal nuclear accumulation (149). The CKII and *cdc2* sites appear to function completely independently of one another in terms of both regulating T-ag nuclear transport and influencing phosphorylation at the other site (149); that is, CKII phosphorylation does not enhance nuclear import by reducing phosphorylation at the inhibitory *cdc2* site, nor does Thr-124 phosphorylation inhibit nuclear transport by impairing Ser-112 phosphorylation (149). We have named this regulatory module for T-ag nuclear transport, comprising CKII ("C") and cdk/cdc2 ("c") sites and the NLS ("N"), the "CcN motif" (149) (see Fig. 5).

The effects on nuclear transport of phosphorylation at either CKII or *cdc2* site can be simulated by replacing the phosphorylated residues with aspartic acid residues (149, 150), implying that the phosphorylated sites represent signals in themselves that are recognized by components of the cellular nuclear transport machinery. The results with Asp-substituted proteins also indicate that phosphatase activity at either kinase site has no direct role in the nuclear transport process, i.e., nuclear dephosphorylation at either the CKII or *cdc2* sites is not involved in T-ag retention in the nucleus (149, 150). Similarly, phosphorylation in the nucleus at either kinase site to effect nuclear retention is not the mechanism of nuclear accumulation, as shown by mutational analyses (150, 283). Both CKII and cdk site phosphorylations almost certainly occur in the cytoplasm *in vivo* (see Ref. 150), which im-

Element:	Casein Kinase II site	cdk/cdc2 site	NLS
Regulation:	Proliferative Signals	Cell Cycle	K ¹²⁸ critical residue
Effect on Transport:	Positive Accelerates nuclear import ($t_{1/2}$)	Negative Reduces maximal accumulation (V_{max})	Essential for Nuclear Localization
Putative Mechanism:	Phosphorylation increases affinity for nuclear envelope/NLSBP	Phosphorylation increases affinity for cytoplasmic retention factor	Interaction with NLSBP

FIG. 5. Summary of regulation of SV40 T-ag nuclear protein transport by CcN motif. Single letter amino acid code is used, with phosphorylated residues numbered and CKII and cdk sites and NLS (bold) boxed. *Xenopus* phosphoprotein nucleoplasmin and yeast transcription factor SWI5 show identical regulation by CKII and cdk sites, respectively, of their CcN motifs in terms of half-life ($t_{1/2}$; Ref. 358) and maximum velocity (V_{max} ; Refs. 151, 225), respectively (see Tables 3 and 4). NLSBP, NLS binding proteins.

plies that the initial regulatory events determining nuclear import kinetics are cytoplasmic and precede those delineated in Figure 2.

As alluded to above, the mechanism of *cdc2*-mediated inhibition of the maximal level of nuclear transport appears to be through cytoplasmic retention (149), whereas that of CKII phosphorylation-mediated enhancement of nuclear transport may revolve around increasing the affinity/rate of association with the karyopherin complex, resulting in enhancement of the rate of docking at the NPC. Figure 6 represents the events thought to be involved in the regulation of T-ag nuclear import by the CcN motif, involving *cdc2* regulation of cytoplasmic retention and CKII regulation of the kinetics of docking at the NPC.

The existence of a complex regulatory system for SV40 T-ag nuclear localization involving two different kinases reinforces the concept of there being specific mechanisms regulating nuclear entry (147, 150). The NLS is clearly not the sole determinant of nuclear localization; rather, the kinetics of NLS-dependent nuclear import are regulated by phosphorylation in the vicinity of the NLS. Through a potentially signal transduction responsive CKII site (see Refs. 147, 150, 220, 264, 271) positively regulating the rate of nuclear transport, and a cell cycle-dependent cdk/cdc2 site negatively regulating the maximal extent of accumulation, the level of T-ag present in the nucleus can be precisely regulated as required with respect to the eukaryotic cell cycle and stages of the viral lytic cycle.

Significantly, a variety of proteins other than T-ag possess putative CcN motifs (see Refs. 147, 149; Table 4), implying a general role for the CcN motif in regulating nuclear protein transport. These include oncogene products, viral

proteins, and TFs (149), as well as the *D. melanogaster* "Notch" group of genes and human homologues, the yeast TF SNF2 and human and *Drosophila* homologues, the family

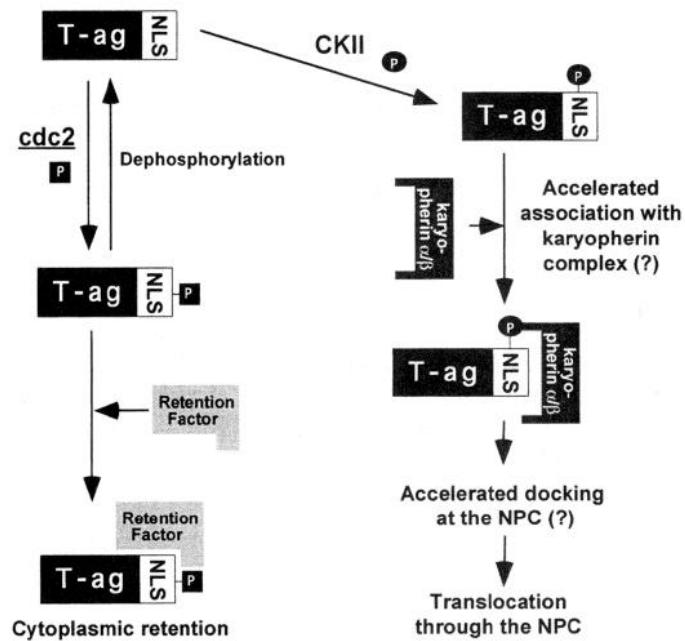


FIG. 6. SV40 T-ag nuclear transport is regulated by phosphorylation and CcN motif. Phosphorylation by *cdc2* results in cytoplasmic retention of T-ag (149), probably through increasing affinity for a cytoplasmic anchor protein. Phosphorylation at CKII site increases nuclear import rate (150, 283), probably by increasing kinetics of docking at the nuclear envelope/NPC through regulating interactions with the karyopherin complex. The two CcN motif kinase sites are completely independent in terms of both phosphorylation at respective sites and their effects on nuclear import (149).

TABLE 4. Potential and confirmed CKII and cdk kinase phosphorylation sites in the vicinity of the NLS of various nuclear proteins

Protein	CKII Site(s)	cdk Site(s)	NLS
SV40 T-ag	S ¹¹¹ S ¹¹² DDE ⁺ (105, 302, 303)	ST ¹²⁴ PPK ⁺ (215, 302, 303)	PKKKRKV ¹³² (155, 156, 177, 284)
Polyoma T-ag	SS ¹⁵⁶ PTD ES ²⁶¹ ENE T ²⁶⁵ EED ⁺ (366)	PRT ¹⁸⁷ PVS AT ²⁷⁸ PPK	VSRKRPRP ¹⁹⁶ PPKKARED ²⁸⁶ (282) KVNDRKRRK ¹⁴ (366) ^a
CTP phosphocholine cytidylyltransferase			
p53 (human)	GPD ³⁸⁹ D ⁺ (24, 216)	SS ³¹² PQP ⁺ (24, 25)	PQPKKKP ³¹⁹ (38, 311) LTKKGQ ³⁷² (NLS II; 311) SRHKKTM ³⁸¹ (NLS III; 311) PAAKRVKL ³²⁶ (52) RQRRNELKRS ³⁷³ (52) LKRQRKRRV ⁴¹⁸ (342) KRKPGLRRSPKKVRKS ⁵⁸⁶ (342) SALIKKKKKMAP ⁶³¹ (301, 359)
c-myc (human)	SS ³⁴⁸ DTEE ⁺ (202)	PST ³¹⁵ RKP TS ³⁴⁴ PRS	
B-myb (human)	S ⁴³¹ FLD	T ⁴⁴⁰ PKST ⁴⁴⁴ PVK (201) TS ⁵⁶⁰ PVR (201)	
c-abl IV (mouse)	ESS ³²² ISDE	APDT ⁵⁶⁶ PEL ⁺ (163) PAVS ⁵⁸⁸ PLL ⁺ (163)	
p110 ^{RB1} (mouse)	S ⁸⁹⁴ IGE		KRSAEFFNPPKPLKKLR ⁸⁶⁹ (395) ^b
Lamin A/C	S ¹⁶⁸ NED	SPS ³⁹² PTS ⁺ (368)	SVTKKRKLE ⁴¹² (199)
Nucleoplasmin (<i>Xenopus</i>)	EDES ¹⁷⁷ S ¹⁷⁸ EED	S ¹⁸² PTKK S ¹⁴⁹ PPK	KRPAATKKAGQAKKKL ¹⁷¹ (38, 64, 65, 178, 286)
N1/N2 (<i>Xenopus</i>)	T ⁸³⁷ EEE	S ⁵⁴¹ PLK	RKKRK-12 amino acid spacer-KKSQKEP ⁵⁶⁴ (165)
SW15 (<i>S. cerevisiae</i>)		KRS ⁶⁴⁶ PRK ⁺ (151, 225)	KKYENVVIRKSPRKRGRPRK ⁶⁵⁵ (151, 252)
Opaque-2 (maize)	ES ²³⁴ NRES	SSS ⁶⁶⁴ PIK ⁺ (151, 225)	RKRKESNRESARRSRYRK ²⁴⁷ (362)
<i>Agrobacterium tumefaciens</i>			
VirE2 protein	S ⁹⁰⁰ DTE		RAIKTKYGSDEIQLKSK ³⁰⁹ (42)
VirD2 protein (octopine)	DES ⁷¹ QS ⁷³ DDD EQDT ⁹⁷² RDD	S ³⁹⁴ PKR	EYLSRKKGKLEL ³⁶ (346) KRPRDRHDGELGGRKRAR ⁴¹³ (136, 346)

Single letter amino acid code is used. Casein kinase II (CKII) consensus site comprises an acidic amino acid 3 residues COOH-terminal to phosphorylatable S/T, with an elevated number of acidic residues in the vicinity of the site increasing its affinity for CKII (169, 172, 271); cdks phosphorylate S/T residues NH₂-terminal to proline residues, with a basic amino acid 1 or 2 residues COOH-terminal to proline. References apply as indicated to confirmed in vitro or in vivo (indicated by +) phosphorylation sites for CKII, cdk, and NLS. Phosphorylated residues are numbered.

^a Nuclear localization is dependent on amino acids 6–28, i.e., including CKII site. Both nuclear transport (no kinetic analysis) and phosphorylation are reduced if CKII site is absent (366). ^b A second putative CcN motif is present in amino acids 601–621 (see Table 3).

of interferon-induced TFs including IFI204 and IFI16, the Swi4 family of mismatch repair enzymes, various DNA repair and processing enzymes, the protein tyrosine phosphatase PEP [PEST (proline-, glutamic acid-, serine-, and threonine)-enriched phosphatase], and various other proteins including kinases and TFs (see Ref. 147). The p85^{s6k} (s6 kinase) is interesting in that, in contrast to the alternatively spliced p70^{s6k} variant, it possesses a putative CcN motif, whereby a putative CKII site (S⁴⁰EDELEE) is upstream of the NH₂-terminal NLS (280; see sect. III D3). Whereas the CKII site does not appear to be absolutely necessary for nuclear localization in this case (280), analysis of the rate of nuclear transport in its presence or absence has not been performed. Putative CcN motifs are also found in the sequences of proteins localizing in the plant cell nucleus (see Table 4; Ref. 147).

Of the proteins listed in Table 4 and elsewhere (147, 149), very few have been examined in terms of nuclear import kinetics. A few examples of cell cycle-dependent phosphorylation inhibiting nuclear transport are known (see 151 and Table 3), the best being the inhibition of NLS-dependent nuclear import exerted by cdk sites in the vicinity of the SWI5 NLS (151, 225; see Tables 3 and 4

and Fig. 4). In this case, the maximal level of nuclear accumulation [and not half-life (*t*_{1/2})] is affected by cdk phosphorylation (151), completely consistent with the results for cdk phosphorylation of T-ag (149). Demonstration of CKII-mediated effects on transport other than for T-ag has thus far been restricted to nucleoplasmin (355, see Tables 3 and 4 and Fig. 4), where CKII phosphorylation accelerates nuclear import (*t*_{1/2}) in identical fashion to its effect on T-ag. These results strongly support the idea of the CcN motif being a specialized prNLS, mediating two parameters of nuclear import very specifically in response to cell cycle or proliferative signals. The hypothesis that the CcN motif may have a centrally important role in nuclear protein import in general, however, requires a great deal more experimental evidence.

VI. NUCLEAR PROTEIN EXPORT

A. Unidirectionality of Nuclear Localization Signal-Dependent Nuclear Protein Transport

At least in the short term (up to 4 h), NLS-dependent nuclear protein transport appears to be strictly unidirec-

tional (69, 89, 284, 310), with the nuclear envelope not only representing a barrier to protein entry into the nucleus, but also restricting the exit of macromolecules from the nucleus. We have demonstrated this unidirectionality convincingly *in vitro* (310), where the maximal level of nuclear accumulation of T-ag CcN motif-containing transport substrate interestingly appears to be directly proportional to its concentration in the cytoplasm. Unidirectionality of transport could be demonstrated by incubating nuclei with various concentrations of transport substrate until steady-state levels of nuclear accumulation were attained, and then the cytosolic concentration of transport substrate either increased or reduced to zero (310). After incubation for an additional hour, no export of accumulated protein could be detected; the level of nuclear accumulation only changed (increased) if the concentration of T-ag protein exceeded that during the first incubation, indicating that T-ag nuclear import is strictly unidirectional in the import direction over 2 h at 37°C. These and other results (see Ref. 310) led to the conclusion that an inhibitory feedback loop may regulate maximal nuclear accumulation, whereby the cytosolic concentration of transport substrate determines the precise end point of nuclear accumulation (see Ref. 310). The results are clearly insufficient to enable conclusions as to their wider significance, but one can speculate at this stage that such a regulatory feedback loop may represent an important third step of nuclear protein import, subsequent to those of docking and translocation, at least in the case of T-ag (310).

B. Protein Export From the Nucleus and Nuclear Export Signals

Certain proteins such as ribosomal subunits are known to be specifically exported from the nucleus through a process that is saturable, temperature dependent, energy dependent, and inhibitible by WGA (16, 306). Nuclear protein export can be reconstituted *in vitro* in digitonin-permeabilized cells and, like nuclear protein import, can be shown to require the GTP-binding protein Ran/TC4 (233). Experiments using an NLS-peptide-human serum albumin conjugate indicate that nuclear protein export is blocked by fraction A (i.e., karyopherin α/β). It is inhibitible by nonhydrolyzable GTP but not by ATP analogues, thus appearing to be entirely GTP driven (233). Consistent with this, a GTPase mutant of Ran/TC4 is unable to reconstitute nuclear protein export in digitonin-permeabilized cells (233).

Recent results (86, 92, 372) suggest that the process may be signal mediated, with specific nuclear export signals (NESs), analogous to nuclear import signals (NLSs), having been identified; NESs are defined as the sequences sufficient and necessary to confer rapid and active export

from the nucleus. The two NESs thus far characterized are those of the PKA specific inhibitor protein PKI (Leu-Ala-Leu-Lys-Leu-Ala-Gly-Leu-Asp-Ile-Asp⁴⁷; Ref. 372), and of the HIV-1 Rev protein (Leu-Pro-Pro-Leu-Glu-Arg-Leu-Thr-Leu-Asn⁸⁴; Ref. 86). Both NESs are functional in effecting nuclear export of heterologous proteins when present either as covalently attached peptides (86) or within fusion protein sequences (372). It has even been speculated that proteins functioning as NES receptors, analogous to importin/karyopherin in the case of NLSs, are required (see Ref. 92). A combination of the T-ag NLS and the PKI NES resulted in a steady-state nuclear localization (372), implying that the T-ag NLS is a stronger targeting signal.

C. Shutting Proteins

Several proteins have been characterized that appear to "shuttle" between the nucleolus and the cytoplasm, including the nucleolar proteins B23 (NO38) (30) and nucleolin (30, 306), HSC70 (207), various heterogeneous nuclear ribonucleoprotein particle (hnRNP) protein components (272, 369), and the NLSBP Nopp140 (218). These proteins appear to have roles in RNA transport processes, with the exception of HSC70 and Nopp140 which have putative NLSBP roles in nuclear protein import (see sect. IV C); nucleolin may also have a role in the latter through its apparent ability to bind T-ag and histone H2B type NLSs specifically (383). Shutting in these studies has been shown by monitoring the internuclear migration of proteins in interspecies hybrids (31) or by demonstrating nuclear/nucleolar colocalization of antibodies microinjected into the cytoplasm via a piggy back mechanism in the presence of inhibitors of de novo protein synthesis (30, 218, 272).

Bidirectional movement between nucleus and cytoplasm has been reported for the influenza virus ribonucleoprotein M1 (211) and for the yeast RNA binding protein Npl3 (89). Energy-dependent shuttling between nucleus and cytoplasm of the progesterone, estrogen, and glucocorticoid hormone receptors has also been proposed (112, 113, 137, 266), whilst recent experiments suggest that even T-ag may be able to shuttle (112, 306), albeit over a longer time frame (see also Ref. 89). Flach et al. (89) show that while T-ag- and histone H2B-NLS-fusion proteins are strictly nuclear over 3 h, Npl3 can shuttle through the action of specific but as yet unidentified sequences distinct from its NLS-functioning COOH-terminal which is also required. There is limited evidence that functional NLSs are required for shuttling (89, 112).

Based on the analysis of three proteins (lamin B2, nucleolin, and a T-ag NLS-BSA conjugate), Schmidt-Zachmann et al. (306) propose that nucleocytoplasmic shuttling is inversely related to the affinity of binding in the

nucleus ("nuclear retention"), whereby proteins which bind tightly in the nucleus or nucleolus are less likely to shuttle (see also Ref. 174). This occurs through the RNP binding domain in the case of nucleolin; and through polymerization in the case of lamin B2, whereby deletion of the NH₂-terminal 27 amino acids prevents oligomerization and allows it to shuttle (which is not normally the case) (306). Difficulties in interpreting the results, however, are caused by the fact that nuclear injected BSA, which has no NLS or specific domains conferring binding to nuclear components, remains nuclear in the experimental system. Also, since the proteins analyzed can hardly be regarded as "typical," lamin B2 is a constitutively nuclear structural protein and nucleolin is nucleolar rather than nuclear (where binding and retention certainly play a role; see sect. III E 1). Conclusions cannot be drawn with respect to a general mechanism.

A comparable study to that of Schmidt-Zachmann et al. shows that, in contrast to the results for nucleolin where the RNP domain increases nuclear retention, export of the hnRNP A1 protein (whose nuclear entry requires an NLS) requires at least one RNP binding domain (369). This indicates that nuclear exit may occur through binding to mRNA. It is interesting that almost all of the proteins listed above as shuttling between the nucleus (or nucleolus) and the cytoplasm indeed possess RNA-binding domains, consistent with the idea that nuclear protein export may occur through piggy backing on RNA exiting from the nucleus. What seems clear is that shuttling between nucleus and cytoplasm is a slower process than either NLS-dependent nuclear import or NES-dependent nuclear export (see Refs. 69, 92, 284, 310). It thus probably relates more closely to long-term responses in terms of gene regulation rather than to rapid responses stimulated by hormones or other signal transduction pathways.

VII. SUMMARY AND FUTURE PROSPECTS

Although the mechanism of nuclear protein import, its essential cellular components, and precise signal sequence/phosphorylation site requirements for nuclear localization may not yet be fully understood, a general picture seems to have emerged. Multiple factors at all levels are necessary for active NLS-dependent nuclear protein import, including nucleoporins, specific docking proteins, molecular chaperones, GTP-binding protein complexes, and, in addition, all of the protein factors specific to regulating nuclear import, such as cytoplasmic retention factors and kinases/phosphatases (see sect. V). This complex array of factors is responsible for the high specificity of the cellular nuclear transport machinery, which is so essential to eukaryotic cell function. This is epitomized by the intricate complexity of structure of the NPC, which

enables it to transport both small molecules passively and larger molecules in active NLS-dependent fashion.

Results for the kinetics of nuclear transport of TFs such as T-ag, SWI5, or nucleoplasmin show that both the amount of a particular protein in the nucleus and also the time and the rate at which nuclear entry occurs, according to the stage of the cell cycle or in response to hormonal or growth factor signals, can be precisely regulated. The mechanisms regulating nuclear protein transport, such as CcN motifs, cytoplasmic retention factors, intra- and intermolecular NLS masking, and the masking of NLSs by phosphorylation, are highly specific and appear to be conserved across eukaryotes (e.g., the conservation of structure and function of the IκB and cactus cytoplasmic retention factor proteins, together with the prNLSs of their specific TF partners). The implication is that the signals regulating nuclear protein transport may be as universal as the NLSs themselves (147, 151).

Nuclear localization sequences from plants, yeast, and higher mammals have been shown to be functionally interchangeable, e.g., the T-ag NLS is functional in yeast and plant cells (318; see Ref. 277), the bipartite NLS of the yeast TF SWI5 is functional in mammalian cells (151), and the plant-cell NLS of *Agrobacterium tumefaciens* VirD2 protein is functional in yeast cells (346). Furthermore, recent results for SWI5 (151) show that not only a yeast bipartite NLS, but also, fascinatingly, its regulatory mechanisms, is functional in higher eukaryotes, implying the universal nature of regulatory signals for protein transport to the nucleus. This fascinating conservation of function at the level of NLS and NLS-modifying sequences clearly reflects the conservation of structure and function of the entire eukaryotic cell nuclear transport apparatus. A fuller understanding of the precise details of nuclear protein transport at all levels should help to establish the central importance of nuclear protein transport in the process of gene regulation, which is so integral to all aspects of eukaryotic cell function.

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REFERENCES

- ABATE, C., L. PATEL, F. J. RAUSCHER III, AND T. CURRAN. Redox regulation of fos and jun DNA-binding activity in vitro. *Science Wash. DC* 249: 1157–1161, 1990.
- ABE, H., R. NAGAOKA, AND T. OBINATA. Cytoplasmic localization and nuclear transport of cofilin in cultured myotubes. *Exp. Cell Res.* 206: 1–10, 1993.
- ACKERMAN, P., C. V. C. GLOVER, AND N. OSHERHOFF. Stimulation of casein kinase II by epidermal growth factor: relationship between the physiological activity of the kinase and the phosphorylation state of its beta subunit. *Proc. Natl. Acad. Sci. USA* 87: 821–825, 1990.
- ADAM, E. J. H., AND S. A. ADAM. Identification of cytosolic factors

- required for nuclear location sequence-mediated binding to the nuclear envelope. *J. Cell Biol.* 125: 547–555, 1994.
5. ADAM, S. A., AND L. GERACE. Cytosolic factors that specifically bind nuclear location signals are receptors for nuclear transport. *Cell* 66: 837–847, 1991.
 6. ADAM, S. A., T. J. LOBL, M. A. MITCHELL, AND L. GERACE. Identification of specific binding proteins for a nuclear location sequence. *Nature Lond.* 227: 276–279, 1989.
 7. ADAM, S. A., R. E. STERNE-MARR, AND L. GERACE. Nuclear import in permeabilized mammalian cells requires soluble cytoplasmic factors. *J. Cell Biol.* 111: 807–816, 1990.
 8. AGUTTER, P. S., AND D. PROCHNOW. Nucleocytoplasmic transport. *Biochem. J.* 300: 609–618, 1994.
 9. AKEY, C. W. Visualization of transport-related configurations of the nuclear pore transporter. *Biophys. J.* 58: 341–355, 1990.
 10. AKEY, C. W., AND D. S. GOLDFARB. Protein import through the nuclear pore complex is a multistep process. *J. Cell Biol.* 109: 971–982, 1989.
 11. AUWERX, J., B. STAELS, AND P. SASSONE-CORSI. Coupled and uncoupled induction of fos and jun transcription by different second messengers in cells of hematopoietic origin. *Nucleic Acids Res.* 18: 221–228, 1990.
 12. BAGLIA, F. A., AND G. G. MAUL. Nuclear ribonucleoprotein release and nucleoside triphosphatase activity are inhibited by antibodies directed against one nuclear matrix glycoprotein. *Proc. Natl. Acad. Sci. USA* 80: 2285–2289, 1983.
 13. BAKER, S. J., T. K. KERPPOLA, D. LUK, M. T. VANDENBERG, D. R. MARSHAK, T. CURRAN, AND C. ABATE. Jun is phosphorylated by several protein kinases at the same sites that are modified in serum-stimulated fibroblasts. *Mol. Cell. Biol.* 12: 4694–4705, 1992.
 14. BALDIN, V., AND B. DUCOMMUN. Subcellular localisation of human wee1 is regulated during the cell cycle. *J. Cell Sci.* 108: 2425–2432, 1995.
 15. BANDARA, L. R., J. P. ADAMCZEWSKI, T. HUNT, AND N. B. LA THANGUE. Cyclin A and the retinoblastoma gene product complex with a common transcription factor. *Nature Lond.* 352: 249–251, 1991.
 16. BATAILLE, N., T. HELSER, AND H. M. FRIED. Cytoplasmic transport of ribosomal subunits microinjected into the *Xenopus laevis* oocyte nucleus: a generalized, facilitated process. *J. Cell Biol.* 111: 1571–1582, 1990.
 17. BEG, A. A., T. S. FINCO, P. V. NANTERMET, AND A. S. BALDWIN, JR. Tumor necrosis factor and interleukin-1 lead to phosphorylation and loss of IκBα: a mechanism for NF-κB activation. *Mol. Cell. Biol.* 13: 3301–3310, 1993.
 18. BEG, A. A., S. M. RUBEN, R. I. SCHEINMAN, S. HASKILL, C. A. ROSEN, AND A. S. BALDWIN, JR. IκB interacts with the nuclear localization sequences of the subunits of NF-κB: a mechanism for cytoplasmic retention. *Genes Dev.* 6: 1899–1913, 1992.
 19. BELLINI, M., J.-C. LACROIX, AND J. G. GALL. A putative zinc-binding protein on lampbrush chromosome loops. *EMBO J.* 12: 107–114, 1993.
 20. BENDITT, J. O., C. MEYER, H. FASOLD, F. C. BARNARD, AND N. RIEDEL. Interaction of a nuclear location signal with isolated nuclear envelopes and identification of signal-binding proteins by photolabelling. *Proc. Natl. Acad. Sci. USA* 86: 9327–9331, 1989.
 21. BENSON, D. L., P. J. JACKSON, C. M. GALL, AND E. G. JONES. Differential effects of mononuclear deprivation on glutamic acid decarboxylase and type II calcium-calmodulin dependent protein kinase determined by molecular cloning. *J. Neurosci.* 11: 31–47, 1991.
 22. BERRIOS, M., AND P. A. FISHER. A myosin heavy chain-like polypeptide is associated with the nuclear envelope in higher eukaryotic cells. *J. Cell Biol.* 103: 711–724, 1986.
 23. BERRIOS, M., P. A. FISHER, AND E. C. MATZ. Localization of a myosin heavy chain-like polypeptide to *Drosophila* nuclear pore complexes. *Proc. Natl. Acad. Sci. USA* 88: 219–223, 1991.
 24. BISCHOFF, J. R., D. CASSO, AND D. BEACH. Human p53 inhibits growth in *Schizosaccharomyces pombe*. *Mol. Cell. Biol.* 12: 1405–1411, 1992.
 25. BISCHOFF, J. R., P. N. FRIEDMAN, D. R. MARSHAK, C. PRIVES, AND D. BEACH. Human p53 is phosphorylated by p60-cdc2 and cyclin B-cdc2. *Proc. Natl. Acad. Sci. USA* 87: 4766–4770, 1990.
 26. BISCHOFF, F. R., C. KLEBE, J. KRETSCHMER, A. WITTINGHOFER, AND H. PONSTINGL. RanGAP1 induces GTPase activity of nuclear Ras-related Ran. *Proc. Natl. Acad. Sci. USA* 91: 2587–2591, 1994.
 27. BLANK, V., P. KOURILSKY, AND A. ISRAEL. Cytoplasmic retention, DNA binding and processing of the NF-κB p50 precursor are controlled by a small region in its C-terminus. *EMBO J.* 10: 4159–4167, 1991.
 28. BOGERD, A. M., J. A. HOFFMAN, D. C. AMBERG, G. R. FINK, AND L. I. DAVIS. *nup1* mutants exhibit pleiotropic defects in nuclear pore complex function. *J. Cell Biol.* 127: 319–332, 1994.
 29. BOOHER, R. N., C. E. ALFA, J. S. HYAMS, AND D. H. BEACH. The fission yeast cdc2/cdc13/suc1 protein kinase: regulation of catalytic activity and nuclear localization. *Cell* 58: 485–497, 1989.
 30. BORER, R. A., C. F. LEHNER, H. M. EPPENBERGER, AND E. A. NIGG. Major nucleolar proteins shuttle between nucleus and cytoplasm. *Cell* 56: 379–390, 1989.
 31. BOULUKOS, K. E., P. POGNONEC, B. RABAULT, A. BEGUE, AND J. GHYSAEL. Definition of an *Ets1* protein domain required for nuclear localization in cells and DNA-binding activity in vitro. *Mol. Cell. Biol.* 9: 5718–5721, 1989.
 32. BREEUWER, M., AND D. S. GOLDFARB. Facilitated nuclear transport of histone H1 and other small nucleophilic proteins. *Cell* 60: 999–1008, 1990.
 33. CADEPOND, F., J.-M. GASC, F. DELAHAYE, N. JIBAUD, G. SCHWEIZER-GROYER, I. SEGARD-MAUREL, R. EVANS, AND E.-E. BAULIEU. Hormonal regulation of the nuclear localization signals of the human glucocorticosteroid receptor. *Exp. Cell Res.* 201: 99–108, 1992.
 34. CAPOBIANCO, A. J., D. L. SIMMONS, AND T. D. GILMORE. Cloning and expression of a chicken *c-rel* cDNA: unlike p59^{v-rel}, p68^{c-rel} is a cytoplasmic protein in chicken embryo fibroblasts. *Oncogene* 5: 584–591, 1990.
 35. CARMO-FONSECA, M., H. KERN, AND E. C. HURT. Human nucleoporin p62 and the essential yeast nuclear pore protein NSP1 show sequence homology and a similar domain organization. *Eur. J. Biol.* 55: 17–30, 1991.
 36. CARMO-FONSECA, M., D. TOLLERVEY, R. PEPPERKOK, S. M. L. BARABINO, A. MERDES, C. BRUNNER, P. ZAMORE, M. R. GREEN, E. HURT, AND A. I. LAMOND. Mammalian nuclei contain foci which are highly enriched in components of the pre-mRNA splicing machinery. *EMBO J.* 10: 195–206, 1991.
 37. CHANG, M.-F., S. C. CHANG, C.-I. CHANG, K. WU, AND H.-Y. KANG. Nuclear localization signals, but not putative leucine zipper motifs, are essential for nuclear transport of hepatitis delta antigen. *J. Virol.* 66: 6019–6027, 1992.
 38. CHELSKY, D., R. RALPH, AND G. JONAK. Sequence requirements for synthetic peptide-mediated translocation to the nucleus. *Mol. Cell. Biol.* 9: 2487–2492, 1989.
 39. CHEN, R.-H., C. SARNECKI, AND J. BLENIS. Nuclear localization and regulation of erk- and rsk-encoded protein kinases. *Mol. Cell. Biol.* 12: 915–927, 1992.
 40. CHI, N. C., E. J. H. ADAM, AND S. A. ADAM. Sequence and characterization of cytoplasmic nuclear protein import factor p97. *J. Cell Biol.* 130: 265–274, 1995.
 41. CHIDA, K., AND P. K. VOGT. Nuclear translocation of viral Jun but not of cellular Jun is cell cycle dependent. *Proc. Natl. Acad. Sci. USA* 89: 4290–4294, 1992.
 42. CITOVSKY, V., J. ZUPAN, D. WARNICK, AND P. ZAMBRYSKI. Nuclear localization of *Agrobacterium* VirE2 protein in plant cells. *Science Wash. DC* 256: 1802–1805, 1992.
 43. CLEVER, H., AND K. KASAMATSU. Simian virus 40 Vp2/3 small structural proteins harbor their own nuclear transport signal. *Virology* 181: 78–90, 1991.
 44. COGHLAN, V., B. A. PERRINO, M. HOWARD, L. K. LANGEBERG, J. B. HICKS, W. M. GALLATIN, AND J. D. SCOTT. Association of protein kinase A and protein phosphatase 2B with a common anchoring protein. *Science Wash. DC* 267: 108–111, 1995.
 45. COLLEDGE, W. H., W. D. RICHARDSON, M. D. EDGE, AND A. E. SMITH. Extensive mutagenesis of the nuclear localization signal of simian virus 40 large-T antigen. *Mol. Cell. Biol.* 6: 4136–4139, 1986.

46. CORTES, P., Z.-S. YE, AND D. BALTIMORE. RAG-1 interacts with the repeated amino acid motif of the human homologue of the yeast protein SRP-1. *Proc. Natl. Acad. Sci. USA* 92: 1769–1773, 1995.
47. COUTAVAS, E., M. REN, J. D. OPPENHEIM, P. D'EUSTACHIO, AND M. G. RUSH. Characterization of proteins that interact with the cell-cycle regulatory protein Ran/TC4. *Nature Lond.* 366: 585–587, 1993.
48. COWIE, A., J. DE VILLIERS, AND R. KAMEN. Immortalization of rat embryo fibroblasts by mutant polyomavirus large T antigens deficient in DNA binding. *Mol. Cell. Biol.* 6: 4344–4352, 1986.
49. CUOMO, C. A., S. A. KIRCH, J. GYURIS, R. BRENT, AND M. A. OETTINGER. RCH1, a protein that specifically interacts with the RAG-1 recombination-activating protein. *Proc. Natl. Acad. Sci. USA* 91: 7633–7637, 1994.
50. DABAUVILLE, M.-C., R. BENAVENTE, AND N. CHALY. Monoclonal antibodies to a M_r 68,000 pore complex glycoprotein interfere with nuclear protein uptake in *Xenopus* oocytes. *Chromosoma Berlin* 97: 193–197, 1988.
51. DABAUVILLE, M.-C., B. SCHULTZ, U. SCHEER, AND R. PETERS. Inhibition of nuclear accumulation of karyophilic proteins in liver cells by microinjection of the lectin wheat germ agglutinin. *Exp. Cell Res.* 174: 291–296, 1988.
52. DANG, C. V., AND W. M. F. LEE. Identification of the human c-myc protein nuclear translocation signal. *Mol. Cell. Biol.* 8: 4048–4054, 1988.
53. DANG, C. V., AND W. M. F. LEE. Nuclear and nucleolar targeting sequences of c-erb-A, c-myb, N-myc, p53, HSP70, and HIV tat proteins. *J. Biol. Chem.* 264: 18019–18023, 1989.
54. DARGEMONT, C., M. S. SCHMIDT-ZACHMANN, AND L. C. KÜHN. Direct interaction of nucleoporin p62 with mRNA during its export from the nucleus. *J. Cell Sci.* 108: 257–263, 1995.
55. DAVEY, J., N. J. DIMMOCK, AND A. COLMAN. Identification of the sequence responsible for nuclear accumulation of influenza virus nucleoprotein in *Xenopus* oocytes. *Cell* 40: 667–675, 1985.
56. DAVID, M., P. M. GRIMLEY, D. S. FINBLOOM, AND A. C. LARNER. A nuclear tyrosine phosphatase downregulates interferon-induced gene expression. *Mol. Cell. Biol.* 13: 7515–7521, 1993.
57. DAVIS, L. I., AND G. R. FINK. The NUP1 gene encodes an essential component of the yeast nuclear pore complex. *Cell* 61: 965–978, 1990.
58. DAVIS, N., S. GHOSH, D. L. SIMMONS, P. TEMPST, H. LIOU, D. BALTIMORE, AND H. R. BOSE. Rel-associated pp40: an inhibitor of the rel family of transcription factors. *Science Wash. DC* 253: 1268–1271, 1991.
59. DE FRANCO, D. B., M. QI, K. C. BORROR, M. J. GARABEDIAN, AND D. L. BRAUTIGAN. Protein phosphatase types 1 and/or 2A regulate nucleocytoplasmic shuttling of glucocorticoid receptors. *Mol. Endocrinol.* 5: 1215–1228, 1991.
60. DIAZ-MECO, M. T., I. DOMINGUEZ, L. SANZ, P. DENT, J. LOZANO, M. M. MUNICINO, E. BERRA, R. T. HAY, T. W. STURGILL, AND J. MOSCAT. Zeta PKC induces phosphorylation and inactivation of I kappa B-alpha in vitro. *EMBO J.* 13: 2842–2848, 1994.
61. DINGWALL, C., AND R. A. LASKEY. Nuclear targeting sequences—a consensus? *Trends Biochem. Sci.* 16: 478–481, 1991.
62. DINGWALL, C., AND R. A. LASKEY. The nuclear membrane. *Science Wash. DC* 258: 942–947, 1992.
63. DINGWALL, C., S. KANDELS-LEWIS, AND B. SERAPHIN. A family of Ran binding proteins that includes nucleoporins. *Proc. Natl. Acad. Sci. USA* 92: 7525–7529, 1995.
64. DINGWALL, C., J. ROBBINS, S. M. DILWORTH, B. ROBERTS, AND W. D. RICHARDSON. The nucleoplasmmin nuclear location sequence is larger and more complex than that of SV40 large T-antigen. *J. Cell Biol.* 107: 841–849, 1988.
65. DINGWALL, C., S. V. SHARNICK, AND R. A. LASKEY. A polypeptide domain that specifies migration of nucleoplasmmin into the nucleus. *Cell* 30: 449–458, 1982.
66. DOYE, V., R. WEPPF, AND E. C. HURT. A novel nuclear pore protein Nup133p with distinct roles in poly(A)⁺ RNA transport and nuclear pore distribution. *EMBO J.* 13: 6062–6075, 1994.
67. DUVERGER, E., V. CARPENTIER, A.-C. ROCHE, AND M. MONSIGNY. Sugar-dependent nuclear import of glycoconjugates is distinct from the classical NLS pathway. *Exp. Cell Res.* 207: 197–201, 1993.
68. DUVERGER, E., C. PELLERIN-MENDES, R. MAYER, A.-C. ROCHE, AND M. MONSIGNY. Nuclear import of glycoconjugates from the cytosol. *J. Cell Sci.* 108: 1325–1332, 1995.
69. DWORETZKY, S. I., R. E. LANFORD, AND C. M. FELDHERR. The effects of variations in the number and sequence of targeting signals on nuclear uptake. *J. Cell Biol.* 107: 1279–1287, 1988.
70. ELDAR, H., J. BEN-CHAIM, AND E. LIVNEH. Deletions in the regulatory or kinase domains of protein kinase C-alpha cause association with the cell nucleus. *Exp. Cell Res.* 202: 259–266, 1992.
71. ENENKEL, C., G. BLOBEL, AND M. REXACH. Identification of a yeast karyopherin heterodimer that targets import substrate to mammalian nuclear pore complexes. *J. Biol. Chem.* 270: 16499–16502, 1995.
72. ETTEHADIEH, E., J. S. SANGHERA, S. L. PELECH, D. HESS-BIENZ, J. WATTS, N. SHAstri, AND R. AEBERSOLD. Tyrosyl phosphorylation and activation of MAP kinases by p56lck. *Science Wash. DC* 255: 853–855, 1992.
73. FABRE, E., W. C. BOELENS, C. WIMMER, I. W. MATTJAJ, AND E. C. HURT. Nup145p is required for nuclear export of mRNA and binds homopolymeric RNA in vitro via a novel conserved motif. *Cell* 78: 275–289, 1994.
74. FABRE, E., AND E. C. HURT. Nuclear transport. *Curr. Opin. Cell Biol.* 6: 335–342, 1994.
75. FANTOZZI, D. A., A. T. HAROOTUNIAN, W. WEN, S. S. TAYLOR, J. R. FERAMISCO, R. Y. TSIEN, AND J. L. MEINKOTH. Thermostable inhibitor of cAMP-dependent protein kinase enhances the rate of export of the kinase catalytic subunit from the nucleus. *J. Biol. Chem.* 269: 2676–2686, 1994.
76. FANTOZZI, D. A., S. S. TAYLOR, P. W. HOWARD, R. A. MAURER, J. R. FERAMISCO, AND J. L. MEINKOTH. Effect of the thermostable protein kinase inhibitor on intracellular localization of the catalytic subunit of cAMP-dependent protein kinase. *J. Biol. Chem.* 267: 16824–16828, 1992.
77. FEATHERSTONE, C., M. K. DARBY, AND L. GERACE. A monoclonal antibody against the nuclear pore complex inhibits nucleocytoplasmic exchange of protein and RNA in vivo. *J. Cell Biol.* 107: 1289–1297, 1988.
78. FELDHERR, C. M., AND D. AKIN. Signal-mediated nuclear transport in proliferating and growth-arrested BALB/c 3T3 cells. *J. Cell Biol.* 115: 933–939, 1991.
79. FELDHERR, C. M., E. KALLEBACH, AND N. SCHULTZ. Movement of a karyophilic protein through the nuclear pore of oocytes. *J. Cell Biol.* 99: 2216–2222, 1984.
80. FINBLOOM, D. S., III, E. F. PETRICOIN, R. H. HACKETT, M. DAVID, G. M. FELDMAN, K. IGARISHI, E. FIBACH, M. J. WEBER, M. O. THORNER, C. M. SILVA, AND A. C. LARNER. Growth hormone and erythropoietin differentially activate DNA-binding proteins by tyrosine phosphorylation. *Mol. Cell. Biol.* 14: 2113–2118, 1994.
81. FINLAY, D. R., AND D. J. FORBES. Reconstitution of biochemically altered nuclear pores: transport can be eliminated and restored. *Cell* 60: 17–29, 1990.
82. FINLAY, D. R., E. MEIER, P. BRADLEY, J. HORECKA, AND D. J. FORBES. A complex of nuclear pore proteins required for pore function. *J. Cell Biol.* 114: 169–183, 1991.
83. FINLAY, D. R., D. D. NEWMAYER, T. M. PRICE, AND D. J. FORBES. Inhibition of in vitro nuclear import by a lectin that binds to nuclear pores. *J. Cell Biol.* 104: 189–200, 1987.
84. FISCHER, U., E. DARZYNKIEWICZ, S. M. TAHARA, N. A. DATHAN, R. LÜHRMANN, AND I. W. MATTJAJ. Diversity in the signals required for nuclear accumulation of U snRNPs and variety in the pathways of nuclear transport. *J. Cell Biol.* 113: 705–714, 1991.
85. FISCHER, U., J. HEINRICH, K. VAN ZHEE, E. FANNING, AND R. LÜHRMANN. Nuclear transport of U1 snRNP in somatic cells: differences in signal requirement compared with *Xenopus* oocytes. *J. Cell Biol.* 12: 971–980, 1994.
86. FISCHER, U., J. HUBER, W. C. BOELENS, I. W. MATTJAJ, AND R. LÜHRMANN. 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, 1995.
87. FISCHER, U., V. SUMPTER, M. SEKINE, T. SATOH, AND R. LÜHR-

- MANN. Nucleo-cytoplasmic transport of U snRNPs: definition of a nuclear localization signal in the Sm core domain that binds a transport receptor independently of the m₅G cap. *EMBO J.* 13: 222–231, 1993.
88. FISCHER-FANTUZZI, L., AND C. VESCO. Cell-dependent efficiency of reiterated nuclear signals in a mutant simian virus 40 oncoprotein targeted to the nucleus. *Mol. Cell. Biol.* 8: 5495–5503, 1988.
89. FLACH, J., M. BOSSIE, J. VOGEL, A. CORBETT, T. JINKS, D. A. WILLINS, AND P. A. SILVER. A yeast RNA-binding protein shuttles between the nucleus and the cytoplasm. *Mol. Cell. Biol.* 14: 8399–8407, 1994.
90. GAO, M., AND D. M. KNIFE. Distal protein sequences can affect the function of a nuclear localization signal. *Mol. Cell. Biol.* 12: 1330–1339, 1992.
91. GARCIA-BUSTOS, J., J. HEITMAN, AND M. N. HALL. Nuclear protein localization. *Biochim. Biophys. Acta* 1071: 83–101, 1991.
92. GERACE, L. Nuclear export signals and the fast track to the cytoplasm. *Cell* 82: 341–344, 1995.
93. GHOSH, S., AND D. BALTIMORE. Activation in vitro of NF- κ B by phosphorylation of its inhibitor I κ B. *Nature Lond.* 344: 678–682, 1990.
94. GILLESPIE, S. K. H., AND S. A. WASSERMAN. Dorsal, a *Drosophila* Rel-like protein, is phosphorylated upon activation of the transmembrane protein Toll. *Mol. Cell. Biol.* 14: 3559–3568, 1994.
95. GILMORE, T. D., AND H. M. TEMIN. v-Rel oncoproteins in the nucleus and cytoplasm of transform chicken spleen cells. *J. Virol.* 62: 733–741, 1988.
96. GIRDHAM, C. H., AND D. M. GLOVER. Chromosome tangling and breakage at anaphase result from mutations in Iodestar, a *Drosophila* gene encoding a putative nucleoside triphosphate-binding protein. *Genes Dev.* 5: 1786–1799, 1991.
97. GOLDFARB, D. S., J. GARIÉPY, G. SCHOOLNIK, AND R. D. KORNBURG. Synthetic peptides as nuclear localization signals. *Nature Lond.* 322: 641–644, 1986.
98. GÖRLICH, D., S. KOSTKA, R. KRAFT, C. DINGWALL, R. A. LASKEY, E. HARTMANN, AND S. PREHN. Two different subunits of importin cooperate to recognize nuclear localization signals and bind them to the nuclear envelope. *Curr. Biol.* 5: 383–392, 1995.
99. GÖRLICH, D., S. PREHN, R. A. LASKEY, AND E. HARTMANN. Isolation of a protein that is essential for the first step of nuclear protein import. *Cell* 79: 767–778, 1994.
100. GÖRLICH, D., F. VOGEL, A. D. MILLS, E. HARTMANN, AND R. A. LASKEY. Distinct functions for the two importin subunits in nuclear protein import. *Nature Lond.* 377: 246–248, 1995.
101. GORSCH, L. C., T. C. DOCKENDORFF, AND C. N. COLE. A conditional allele of the novel repeat-containing nucleoporin RAT7/NUP159 causes both rapid cessation of mRNA export and reversible clustering of nuclear pore complexes. *J. Cell Biol.* 129: 939–955, 1995.
102. GOVIND, S., AND R. STEWARD. Dorsoventral pattern formation in *Drosophila*: signal transduction and nuclear targeting. *Trends Genet.* 7: 119–125, 1991.
103. GRANDI, P., V. DOYE, AND E. C. HURT. Purification of NSP1 reveals complex formation with “GLFG” nucleoporins and a novel nuclear pore protein NIC96. *EMBO J.* 12: 3061–3071, 1993.
104. GRANDI, P., N. SCHLAICH, H. TEKOTTE, AND E. C. HURT. Functional interaction of Nic96p with a core nucleoporin complex consisting of Nsp1p, Nup49p and a novel protein Nup57p. *EMBO J.* 14: 76–87, 1995.
105. GRÄSSER, F. A., K.-H. SCHEIDTMAN, P. T. TUAZON, T. A. TRAUGH, AND G. WALTER. In vitro phosphorylation of SV40 large T antigen. *Virology* 165: 13–22, 1988.
106. GREBER, U. F., AND L. GERACE. Nuclear protein import is inhibited by an antibody to a luminal epitope of a pore complex glycoprotein. *J. Cell Biol.* 116: 15–30, 1992.
107. GREBER, U. F., AND L. GERACE. Depletion of calcium from the lumen of endoplasmic reticulum reversibly inhibits passive diffusion and signal-mediated transport into the nucleus. *J. Cell Biol.* 128: 5–14, 1995.
108. GREENSPAN, D., P. PALESE, AND M. KRYSZTAŁ. Two nuclear location signals in the influenza virus NS1 nonstructural protein. *J. Virol.* 62: 3020–3026, 1988.
109. GRENFELL, S. J., J. S. TRAUSCH-AZAR, P. M. HANDLEY-GEAR, HART, A. CIECHANOVER, AND A. L. SCHWARTZ. Nuclear localization of the ubiquitin-activating enzyme, E1, is cell-cycle-dependent. *Biochem. J.* 300: 701–708, 1994.
110. GROßHANS, J., A. BERGMANN, P. HAFTER, AND C. NUSSLIN-VOLHARD. Activation of the kinase *Pelle* by *Tube* in the dorsoventral signal transduction pathway of the *Drosophila* embryo. *Nature Lond.* 372: 563–566, 1994.
111. GRONOWSKI, A. M., AND P. ROTWEIN. Rapid changes in nuclear protein tyrosine phosphorylation after growth hormone treatment in vivo. Identification of phosphorylated mitogen-activated protein kinase and STAT91. *J. Biol. Chem.* 269: 7874–7878, 1994.
112. GUIOCHON-MANTEL, A., K. DELABRE, P. LESCOP, AND E. MILGROM. Nuclear localization signals also mediate the outward movement of proteins from the nucleus. *Proc. Natl. Acad. Sci. USA* 91: 7179–7183, 1994.
113. GUIOCHON-MANTEL, A., P. LESCOP, S. CHRISTIN-MAITRE, H. LOOSFELT, M. PERROT-APPLANAT, AND E. MILGROM. Nucleocytoplasmic shuttling of the progesterone receptor. *EMBO J.* 10: 3851–3859, 1991.
114. GUIOCHON-MANTEL, A., H. LOOSFELT, P. LESCOP, S. SAR, M. ATGER, M. PERROT-APPLANAT, AND E. MILGROM. Mechanism of nuclear localization of the progesterone receptor: evidence for interaction between monomers. *Cell* 57: 1147–1154, 1989.
115. GÜSSE, M., J. GHYSDAEL, G. EVAN, T. SOUSSI, AND M. MECHALI. Translocation of a store of maternal cytoplasmic c-myc protein into nuclei during early development. *Mol. Cell. Biol.* 9: 5395–5403, 1989.
116. GUTCH, M. J., C. DALY, AND N. C. REICH. Tyrosine phosphorylation is required for activation of an alpha interferon-stimulated transcription factor. *Proc. Natl. Acad. Sci. USA* 89: 11411–11415, 1992.
117. HALL, N. M., C. CRAIK, AND Y. HIRAKAWA. Homeodomain of yeast repressor alpha 2 contains a nuclear localization signal. *Proc. Natl. Acad. Sci. USA* 87: 6954–6958, 1990.
118. HALL, N. M., L. HEREFORD, AND I. HERSKOWITZ. Targeting of *E. coli* beta-galactosidase to the nucleus in yeast. *Cell* 36: 1057–1065, 1984.
119. HALLBERG, E., R. W. WOZNIAK, AND G. BLOBEL. An integral membrane protein of the pore membrane domain of the nuclear envelope contains a nucleoporin-like region. *J. Cell Biol.* 122: 513–521, 1993.
120. HAMEL, P. A., B. L. COHEN, L. M. SORCE, B. L. GALLIE, AND R. A. PHILLIPS. Hyperphosphorylation of the retinoblastoma gene product is determined by domains outside the simian virus 40 large-T-binding regions. *Mol. Cell. Biol.* 10: 6586–6595, 1990.
121. HAMM, J., E. DARZYNIEWICZ, S. M. TAHLARA, AND I. W. MATTAJ. The trimethylguanosine cap structure of U1 snRNP is a component of a bipartite nuclear targeting signal. *Cell* 62: 569–577, 1990.
122. HAMM, J., AND I. W. MATTAJ. Monomethylated cap structures facilitate RNA export from the nucleus. *Cell* 63: 109–118, 1990.
123. HANNINK, M., AND D. J. DONOGHUE. Biosynthesis of the v-sis gene product: signal sequence cleavage, glycosylation, and proteolytic processing. *Mol. Cell. Biol.* 6: 1343–1348, 1986.
124. HANOVER, J. A. The nuclear pore: at the crossroads. *FASEB J.* 6: 2288–2295, 1992.
125. HASKILL, S., A. A. BEG, S. M. TOMPKINS, J. S. MORRIS, A. D. YUROCHKO, A. SAMPSION-JOHANNES, K. MONDAL, P. RALPH, AND A. S. BALDWIN, JR. Characterization of an immediate-early gene induced in adherent monocytes that encodes I kappa B-like activity. *Cell* 65: 1281–1289, 1991.
126. HATADA, E. N., A. NIETERS, F. G. WULCZYN, M. NAUMANN, R. MEYER, G. NUCIFORA, T. W. MCKEITHAN, AND C. SCHEIDEREIT. The ankyrin repeat domains of the NF-kappa B precursor p105 and the protooncogene bcl-3 act as specific inhibitors of NF-kappa B DNA binding. *Proc. Natl. Acad. Sci. USA* 89: 2489–2493, 1992.
127. HEALD, R., AND F. McKEON. Mutations of phosphorylation sites in lamin A that prevent nuclear lamina disassembly in mitosis. *Cell* 61: 579–589, 1990.
128. HEGDE, J., AND E. C. STEPHENSON. Distribution of swallow protein in egg chambers and embryos of *Drosophila melanogaster*. *Development* 119: 457–470, 1993.
129. HEI, Y. J., X. CHEN, J. DIAMOND, AND J. H. MCNEILL. Distribution

- of MAP kinase, S6 kinase, and casein kinase II in rat tissues: activation by insulin in spleen. *Biochem. Cell Biol.* 72: 49–53, 1994.
130. HENKEL, T., U. ZABEL, K. VAN ZEE, J. M. MÜLLER, E. FANNING, AND P. A. BAEUERLE. Intramolecular masking of the nuclear localization signal and dimerization domain in the precursor for the p50 NF- κ B subunit. *Cell* 68: 1121–1133, 1992.
 131. HENNEKES, H., M. PETER, K. WEBER, AND E. A. NIGG. Phosphorylation of protein kinase C sites inhibits nuclear import of Lamin B₂. *J. Cell Biol.* 120: 1293–1304, 1993.
 132. HIGASHI, K., AND H. OGAWARA. Daidzein inhibits insulin- or insulin-like growth factor-1-mediated signaling in cell cycle progression of Swiss 3T3 cells. *Biochim. Biophys. Acta* 1221: 29–35, 1994.
 133. HIRAI, H., T. SUZUKI, J. FUJISAWA, J. INOUE, AND M. YOSHIDA. Tax protein of human T-cell leukemia virus type I binds to the ankyrin motifs of inhibitory factor kappa B and induces nuclear translocation of transcription factor NF-kappa B proteins for transcriptional activation. *Proc. Natl. Acad. Sci. USA* 91: 3584–3588, 1994.
 134. HOCEVAR, B. A., AND A. P. FIELDS. Selective translocation of b₁-protein kinase C to the nucleus of human promyeolocytic (HL60) leukemia cells. *J. Biol. Chem.* 266: 28–33, 1991.
 135. HOCEVAR, B. A., D. M. MORROW, M. L. TYKOCINSKI, AND A. P. FIELDS. Protein kinase C isotypes in human erythroleukemia cell proliferation and differentiation. *J. Cell Sci.* 101: 671–679, 1992.
 136. HOWARD, E. A., J. R. ZUPAN, V. CITOVSKY, AND P. ZAMBRYSKI. The VirD2 protein of *A. tumefaciens* contains a C-terminal bipartite nuclear localization signal: implications for nuclear uptake of DNA in plant cells. *Cell* 68: 109–118, 1992.
 137. HSU, S.-C., M. QI, AND D. B. DE FRANCO. Cell cycle regulation of glucocorticoid receptor function. *EMBO J.* 11: 3457–3468, 1992.
 138. IIURT, E. C. Targeting of a cytosolic protein to the nuclear periphery. *J. Cell Biol.* 111: 2829–2837, 1990.
 139. IGARSHI, K., M. DAVID, D. S. FINNBLOOM, AND A. C. LARNER. In vitro activation of the transcription factor gamma interferon activation factor by gamma interferon: evidence for a tyrosine phosphatase/kinase signaling cascade. *Mol. Cell. Biol.* 13: 1634–1640, 1993.
 140. IMAMOTO, N., T. SHIMAMOTO, T. TAKAO, T. TACHIBANA, S. KOSE, M. MATSUBAE, T. SEKIMOTO, Y. SHIMONISHI, AND Y. YONEDA. In vivo evidence for involvement of a 58 kDa component of nuclear pore-targeting complex in nuclear protein import. *EMBO J.* 14: 3617–3626, 1995.
 141. IMAMOTO-SONOBE, N., Y. MATSUOKA, T. KURIHARA, K. KOHNO, M. MIYAGI, F. SAKIYAMA, Y. OKADA, S. TSUNASAWA, AND Y. YONEDA. Antibodies to 70kD heat shock cognate protein inhibit mediated nuclear import of karyophilic proteins. *J. Cell Biol.* 119: 1047–1061, 1992.
 142. IMAMOTO-SONOBE, N., Y. MATSUOKA, T. SEMBA, Y. OKADA, T. UCHIDA, AND Y. YONEDA. A protein recognized by antibodies to Asp-Asp-Asp-Glu-Asp shows specific binding activities to heterogeneous nuclear transport signals. *J. Biol. Chem.* 265: 16504–16508, 1990.
 143. INOUE, J., L. D. KERR, A. KAKIZUKA, AND I. M. VERMA. I_kB γ , a 70 kd protein identical to the C-terminal half of p110 NF- κ B: a new member of the I_kB family. *Cell* 68: 1109–1120, 1992.
 144. JAMES, G., AND E. OLSON. Deletion of the regulatory domain of protein kinase C α exposes regions in the hinge and catalytic domains that mediate nuclear targeting. *J. Cell Biol.* 116: 863–874, 1989.
 145. JANS, D. A. The mobile receptor hypothesis revisited: a mechanistic role for hormone receptor lateral mobility in signal transduction. *Biochim. Biophys. Acta* 1113: 271–276, 1992.
 146. JANS, D. A. Nuclear signalling pathways for polypeptide ligands and their membrane-receptors? *FASEB J.* 8: 841–847, 1994.
 147. JANS, D. A. Phosphorylation-mediated regulation of signal-dependent nuclear protein transport—the “CcN motif.” *Membr. Protein Transp.* 2: 161–199, 1995.
 148. JANS, D. A. The regulation of protein transport to the nucleus by phosphorylation. *Biochem. J.* 311: 705–716, 1995.
 149. JANS, D. A., M. ACKERMANN, J. R. BISCHOFF, D. H. BEACH, AND R. PETERS. p34^{cdc2}-mediated phosphorylation at T¹²⁴ inhibits nuclear import of SV40 T-antigen proteins. *J. Cell Biol.* 115: 1203–1212, 1991.
 150. JANS, D. A., AND P. JANS. Negative charge at the casein kinase II site flanking the nuclear localization signal of the SV40 large T-antigen is mechanistically important for enhanced nuclear import. *Oncogene* 9: 2961–2968, 1994.
 151. JANS, D. A., T. MOLL, K. NASMYTH, AND P. JANS. Cyclin-dependent kinase site-regulated signal-dependent nuclear localization of the yeast transcription factor SWI5 in mammalian cells. *J. Biol. Chem.* 270: 17064–17067, 1995.
 152. JARNIK, M., AND U. AEBI. Towards a more complete 3-D structure of the nuclear pore complex. *J. Struct. Biol.* 107: 237–249, 1991.
 153. JIANG, C. K., S. FLANAGAN, M. OHTSUKI, K. SHUAI, M. FREEDBERG, AND M. BLUMENBERG. Disease-activated transcription factor: allergic reactions in human skin cause nuclear translocation of STAT-91 and induce synthesis of keratin K17. *Mol. Cell. Biol.* 14: 4759–4769, 1994.
 154. JOHNSON, A., C.-H. HELDIN, A. WASTESON, B. WESTERMARK, T. F. DEUEL, J. S. HUANG, P. H. SEEBURG, A. GRAY, A. ULLRICH, C. SCRACE, P. STROOBANT, AND W. D. WATERFIELD. The c-sis gene encodes a precursor of the B chain of platelet-derived growth factor. *EMBO J.* 3: 921–928, 1984.
 155. KALDERON, D., W. D. RICHARDSON, A. F. MARKHAM, AND A. E. SMITH. Sequence requirements for nuclear location of simian virus 40 large T-antigen. *Nature Lond.* 311: 109–118, 1984.
 156. KALDERON, D., B. L. ROBERTS, W. D. RICHARDSON, AND A. E. SMITH. A short amino acid sequence able to specify nuclear location. *Cell* 39: 499–509, 1984.
 157. KAMBACH, C., AND I. W. MATTAJ. Nuclear transport of the U2 snRNP-specific U2B protein is mediated by both direct and indirect signalling mechanisms. *J. Cell Sci.* 107: 1807–1816, 1994.
 158. KAMBACH, C., AND I. W. MATTAJ. Intracellular distribution of the U1A protein depends on active transport and nuclear binding to U1 snRNA. *J. Cell Biol.* 118: 11–21, 1992.
 159. KANG, K. I., D. J. DEVIN, F. CADEPOND, N. GIBARD, A. GUIOCHON-MANTEL, E.-E. BAULIEU AND M. G. CATELLI. In vivo functional protein-protein interaction: nuclear targeted hsp90 shifts cytoplasmic steroid receptor mutants into the nucleus. *Proc. Natl. Acad. Sci. USA* 91: 340–344, 1994.
 160. KIDD, S. Characterization of the *Drosophila cactus* locus and analysis of interactions between *cactus* and dorsal proteins. *Cell* 71: 623–635, 1992.
 161. KIEFER, P., P. ACLAND, D. PAPPIN, G. PETERS, AND C. DICKSON. Competition between nuclear localization and secretory signals determines the subcellular fate of a single CUG-initiated form of FGF3. *EMBO J.* 13: 4126–4136, 1994.
 162. KING, M. W., J. M. ROBERTS, AND R. N. EISENMAN. Expression of the c-myc proto-oncogene during development of *Xenopus laevis*. *Mol. Cell. Biol.* 6: 4499–4508, 1986.
 163. KIPREOS, E. T., AND J. Y. WANG. Differential phosphorylation of c-Abl in cell cycle determined by cdc2 kinase and phosphatase activity. *Science Wash. DC* 248: 217–220, 1990.
 164. KITTEN, G. T., AND E. A. NIGG. The CaaX motif is required for isoprenylation, carboxyl methylation and nuclear membrane association of lamin B2. *J. Cell Biol.* 113: 13–23, 1991.
 165. KLEINSCHMIDT, J. A., AND A. SEITER. Identification of domains involved in nuclear uptake and histone binding of nuclear protein N1/N2. *EMBO J.* 7: 1605–1614, 1988.
 166. KORNBLUTH, S., M. DASSO, AND J. NEWPORT. Evidence for a dual role for TC4 protein in regulating nuclear structure and cell cycle progression. *J. Cell Biol.* 125: 705–719, 1994.
 167. KRAEMER, D., R. W. WOZNIAK, G. BLOBEL, AND A. RADU. 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, 1994.
 168. KRAEMER, D. M., C. STRAMBIO-DE-CASTILLIA, G. BLOBEL, AND M. P. ROUT. The essential yeast nucleoporin NUP159 is located on the cytoplasmic side of the nuclear pore complex protein and serves in karyopherin-mediated binding of transport substrate. *J. Biol. Chem.* 270: 19017–19021, 1995.
 169. KREBS, E. G., E. A. EISENMAN, E. A. KUENZEL, D. W. LICHTFIELD, F. J. LOZEMAN, B. LÜSCHER, AND J. SOMMERCORN. Casein kinase II as a potentially important enzyme concerned with

- signal transduction. *Cold Spring Harbor Symp. Quant. Biol.* III: 77–84, 1988.
170. KROHNE, G., S. L. WOLIN, F. D. McKEON, W. W. FRANKE, AND M. W. KIRSCHNER. Nuclear lamin LI of *Xenopus laevis*: cDNA cloning, amino acid sequence and binding specificity of a member of the lamin B subfamily. *EMBO J.* 6: 3801–3808, 1987.
 171. KUBOTA, K., F. J. KEITH, AND N. J. GAY. Relocalization of *Drosophila* dorsal protein can be induced by a rise in cytoplasmic calcium concentration and the expression of constitutively active but not wild-type Toll receptors. *Biochem. J.* 296: 497–503, 1993.
 172. KUENZEL, E. A., J. A. MULLIGAN, J. SOMMERCORN, AND E. G. KREBS. Substrate specificity determinants for casein kinase II as deduced from studies with synthetic peptides. *J. Biol. Chem.* 262: 9136–9140, 1987.
 173. KUESSEL, P., AND M. FRASCH. Pendulin, a *Drosophila* protein with cell cycle-dependent nuclear localization, is required for normal cell proliferation. *J. Cell Biol.* 129: 1491–1507, 1995.
 174. LACASSE, E. C., AND Y. A. LEFEBVRE. Nuclear localization signals overlap DNA- or RNA-binding domains in nucleic acid-binding proteins. *Nucleic Acids Res.* 23: 1647–1656, 1995.
 175. LAMY, F., F. WILKIN, M. BAPTIST, P. P. ROGER, AND J. E. DUMONT. Phosphorylation of mitogen-activated protein kinases is involved in the epidermal growth factor and phorbol ester, but not in the thyrotropin/cAMP, thyroid mitogenic pathway. *J. Biol. Chem.* 268: 8398–8401, 1993.
 176. LANDSMAN, D., AND M. BUSTIN. A signature for the HMG-1 box DNA-binding proteins. *Bioessays* 15: 539–546, 1993.
 177. LANFORD, R. E., AND J. S. BUTEL. Construction and characterization of an SV40 mutant defective in nuclear transport of T-antigen. *Cell* 37: 801–813, 1984.
 178. LANFORD, R. E., C. M. FELDHERR, R. G. WHITE, R. G. DUNHAM, AND P. KANDA. Comparison of diverse transport signals in synthetic peptide-induced nuclear transport. *Exp. Cell Res.* 186: 32–38, 1990.
 179. LANFORD, R. E., P. KANDA, AND R. C. KENNEDY. Induction of nuclear transport with a synthetic peptide homologous to the SV40 T antigen transport signal. *Cell* 46: 575–582, 1986.
 180. LEACH, K. L., E. A. POWERS, V. A. RUFF, S. JAKEN, AND S. KAUFMANN. Type 3 protein kinase C localization to the nuclear envelope of phorbol ester-treated NIH 3T3 cells. *J. Cell Biol.* 109: 685–695, 1992.
 181. LEACH, K. L., V. A. RUFF, M. B. JARPE, L. D. ADAMS, D. FABBRO, AND D. M. RABEN. α -Thrombin stimulates nuclear diglyceride levels and differential nuclear localization of protein kinase C isozymes in IIC9 cells. *J. Biol. Chem.* 267: 21816–21822, 1992.
 182. LEE, W.-C., AND T. MELESE. Identification and characterization of a nuclear localization sequence-binding protein in yeast. *Proc. Natl. Acad. Sci. USA* 86: 8808–8812, 1989.
 183. LEE, W.-C., Z. XUE, AND T. MELESE. The NSR1 gene encodes a protein that specifically binds nuclear localization sequences and has two RNA binding motifs. *J. Cell Biol.* 113: 1–12, 1991.
 184. LEE, W.-C., D. ZABETAKIS, AND T. MELESE. NSR1 is required for pre-rRNA processing and for the proper maintenance of steady-state levels of ribosomal subunits. *Mol. Cell. Biol.* 12: 3865–3871, 1992.
 185. LENARDO, M. J., AND D. BALTIMORE. NF-kappa B: a pleiotropic mediator of inducible and tissue-specific gene control. *Cell* 58: 227–229, 1989.
 186. LEVY, D. E., D. S. KESSLER, R. PINE, AND J. E. DARRELL. Cytoplasmic activation of ISGF3, the positive regulator of interferon-alpha-stimulated transcription, reconstituted in vitro. *Genes Dev.* 3: 1362–1371, 1989.
 187. LEWIS, M. J., AND H. R. PELHAM. Involvement of ATP in the nuclear and nucleolar functions of the 70 kd heat shock protein. *EMBO J.* 4: 3137–3143, 1985.
 188. LI, H., AND P. M. BINGHAM. Arginine-serine-rich domains of the *su(w^a)* and *tra* RNA processing regulators target proteins to a subnuclear compartment implicated in splicing. *Cell* 67: 335–342, 1991.
 189. LI, J.-M., A. K. HOPPER, AND N. C. MARTIN. *N*₂,*N*₂-dimethylguanosine-specific tRNA methyltransferase contains both nuclear and mitochondrial targeting signals in *Saccharomyces cerevisiae*. *J. Cell Biol.* 109: 1411–1419, 1989.
 190. LI, R., Y. SHI, AND J. O. THOMAS. Intracellular distribution of a nuclear localization signal binding protein. *Exp. Cell Res.* 202: 355–365, 1992.
 191. LI, R., AND J. O. THOMAS. Identification of a human protein that interacts with nuclear localization signals. *J. Cell Biol.* 109: 2623–2632, 1989.
 192. LI, X., AND L. D. ETKIN. *Xenopus* nuclear factor 7 (xnf7) possesses an NLS that functions efficiently in both oocytes and embryos. *J. Cell Sci.* 105: 389–395, 1993.
 193. LI, X., W. SHOU, B. A. REDDY, M. KLOC, AND L. D. ETKIN. Cytoplasmic retention of *Xenopus* nuclear factor 7 before the mid blastula transition uses a unique anchoring mechanism involving a retention domain and several phosphorylation sites. *J. Cell Biol.* 124: 1–17, 1994.
 194. LIN, Y.-C., K. BROWN, AND U. SIEBENLIST. Activation of NF- κ B requires proteolysis of the inhibitor I κ B- α ; signal induced phosphorylation of I κ B- α alone does not release NF- κ B. *Proc. Natl. Acad. Sci. USA* 92: 552–556, 1995.
 195. LIOU, H.-C., G. P. NOLAN, S. GHOSH, T. FUJITA, AND D. BALTIMORE. The NF-kappa B p50 precursor, p105, contains an internal I kappa B-like inhibitor that preferentially inhibits p50. *EMBO J.* 11: 3003–3009, 1992.
 196. LIU, J. FK506 and cyclosporin: molecular probes for studying intracellular signal transduction. *Trends Pharmacol. Sci.* 14: 182–188, 1993.
 197. LLAMAZARES, S., A. MOREIRA, A. TAVARES, C. GIRDHAM, B. A. SPRUCE, C. GONZALEZ, R. E. KARESS, D. M. GLOVER, AND C. E. SUNKEL. Polo encodes a protein kinase homolog required for mitosis in *Drosophila*. *Genes Dev.* 5: 2153–2165, 1991.
 198. LOBIE, P. E., T. J. J. WOOD, C. M. CHEN, M. J. WATERS, AND G. J. NORSTEDT. Nuclear translocation and anchorage of the growth hormone receptor. *J. Biol. Chem.* 269: 31735–31746, 1994.
 199. LOEWINGER, L., AND F. McKEON. Mutations in the nuclear lamin proteins resulting in their aberrant assembly in the cytoplasm. *EMBO J.* 7: 2301–2309, 1988.
 200. LORENZ, P., R. PEPPERKOK, W. ANSORGE, AND W. PYERIN. Cell biological studies with monoclonal and polyclonal antibodies against human casein kinase II subunit beta demonstrate participation of the kinase in mitogenic signaling. *J. Biol. Chem.* 268: 2733–2739, 1993.
 201. LÜSCHER, B., AND R. N. EISENMAN. Mitosis-specific phosphorylation of the nuclear oncoproteins Myc and Myb. *J. Cell Biol.* 118: 775–784, 1992.
 202. LÜSCHER, B., E. A. KÜNZEL, E. G. KREBS, AND R. N. EISENMAN. Myc oncoproteins are phosphorylated by casein kinase II. *EMBO J.* 8: 1111–1119, 1989.
 203. LYONS, R. H., B. Q. FERGUSON, AND M. ROSENBERG. Pentapeptide nuclear localization signal in adenovirus E1A. *Mol. Cell Biochem.* 7: 2451–2456, 1987.
 204. MACAULAY, C., E. MEIER, AND D. J. FORBES. Differential mitotic phosphorylation of proteins of the nuclear pore complex. *J. Biol. Chem.* 270: 254–262, 1995.
 205. MAEDA, Y., K. HISATAKE, T. KONDO, K. HANADA, C.-Z. SONG, T. NISHIMURA, AND M. MURAMATSU. Mouse rRNA gene transcription factor mUBF requires both HMG-box1 and an acidic tail for nucleolar accumulation: molecular analysis of the nucleolar targeting mechanism. *EMBO J.* 11: 3695–3704, 1992.
 206. MAHER, D. W., B. A. LEE, AND D. J. DONOGHUE. The alternatively spliced exon of the platelet-derived growth factor A chain encodes a nuclear targeting signal. *Mol. Cell. Biol.* 9: 2251–2253, 1989.
 207. MANDELL, R. B., AND C. M. FELDHERR. Identification of two HSP70-related *Xenopus* oocyte proteins that are capable of recycling across the nuclear envelope. *J. Cell Biol.* 111: 1775–1784, 1991.
 208. MANDELL, R. B., AND C. M. FELDHERR. The effect of carboxy terminal deletions on the nuclear transport rate of rat hsc70. *Exp. Cell Res.* 198: 164–169, 1992.
 209. MANROW, R. E., A. R. SBURLATI, J. A. HANOVER, AND S. L. BERGER. Nuclear targeting of prothymosin alpha. *J. Biol. Chem.* 266: 3916–3924, 1991.
 210. MARSHALLSAY, C., AND R. LÜHRMANN. In vitro nuclear import of snRNPs: cytosolic factors mediate m₉G-cap dependence of U3 and U2 snRNP transport. *EMBO J.* 13: 222–231, 1994.

211. MARTIN, K., AND A. HELENIUS. Nuclear transport of influenza virus ribonucleoproteins: the viral matrix protein (M1) promotes export and inhibits import. *Cell* 67: 117–130, 1991.
212. MARTINEZ, J., I. GEORGOFF, J. MARTINEZ, AND A. J. LEVINE. Cellular localization and cell cycle regulation by a temperature-sensitive p53 protein. *Genes Dev.* 5: 151–159, 1991.
213. MATHENY, C., M. C. DAY, AND J. MILBRANDT. The nuclear localization signal of NGF1-A is located within the Zinc finger binding domain. *J. Biol. Chem.* 269: 8176–8181, 1994.
214. McKEON, F. D. Nuclear lamin proteins: domains required for nuclear targeting, assembly, and cell-cycle regulated dynamics. *Curr. Opin. Cell Biol.* 3: 82–86, 1991.
215. MCVEY, D., L. BRIZUELA, I. MOHR, D. R. MARSHAK, Y. GLUZMAN, AND D. BEACH. Phosphorylation of large tumour antigen by cdc2 stimulates SV40 DNA replication. *Nature Lond.* 341: 503–507, 1989.
216. MEEK, D. W., S. SIMON, U. KIKKAWA, AND W. ECKHART. The p53 tumour suppressor protein is phosphorylated at serine 389 by casein kinase II. *EMBO J.* 9: 3253–3260, 1990.
217. MEIER, U. T., AND G. BLOBEL. A nuclear localization signal binding protein in the nucleolus. *J. Cell Biol.* 111: 2235–2245, 1990.
218. MEIER, U. T., AND G. BLOBEL. Nopp140 shuttles on tracks between nucleolus and cytoplasm. *Cell* 70: 127–138, 1992.
219. MEINKOTH, J. L., Y. JI, S. S. TAYLOR, AND J. R. FERAMISCO. Dynamics of the distribution of cyclic AMP-dependent protein kinase in living cells. *Proc. Natl. Acad. Sci. USA* 87: 9595–9599, 1990.
220. MEISNER, H., AND M. P. CZECH. Phosphorylation of transcriptional factors and cell-cycle-dependent proteins by casein kinase II. *Curr. Opin. Cell Biol.* 3: 474–483, 1991.
221. MELCHIOR, F., B. PASCHAL, J. EVANS, AND L. GERACE. 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, 1993.
222. METZ, R., AND E. ZIFF. cAMP stimulates the C/EBP-related transcription factor rNFIL-6 to translocate to the nucleus and induce c-fos transcription. *Genes Dev.* 5: 1754–1766, 1991.
223. MICHAUD, N., AND D. GOLDFARB. Microinjected U snRNPs are imported into oocyte nuclei via the nuclear pore complex by three distinguishable targeting pathways. *J. Cell Biol.* 116: 851–861, 1992.
224. MIYAMOTO, S., M. MAKI, M. J. SCHMITT, M. HATANAKA, AND I. M. VERMA. Tumor necrosis factor α -induced phosphorylation of IkB α is a signal for its degradation but not dissociation from NF- κ B. *Proc. Natl. Acad. Sci. USA* 91: 12740–12744, 1994.
225. MOLL, T., G. TEBB, U. SURANA, H. ROBITSCH, AND K. NASYMTA. 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, 1991.
226. MONTECUCCO, A., E. SAVINI, F. WEIGHARDT, R. ROSSI, G. CIARROCCHI, A. VILLA, AND G. BIAMONTI. The N-terminal domain of human DNA ligase I contains the nuclear localization signal and directs the enzyme to sites of DNA replication. *EMBO J.* 14: 5379–5386, 1995.
227. MOORE, M. S., AND G. BLOBEL. 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, 1992.
228. MOORE, M. S., AND G. BLOBEL. The GTP-binding protein Ran/TC4 is required for protein import into the nucleus. *Nature Lond.* 365: 661–663, 1993.
229. MOORE, M. S., AND G. BLOBEL. Purification of a Ran-interacting protein that is required for protein import into the nucleus. *Proc. Natl. Acad. Sci. USA* 91: 10212–10216, 1994.
230. MOORE, M. S., AND G. BLOBEL. A G protein involved in nucleocytoplasmic transport: the role of Ran. *Trends Biochem. Sci.* 19: 211–216, 1994.
231. MORELAND, R. B., H. G. LAM, L. M. HEREFORD, AND H. M. FRIED. Identification of a nuclear localization signal of a yeast ribosomal protein. *Proc. Natl. Acad. Sci. USA* 82: 6561–6565, 1985.
232. MORELAND, R. B., G. L. LANGEVIN, R. H. SINGER, R. L. GARCEA, AND L. M. HEREFORD. Amino acid sequences that determine the nuclear localization of yeast histone 2B. *Mol. Cell. Biol.* 7: 4048–4057, 1987.
233. MORIANU, J., AND G. BLOBEL. Protein export from the nucleus requires the GTPase Ran and GTP hydrolysis. *Proc. Natl. Acad. Sci. USA* 92: 4318–4322, 1995.
234. MORIANU, J., G. BLOBEL, AND A. RADU. 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, 1995.
235. MORIANU, J., M. HIIKATA, G. BLOBEL, AND A. RADU. Mammalian karyopherin $\alpha_1\beta$ and $\alpha_2\beta$ heterodimers: α_1 or α_2 subunit binds nuclear localization signal and β subunit interacts with peptide repeat-containing nucleoporins. *Proc. Natl. Acad. Sci. USA* 91: 6592–6596, 1995.
236. MORIN, N., C. DELSERT, AND D. F. KLESSIG. Nuclear localization of the adenovirus DNA-binding protein: requirement for two signals and complementation during viral infection. *Mol. Cell. Biol.* 9: 4372–4380, 1989.
237. MOSIALOS, G., P. HAMER, A. J. CAPOBIANCO, R. A. LAURSEN, AND T. D. GILMORE. A protein-kinase-A recognition sequence is structurally linked to transformation by p59^{rel} and cytoplasmic retention of p68^{rel}. *Mol. Cell. Biol.* 11: 5867–5877, 1991.
238. MUKAIGAWA, J., AND D. P. NAYAK. Two signals mediate nuclear localization of influenza virus (A/WSN/33) polymerase basic protein 2. *J. Virol.* 65: 245–253, 1991.
239. NAGAI, Y., S. KANEDA, K. NOMURA, H. YASUDA, T. SENO, AND F. YAMO. Ubiquitin-activating enzyme, E1, is phosphorylated in mammalian cell by the protein kinase cdc2. *J. Cell Sci.* 108: 2145–2152, 1995.
240. NATH, S. T., AND D. P. NAYAK. Function of two discrete regions is required for nuclear localization of polymerase basic protein 1 of A/WSN33 influenza virus (H1N1). *Mol. Cell. Biol.* 10: 4139–4145, 1990.
241. NEHRBASS, U., H. KERN, A. MUTVEI, B. HORSTMANN, B. MARSHALLSAY, AND E. C. HURT. NSP1: a yeast nuclear envelope protein localized at the nuclear pores exerts its essential function by its carboxy-terminal domain. *Cell* 61: 979–989, 1990.
242. NELSON, M., AND P. A. SILVER. Context affects nuclear protein localization in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 9: 384–389, 1989.
243. NEWMEYER, D. D., D. R. FINLAY, AND D. J. FORBES. In vitro transport of a fluorescent nuclear protein and exclusion of non-nuclear proteins. *J. Cell Biol.* 103: 2091–2102, 1986.
244. NEWMEYER, D. D., AND D. J. FORBES. Nuclear import can be separated into distinct steps in vitro: nuclear pore binding and translocation. *Cell* 52: 641–653, 1988.
245. NEWMEYER, D. D., AND D. J. FORBES. An N-ethylmaleimide-sensitive cytosolic factor necessary for nuclear protein import: requirement in signal-mediated binding to the nuclear pore. *J. Cell Biol.* 110: 547–557, 1990.
246. NIETO, A., S. DE LA LUNA, J. BARCENA, A. PORTELA, AND J. ORTIN. Complex structure of the nuclear translocation signal of influenza virus polymerase PA subunit. *J. Gen. Virol.* 75: 29–36, 1994.
247. NIGG, E. A., H. HILZ, H. M. EPPENBERGER, AND F. DULTY. Rapid and reversible translocation of the catalytic subunit of cAMP-dependent protein kinase type II from the Golgi complex to the nucleus. *EMBO J.* 4: 2801–2807, 1985.
248. NISHIDA, E., K. IIDA, N. YONEZAWA, S. KOYASU, I. YAHARA, AND H. SAKAI. Cofilin is a component of intranuclear and cytoplasmic actin rods induced in cultured cells. *Proc. Natl. Acad. Sci. USA* 84: 5262–5266, 1987.
249. NOLAN, G. P. NF-AT-AP-1 and Rel-bZIP: hybrid vigor and binding under the influence. *Cell* 77: 795–798, 1994.
250. NORRIS, J. L., AND J. L. MANLEY. Selective nuclear transport of the *Drosophila* morphogen dorsal can be established by a signaling pathway involving the transmembrane protein Toll and protein kinase A. *Genes Dev.* 6: 1654–1667, 1992.
251. NORRIS, J. L., AND J. L. MANLEY. Regulation of dorsal in cultured cells by Toll and tube: tube function involves a novel mechanism. *Genes Dev.* 9: 358–369, 1995.
252. OHNO, H., G. TAKIMOTO, AND T. W. MCKEITHAN. The candidate proto-oncogene bcl-3 is related to genes implicated in cell lineage determination and cell cycle control. *Cell* 60: 991–997, 1990.
253. OHTA, Y., E. NISHIDA, H. SAKAI, AND E. MIYAMOTO. Dephosphor-

- ylation of cofilin accompanies heat shock-induced nuclear accumulation of cofilin. *J. Biol. Chem.* 264: 16143–16148, 1989.
254. O'NEILL, R. E., AND P. PALESE. NPI-1, the human homolog of SRP-1, interacts with influenza virus nucleoprotein. *Virology* 206: 116–125, 1995.
255. OOKATA, K., S. HISANAGA, T. OKANO, K. TCHIBANA, AND T. KISHIMOTO. Relocation and distinct subcellular localization of p34^{cdc2}-cyclin B complex at meiosis reinitiation in starfish oocytes. *EMBO J.* 11: 1763–1772, 1992.
256. OU, J.-H., C.-T. YEH, AND T. S. B. YEN. Transport of hepatitis B virus precursor protein into the nucleus after cleavage of its signal peptide. *J. Virol.* 63: 5238–5243, 1989.
257. PALOMBELLA, V. J., O. J. RANDO, A. L. GOLDBERG, AND T. MANIATIS. The ubiquitin-proteasome pathway is required for processing the NF- κ B1 precursor protein and the activation of NF- κ B. *Cell* 78: 773–785, 1994.
258. PANDEY, S., A. A. KARANDE, K. MISHRA, AND V. K. PARNAIK. Inhibition of nuclear protein import by a monoclonal antibody against a novel class of nuclear pore proteins. *Exp. Cell Res.* 212: 243–254, 1994.
259. PASCHAL, B. M., AND L. GERACE. Identification of NTF2, a cytosolic factor for nuclear import that interacts with nuclear pore complex protein p62. *J. Cell Biol.* 129: 925–937, 1995.
260. PEARSON, D., E. A. NIGG, Y. NAGAMINE, D. A. JANS, AND B. A. HEMMINGS. Mechanisms of cAMP-mediated gene induction: examination of renal epithelial cell mutants affected in the catalytic subunit of the cAMP-dependent protein kinase. *Exp. Cell Res.* 192: 315–318, 1991.
261. PECULIS, B. A., AND J. G. GALL. Localization of the nucleolar protein NO38 in amphibian oocytes. *J. Cell Biol.* 116: 1–14, 1992.
262. PEIFER, M., S. BEG, AND A. B. REYNOLDS. A repeating amino acid motif shared by proteins with diverse roles. *Cell* 76: 789–791, 1994.
263. PEPPERKOK, R., P. LORENZ, W. ANSORGE, AND W. PYERIN. Casein kinase II is required for transition of G₀/G₁, early G₁, and G₁/S phases of the cell cycle. *J. Biol. Chem.* 269: 6986–6991, 1994.
264. PEPPERKOK, R., P. LORENZ, R. JAKOBI, W. ANSORGE, AND W. PYERIN. Cell growth stimulation by EGF: inhibition through antisense-oligodeoxynucleotides demonstrates important role of casein kinase II. *Exp. Cell Res.* 197: 245–253, 1991.
265. PETRUCCO, S., P. K. WELLAUER, AND O. HAGENBÜCHLE. The DNA-binding activity of transcription factor PTF1 parallels the synthesis of pancreas-specific mRNAs during mouse development. *Mol. Cell. Biol.* 10: 254–264, 1990.
266. PICARD, D., V. KUMAR, P. CHAMBON, AND K. R. YAMAMOTO. Signal transduction by steroid hormones: nuclear localization is differentially regulated in estrogen and glucocorticoid receptors. *Mol. Biol. Cell* 1: 291–299, 1990.
267. PICARD, D., S. J. SALSER, AND K. R. YAMAMOTO. A movable and regulatable inactivation function within the steroid binding domain of the glucocorticoid receptor. *Cell* 54: 1073–1080, 1988.
268. PICARD, D., AND K. R. YAMAMOTO. Two signals mediate hormone-dependent nuclear localization of the glucocorticoid receptor. *EMBO J.* 6: 3333–3340, 1987.
269. PINES, J., AND T. HUNTER. Human cyclins A and B1 are differentially located in the cell and undergo cell cycle-dependent nuclear transport. *J. Cell Biol.* 115: 1–17, 1991.
270. PINES, J., AND T. HUNTER. The differential localization of human cyclins A and B is due to a cytoplasmic retention signal in cyclin B. *EMBO J.* 13: 3772–3781, 1994.
271. PINNA, L. A. Casein kinase 2: an "eminence grise" in cellular regulation? *Biochim. Biophys. Acta* 1054: 267–284, 1990.
272. PINOL-ROMA, S., AND G. DREYFUSS. Shuttling of pre-mRNA binding proteins between nucleus and cytoplasm. *Nature Lond.* 355: 730–732, 1992.
273. POULAT, F., F. GIRARD, M.-P. CHEVRON, C. GORE, X. REBIL-LARD, B. CALAS, N. LAMB, AND P. BERTA. Nuclear localization of the testis determining gene product SRY. *J. Cell Biol.* 128: 737–748, 1995.
274. QI, M., B. J. HAMILTON, AND D. DE FRANCO. v-Mos oncproteins affect the nuclear retention and reutilization of glucocorticoid receptors. *Mol. Endocrinol.* 3: 1279–1288, 1989.
275. RADU, A., G. BLOBEL, AND M. S. MOORE. 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, 1995.
276. RADU, A., M. S. MOORE, AND G. BLOBEL. The peptide repeat domain of nucleoporin Nup98 functions as a docking site in transport across the nucleare pore complex. *Cell* 81: 215–222, 1995.
277. RAIKHEL, N. V. Nuclear targeting in plants. *Plant Physiol.* 100: 1627–1632, 1992.
278. RECH, J., I. BARIAT, J. L. VEYRUNE, A. VIE, AND J. M. BLANCHARD. Nuclear import of serum response factor (SRF) requires short amino-terminal nuclear localization sequence and is independent of the casein kinase II phosphorylation site. *J. Cell Sci.* 107: 3029–3036, 1994.
279. REDDY, B. A., M. KLOC, AND L. D. ETKIN. The cloning and characterization of a maternally expressed novel zinc finger nuclear phosphoprotein (xnf7) in *Xenopus laevis*. *Dev. Biol.* 148: 107–116, 1991.
280. REINHARD, C., A. FERNANDEZ, N. J. C. LAMB, AND G. THOMAS. Nuclear localization of p85s6k: functional requirement for entry into S phase. *EMBO J.* 13: 1557–1565, 1994.
281. RICHARDSON, W. D., A. D. MILLS, S. M. DILWORTH, R. A. LASKEY, AND C. DINGWALL. Nuclear protein migration involves two steps: rapid binding at the nuclear envelope followed by slower translocation through nuclear pores. *Cell* 52: 655–664, 1988.
282. RICHARDSON, W. D., B. L. ROBERTS, AND A. E. SMITH. Nuclear location signals in polyoma virus large-T. *Cell* 44: 77–85, 1986.
283. RIHS, H.-P., D. A. JANS, H. FAN, AND R. PETERS. The rate of nuclear cytoplasmic protein transport is determined by a casein kinase II site flanking the nuclear localization sequence of the SV40 T-antigen. *EMBO J.* 10: 633–639, 1991.
284. RIHS, H.-P., AND R. PETERS. Nuclear transport kinetics depend on phosphorylation-site-containing sequences flanking the karyophilic signal of the Simian virus 40 T-antigen. *EMBO J.* 8: 1479–1484, 1989.
285. RIVIERE, Y., V. BLANK, P. KOURILSKY, AND A. ISRAEL. Processing of the precursor of NF- κ B by the HIV-1 protease during acute infection. *Nature Lond.* 350: 625–626, 1991.
286. ROBBINS, J., S. M. DILWORTH, R. A. LASKEY, AND C. DINGWALL. Two interdependent basic domains in nucleoplasmic nuclear targeting sequence: identification of a class of bipartite nuclear targeting sequence. *Cell* 64: 615–623, 1991.
287. ROBERTS, B. Nuclear location signal-mediated protein transport. *Biochim. Biophys. Acta* 1008: 263–280, 1989.
288. ROBERTS, B., W. D. RICHARDSON, AND A. E. SMITH. The effect of protein context on nuclear location signal function. *Cell* 50: 465–475, 1987.
289. ROMAC, J. M.-J., O. H. GRAFF, AND J. D. KEENE. The U1 small nuclear ribonucleoprotein (snRNP) 70K protein is transported independently of U1 snRNP particles via a nuclear localization signal in the RNA-binding domain. *Mol. Cell. Biol.* 14: 4662–4670, 1994.
290. ROMAC, J. M.-J., AND J. D. KEENE. Overexpression of the arginine-rich carboxy-terminal region of U1 snRNP 70K inhibits both splicing and nucleocytoplasmic transport of mRNA. *Genes Dev.* 9: 1400–1410, 1995.
291. ROTH, S., D. STEIN, AND C. NÜSSLIN-VOLHARD. A gradient of nuclear localization of the dorsal protein determines dorsoventral pattern in the *Drosophila* embryo. *Cell* 59: 1189–1202, 1989.
292. ROUX, P., J.-M. BLANCHARD, A. FERNANDEZ, N. LAMB, P. JEANTEUR, AND M. PIECHACZYK. Nuclear localization of c-fos, but not v-fos proteins, is controlled by extracellular signals. *Cell* 63: 341–351, 1990.
293. RUFF, V. A., AND K. L. LEACH. Direct demonstration of NFATp dephosphorylation and nuclear localization in activated HT-2 cells using a specific NFATp polyclonal antibody. *J. Biol. Chem.* 270: 22602–22607, 1995.
294. RUFF-JAMISON, S., K. CHEN, AND S. COHEN. Induction by EGF and interferon-gamma of tyrosine phosphorylated DNA binding proteins in mouse liver nuclei. *Science Wash. DC* 261: 1733–1736, 1993.
295. RUFF-JAMISON, S., K. CHEN, AND S. COHEN. Epidermal growth factor induces the tyrosine phosphorylation and nuclear translocation of Stat-5 in mouse liver. *Proc. Natl. Acad. Sci. USA* 92: 4215–4218, 1995.
296. RUFF-JAMISON, S., Z. ZHONG, Z. WEN, K. CHEN, J. E. DARNELL,

- AND S. COHEN. Induction by EGF and interferon-gamma of tyrosine phosphorylated DNA binding proteins in mouse liver nuclei. *J. Biol. Chem.* 269: 21933–21935, 1994.
297. RUPP, R. A. W., L. SNIDER, AND H. WEINRAUB. *Xenopus* embryos regulate the nuclear localization of XMyoD. *Genes Dev.* 8: 1311–1323, 1994.
298. RUSHLOW, C. A., K. HAN, J. L. MANLEY, AND M. LEVINE. The graded distribution of the dorsal morphogen is initiated by selective nuclear transport in *Drosophila*. *Cell* 59: 1165–1177, 1989.
299. RYAN, J. J., E. PROCHOWNIK, C. A. GOTTLIEB, I. J. APEL, R. MERINO, G. NUNEZ, AND M. F. CLARKE. c-Myc and bcl-2 modulate p53 function by altering p53 subcellular trafficking during the cell cycle. *Proc. Natl. Acad. Sci. USA* 91: 5878–5882, 1994.
300. SADLER, I., A. CHIANG, T. KURIHARA, J. ROTHBLATT, J. WAY, AND P. A. SILVER. A yeast gene important for assembly into the endoplasmic reticulum and the nucleus has homology to DnaJ, an *E. coli* heat shock protein. *J. Cell Biol.* 109: 2665–2675, 1989.
301. SAWYERS, C. L., J. McLAUGHLIN, A. GOGA, M. HAVLIK, AND O. WITTE. The nuclear tyrosine kinase c-Abl negatively regulates cell growth. *Cell* 77: 121–131, 1994.
302. SCHEIDTMAN, K.-H., M. BUCK, J. SCHNEIDER, D. KALDERON, E. FANNING, AND A. E. SMITH. Biochemical characterization of phosphorylation site mutants of simian virus 40 large T antigen: evidence for interaction between amino- and carboxy-terminal domains. *J. Virol.* 65: 1479–1490, 1991.
303. SCHEIDTMAN, K.-H., B. ECHLE, AND G. WALTER. Simian virus 40 large T-antigen is phosphorylated at multiple sites clustered in two separate regions. *Virology* 44: 116–133, 1982.
304. SCHINDLER, C., K. SHUAI, V. R. PREZIOSO, AND J. E. DARNELL, JR. Interferon-dependent tyrosine phosphorylation of a latent cytoplasmic transcription factor. *Science Wash. DC* 257: 809–813, 1992.
305. SCHLENSTEDT, G., C. SAAVEDRA, J. D. LOEB, C. N. COLE, AND P. A. SILVER. 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, 1995.
306. SCHMIDT-ZACHMANN, M. S., C. DARGEMONT, L. C. KÜHN, AND E. A. NIGG. Nuclear export of proteins: the role of nuclear retention. *Cell* 74: 493–504, 1993.
307. SCHMIDT-ZACHMANN, M. S., AND E. A. NIGG. Protein localization to the nucleolus: a search for targeting domains in nucleolin. *J. Cell Sci.* 105: 799–806, 1993.
308. SCHREIBER, J., M. MOLINETE, H. BOEUF, G. DE MURCIA, AND J. MENISSIER-DE MURCIA. The human poly(ADP-ribose) polymerase nuclear localization signal is a bipartite element functionally separate from DNA binding and catalytic activity. *EMBO J.* 11: 3263–3269, 1992.
309. SCHRODER, H. C., M. ROTTMANN, M. BACHMANN, AND W. E. G. MULLER. Purification and characterization of the major nucleoside triphosphatase from rat liver nuclear envelopes. *J. Biol. Chem.* 261: 663–668, 1986.
310. SEYDEL, U., AND D. A. JANS. Evidence for an inhibitory feedback loop regulating SV40 large T-antigen fusion protein nuclear transport. *Biochem. J.* 315: 33–39, 1996.
311. SHAULSKY, G., N. GOLDFINGER, A. BEN-ZE'EV, AND V. ROTTER. Nuclear localization of p53 is mediated by several nuclear localization signals and plays a role in tumorigenesis. *Mol. Cell. Biol.* 10: 6565–6577, 1990.
312. SHAULSKY, G., N. GOLDFINGER, M. S. TOSKY, A. J. LEVINE, AND V. ROTTER. Nuclear localization is essential for the activity of p53 protein. *Oncogene* 6: 2055–2065, 1990.
313. SHEETZ, M. P., E. R. STEUER, AND T. A. SCIROER. The mechanism and regulation of fast axonal transport. *Trends Neurosci.* 12: 474–478, 1989.
314. SHELDON, L. A., AND R. E. KINGSTON. Hydrophobic coiled-coil domains regulate the subcellular localization of the human heat shock factor 2. *Genes Dev.* 7: 1549–1558, 1993.
315. SHELTON, C. A., AND S. A. WASSERMAN. *Pelle* encodes a protein kinase required to establish dorsoventral polarity in the *Drosophila* embryo. *Cell* 72: 515–525, 1993.
316. SHI, Y., AND J. O. THOMAS. The transport of proteins into the nucleus requires the 70-kilodalton heat shock protein or its cytosolic cognate. *Mol. Cell. Biol.* 12: 2186–2192, 1992.
317. SHIEH, M. W., S. R. WESSER, AND N. V. RAIKHEL. Nuclear targeting of the maize R protein requires two nuclear localization sequences. *Plant Physiol.* 101: 353–361, 1993.
318. SHIOZAKI, K., AND M. YANAGIDA. Functional dissection of the phosphorylated termini of fission yeast DNA topoisomerase II. *J. Cell Biol.* 119: 1023–1036, 1992.
319. SHIRAKAWA, F., AND S. B. MIZEL. In vitro activation and nuclear translocation of NF- κ B catalyzed by cyclic AMP-dependent protein kinase and protein kinase C. *Mol. Cell. Biol.* 9: 2424–2430, 1989.
320. SHUAI, K., C. SCHINDLER, V. R. PREZIOSO, AND J. E. DARNELL, JR. Activation of transcription by IFN-gamma: tyrosine phosphorylation of a 91-kD DNA binding protein. *Science Wash. DC* 258: 1808–1812, 1992.
321. SHUAI, K., A. ZIEMIECKI, A. F. WILKS, A. G. HARPUR, H. B. SADOWSKI, M. Z. GILMAN, AND J. E. DARNELL. Polypeptide signalling to the nucleus through tyrosine phosphorylation of Jak and Stat proteins. *Nature Lond.* 366: 580–583, 1993.
322. SILVENNOINEN, O., C. SCHINDLER, J. SCHLESSINGER, AND D. E. LEVY. Ras-independent growth factor signaling by transcription factor tyrosine phosphorylation. *Science Wash. DC* 261: 1736–1739, 1993.
323. SILVER, P., A. CHIANG, AND I. SADLER. Mutations that alter both localization and production of a yeast nuclear protein. *Genes Dev.* 2: 707–717, 1988.
324. SILVER, P., I. SADLER, AND M. A. OSBORNE. Yeast proteins that recognize nuclear localization signals. *J. Cell Biol.* 109: 983–989, 1989.
325. SIMBOLI-CAMPBELL, M., A. M. GAGNON, D. J. FRANKS, AND J.-E. WALSH. 1,25-Dihydroxyvitamin D₃ translocates protein kinase C beta to the nucleus and enhances plasma membrane association of protein kinase C alpha in renal epithelial cells. *J. Biol. Chem.* 269: 3257–3264, 1994.
326. SIMONS, K., AND H. VIRTAN. Perforated MDCK cells support intracellular transport. *EMBO J.* 6: 2241–2247, 1987.
327. SINGLETON, D. R., S. CHEN, M. HITOMI, C. KUMAGI, AND A. M. TARTAKOFF. A yeast protein that bidirectionally affects nucleocytoplasmic transport. *J. Cell Sci.* 108: 265–272, 1995.
328. SIOMI, H., H. SHIDA, S. H. NAM, T. NOSAKA, M. MAKI, AND M. HATANAKA. Sequence requirements for nucleolar localization of human T cell leukemia virus type I pX protein, which regulates viral RNA processing. *Cell* 55: 197–209, 1988.
329. SMITH, M. R., AND W. C. GREENE. Characterization of a novel nuclear localization signal in the HTLV-I tax transactivator protein. *Virology* 187: 316–320, 1992.
330. SOMMER, L., O. HAGENBÜCHLE, P. K. WELLAUER, AND M. STRUBIN. Nuclear targeting of the transcription factor PTF1 is mediated by a protein subunit that does not bind to the PTF1 cognate sequence. *Cell* 67: 987–994, 1991.
331. SRINIVASAN, M., C. F. EDMAN, AND H. SCHULMAN. Alternative splicing introduces a nuclear localization signal that targets multifunctional CaM kinase to the nucleus. *J. Cell Biol.* 126: 839–852, 1994.
332. STANDIFORD, D. M., AND J. D. RICHTER. Analysis of a developmentally regulated nuclear localization signal in *Xenopus*. *J. Cell Biol.* 118: 991–1002, 1992.
333. STARR, C. M., M. D'ONOFRIO, M. K. PARK, AND J. HANOVER. Primary sequence and heterologous expression of nuclear pore glycoprotein p62. *J. Cell Biol.* 110: 1861–1871, 1990.
334. STEWARD, R. Relocalization of the dorsal protein from the cytoplasm to the nucleus correlates with its function. *Cell* 59: 1179–1188, 1989.
335. STOCHAJ, U., M. OSBORNE, T. KURIHARA, AND P. A. SILVER. A yeast protein that binds nuclear localization signals: purification, localization, and antibody inhibition of binding activity. *J. Cell Biol.* 113: 1243–1254, 1991.
336. STOCHAJ, U., AND P. A. SILVER. A conserved phosphoprotein that specifically binds nuclear localization sequences is involved in nuclear import. *J. Cell Biol.* 117: 473–482, 1992.
337. STOCHAJ, U., AND P. A. SILVER. Nucleocytoplasmic traffic of proteins. *Eur. J. Cell Biol.* 59: 1–11, 1993.
338. SUKEGAWA, J., AND G. BLOBEL. A nuclear pore complex protein that contains zinc finger motifs, binds DNA, and faces the nucleoplasm. *Cell* 72: 29–38, 1993.

339. SUZUKI, T., H. HIRAI, T. MURAKAMI, AND M. YOSHIDA. Tax protein of HTLV-1 destabilizes the complexes of NF- κ B and I κ B- α and induces nuclear translocation of NF- κ B for transcriptional activation. *Oncogene* 10: 1199–1207, 1995.
340. TAGAWA, T., T. KUROKI, P. K. VOGT, AND K. CHIDA. The cell cycle-dependent nuclear import of v-jun is regulated by phosphorylation of a serine adjacent to the nuclear localization signal. *J. Cell Biol.* 130: 255–263, 1995.
341. TAKEI, Y., K. TAKAHASHI, Y. KANAHO, AND T. KANADA. Possible involvement of a pertussin toxin-sensitive GTP-binding protein in protein transport into nuclei isolated from rat liver. *J. Biochem.* 115: 578–583, 1994.
342. TAKEMOTO, Y., S. TASHIRO, H. HANNA, AND S. ISHII. Multiple nuclear localization signals of the B-myb gene product. *FEBS Lett.* 350: 55–60, 1994.
343. TEMPLETON, D. J. Nuclear binding of purified retinoblastoma gene product is determined by cell cycle-regulated phosphorylation. *Mol. Cell. Biol.* 12: 435–443, 1992.
344. TEMPLETON, D. J., S. H. PARK, L. LANIER, AND R. A. WEINBERG. Nonfunctional mutants of the retinoblastoma protein are characterized by defects in phosphorylation, viral oncoprotein association, and nuclear tethering. *Proc. Natl. Acad. Sci. USA* 88: 3833–3837, 1991.
345. THOMPSON, J. E., R. J. PHILLIPS, H. ERJUMENT-BROMAGE, P. TEMPST, AND S. GHOSH. I κ B- β regulates the persistent response in biphasic activation of NF- κ B. *Cell* 80: 573–582, 1995.
346. TINLAND, B., Z. KOUKOLIKOVA-NICOLA, M. N. HALL, AND B. HOHN. The T-DNA-linked VirD2 protein contains two distinct functional nuclear localization signals. *Proc. Natl. Acad. Sci. USA* 89: 7442–7446, 1992.
347. TOEROEK, I., D. STRAND, R. SCHMITT, G. TICK, T. TOEROEK, I. KISS, AND B. M. MECHLER. 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, 1995.
348. TOYODA, Y., I. MIWA, S. SATAKE, M. ANAI, AND Y. OKA. Nuclear location of the regulatory protein of glucokinase in rat liver and translocation of the regulator to the cytoplasm in response to high glucose. *Biochem. Biophys. Res. Commun.* 215: 467–473, 1995.
349. TRAECKNER, E. B.-M., H. L. PAHL, T. HENKEL, K. N. SCHMIDT, S. WILK, AND P. A. BAUERLE. Phosphorylation of human I κ B- α on serines 32 and 36 controls I κ B- α proteolysis and NF- κ B activation in response to diverse stimuli. *EMBO J.* 14: 2876–2883, 1995.
350. TRAECKNER, E. B.-M., S. WILK, AND P. A. BAUERLE. A proteasome inhibitor prevents activation of NF- κ B and stabilizes a newly phosphorylated form of I κ B- α that is still bound to NF- κ B. *EMBO J.* 13: 5433–5441, 1994.
351. TRATNER, I., R. OFIR, AND I. M. VERMA. Alteration of a cyclic AMP-dependent protein kinase phosphorylation site in the c-Fos protein augments its transforming potential. *Mol. Cell. Biol.* 12: 998–1006, 1992.
352. TRATNER, I., AND I. M. VERMA. Identification of a nuclear targeting sequence in the Fos protein. *Oncogene* 6: 2049–2053, 1991.
353. TSUNEOKA, M., N. S. IMAMOTO, AND T. UCHIDA. Monoclonal antibody against non-histone chromosomal protein high mobility group 1 co-migrates with High Mobility Group 1 into the nucleus. *J. Biol. Chem.* 261: 1829–1834, 1986.
354. UNDERWOOD, M. R., AND H. M. FRIED. Characterization of nuclear localizing sequences derived from yeast ribosomal protein L29. *EMBO J.* 9: 91–100, 1990.
355. VANCUROVA, I., T. M. PAINE, W. LOU, AND P. L. PAINE. Nucleoplasmic associates with and is phosphorylated by casein kinase II. *J. Cell Sci.* 108: 779–787, 1995.
356. VAN DER KROL, A. R., AND N.-H. CHUA. The basic domain of plant B-ZIP proteins facilitates import of a reporter protein into plant nuclei. *Plant Cell* 3: 667–675, 1991.
357. VANDROMME, M., J.-C. CAVADORE, A. BONNIEU, A. FROESCHLÉ, N. LAMB, AND A. FERNANDEZ. Two nuclear localization signals present in the basic-helix I domains of MyoD promote its active nuclear translocation and can function independently. *Proc. Natl. Acad. Sci. USA* 92: 4646–4650, 1995.
358. VANDROMME, M., G. GARNAC, C. GAUTHIER-ROUVIERE, D. FESQUET, N. LAMB, AND A. FERNANDEZ. Nuclear import of the myogenic factor MyoD requires cAMP-dependent protein kinase activation but not direct phosphorylation of MyoD. *J. Cell Sci.* 107: 631–620, 1994.
359. VAN ETEN, R. A., P. JACKSON, AND D. BALTIMORE. The mouse type IV c-abl gene product is a nuclear protein, and activation of transforming ability is associated with cytoplasmic localization. *Cell* 58: 669–678, 1989.
360. VAN ZHEE, K., F. APPEL, AND E. FANNING. A hydrophobic protein sequence can override a nuclear localization signal independently of protein context. *Mol. Cell. Biol.* 11: 5137–5146, 1991.
361. VARAGONA, M. J., AND N. V. RAIKHEL. The basic domain in the bZIP regulatory protein Opaque 2 serves two independent functions: DNA binding and nuclear localization. *Plant J.* 5: 207–214, 1994.
362. VARAGONA, M. J., R. J. SCHMIDT, AND N. V. RAIKHEL. Nuclear localization signal(s) required for nuclear targeting of the maize regulatory protein opaque-2. *Plant Cell* 4: 1213–1227, 1992.
363. VILLA-MORUZZI, E., AND W. CRABB. Stimulation of FA and casein kinase II by insulin in 3T3-L1 cells. *Biochem. Biophys. Res. Commun.* 177: 1019–1024, 1991.
364. VOIT, R., A. SCHNAPP, A. KUHN, H. ROSENBAUER, P. HIRSCHMANN, H. G. STUNNENBERG, AND I. GRUNT. The nucleolar transcription factor mUBF is phosphorylated by casein kinase II in the C-terminal hyperacidic tail which is essential for transactivation. *EMBO J.* 11: 2211–2218, 1992.
365. WALASCHEWSKI, U., D. A. JANS, D.-C. NEUGEBAUER-GOEDDE, AND R. PETERS. Semi-intact cells for nucleo-cytoplasmic transport studies. *Methods Mol. Cell. Biol.* 5: 87–95, 1995.
366. WANG, Y., J. I. S. MACDONALD, AND C. KENT. Identification of the nuclear localization signal of rat liver CTP: phosphocholine cytidylyltransferase. *J. Biol. Chem.* 270: 354–360, 1995.
367. WANG, X., R. SATO, M. S. BROWN, X. HUA, AND J. L. GOLDSTEIN. SREBP-1, a membrane-bound transcription factor released by sterol-regulated proteolysis. *Cell* 77: 53–62, 1994.
368. WARD, G. E., AND M. W. KIRSCHNER. Identification of cell cycle-regulated phosphorylation sites on nuclear lamin C. *Cell* 61: 561–577, 1990.
369. WEIGHARDT, F., G. BIAMONTI, AND S. RIVA. Nucleo-cytoplasmic distribution of human hnRNP proteins: a search for the targeting domains in hnRNP A1. *J. Cell Sci.* 108: 545–555, 1995.
370. WEIS, K., I. W. MATTAJ, AND A. I. LAMOND. Identification of hSRP1 α as a functional receptor for nuclear localization sequences. *Science Wash. DC* 268: 1049–1053, 1995.
371. WELCH, W. J., AND I. A. MIZZEN. Characterization of the thermotolerant cell. II. Effects on the intracellular distribution of heat-shock protein 70, intermediate filaments, and small nuclear ribonucleoprotein complexes. *J. Cell Biol.* 106: 1117–1130, 1988.
372. WEN, W., J. L. MEINKOTH, R. Y. TSIEN, AND S. S. TAYLOR. Identification of a signal for rapid export of proteins from the nucleus. *Cell* 82: 463–473, 1995.
373. WENTE, S. R., AND G. BLOBEL. 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, 1993.
374. WENTE, S. R., M. P. ROUT, AND G. BLOBEL. A new family of yeast nuclear pore complex proteins. *J. Cell Biol.* 119: 705–723, 1992.
375. WHALEN, A. M., AND R. STEWARD. Dissociation of the dorsal-cactus complex and phosphorylation of the protein correlate with the nuclear localization of dorsal. *J. Cell Biol.* 123: 523–534, 1993.
376. WILKEN, N., U. KOSSNER, J.-L. SENECAL, U. SCHEER, AND M.-C. DABAUVILLE. Nup180, a novel nuclear pore complex protein localizing to the cytoplasmic ring and associated fibrils. *J. Cell Biol.* 123: 1345–1354, 1993.
377. WIMMER, C., V. DOYLE, P. GRANDI, U. NEHRBASS, AND E. C. HURT. A new subclass of nucleoporins that functionally interacts with nuclear pore protein NSP1. *EMBO J.* 11: 5051–5061, 1992.
378. WOZNIAK, R. W., E. BARTNIK, AND G. BLOBEL. Primary structure analysis of an integral membrane protein of the nuclear pore. *J. Cell Biol.* 108: 2083–2092, 1989.
379. WOZNIAK, R. W., AND G. BLOBEL. The single transmembrane segment of gp210 is sufficient for sorting to the pore membrane domain of the nuclear envelope. *J. Cell Biol.* 119: 1441–1449, 1992.
380. WU, J., M. J. MATUNIS, D. KRAEMER, G. BLOBEL, AND E. COUTA-

- VAS. 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, 1995.
381. WULCZYN, F. G., M. NAUMANN, AND C. SCHEIDEREIT. Candidate proto-oncogene *bcl-3* encodes a subunit-specific inhibitor of transcription factor NF- κ B. *Nature Lond.* 358: 597–599, 1992.
382. WYCHOWSKI, C., D. BENICHOU, AND M. GIRARD. A domain of SV40 capsid polypeptide VP1 that specifies migration into the cell nucleus. *EMBO J.* 5: 2569–2576, 1986.
383. XUE, Z., X. SHAN, B. LAPEYRE, AND T. MELESE. The amino terminus of mammalian nucleolin specifically recognises SV40 T-antigen-type nuclear localization sequences. *Eur. J. Cell Biol.* 62: 13–21, 1993.
384. YAMASAKI, L., P. KANDA, AND R. E. LANDFORD. Identification of four nuclear transport signal-binding proteins that interact with diverse transport signals. *Mol. Cell. Biol.* 9: 3028–3036, 1989.
385. YAMASAKI, L., AND R. E. LANDFORD. Nuclear transport: a guide to import receptors. *Trends Cell Biol.* 2: 123–127, 1992.
386. YAN, C., AND T. MÉLESE. Multiple regions of NSR1 are sufficient for accumulation of a fusion protein within the nucleolus. *J. Cell Biol.* 123: 1081–1091, 1993.
387. YANG, J., AND D. B. DE FRANCO. Differential roles of heat shock protein 70 in the in vitro nuclear import of glucocorticoid receptor and simian virus 40 large tumor antigen. *Mol. Cell. Biol.* 14: 5088–5098, 1994.
388. YANO, R., M. OAKES, M. M. TABB, AND M. NOMURA. Yeast SRP1p has homology to armadillo/plakoglobin/ β -catenin and participates in apparently multiple nuclear functions including the maintenance of nucleolar structure. *Proc. Natl. Acad. Sci. USA* 91: 6880–6884, 1992.
389. YANO, R., M. OAKES, M. YAMAGISHI, J. A. DODD, AND M. NOMURA. Cloning and characterization of SRP1, a suppressor of temperature-sensitive RNA polymerase I mutations in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 12: 5640–5651, 1992.
390. YLIKOMI, T., M. T. BOCQUEL, M. BERRY, H. GRONEMEYER, AND P. CHAMBON. Cooperation of proto-signals for nuclear accumulation of estrogen and progesterone receptors. *EMBO J.* 11: 3681–3694, 1992.
391. YOKOYAMA, N., N. HAYASHI, T. SEKI, N. PANTE, T. OHBA, K. NISHII, K. KUMA, T. HAYASHIDA, T. MLYATA, U. AEBL, M. KUKUL, AND T. NISHIMOTO. A giant nucleopore protein that binds Ran/TC4. *Nature Lond.* 376: 184–188, 1995.
392. YONEDA, Y., N. IMAMOTO-SONOBE, Y. MATSUOKA, R. IWAMOTO, Y. KIHO, AND T. UCHIDA. Antibodies to Asp-Asp-Asp-Glu-Asp can inhibit transport of nuclear proteins into the nucleus. *Science Wash. DC* 242: 275–278, 1988.
393. YONEDA, Y., T. SEMBA, Y. KANEDA, R. L. NOBLE, Y. MATSUOKA, T. KURIHARA, Y. OKADA, AND N. IMAMOTO. A long synthetic peptide containing a nuclear localization signal and its flanking sequences of SV40 T-antigen directs the transport of IgM into the nucleus efficiently. *Exp. Cell Res.* 201: 313–320, 1992.
394. ZABEL, U., T. HENKEL, M. DOS SANTOS SILVA, AND P. A. BAEUERLE. Nuclear uptake control of NF- κ B by MAD-3, an I κ B protein present in the nucleus. *EMBO J.* 12: 201–211, 1993.
395. ZACKSENHAUS, E., R. BREMNER, R. A. PHILLIPS, AND B. L. GALLIE. A bipartite nuclear localization signal in the retinoblastoma gene product and its importance for biological activity. *Mol. Cell. Biol.* 13: 4588–4599, 1993.
396. ZERRAHN, J., W. DEPPERT, D. WEIDEMENN, T. PATSCHINSKY, F. RICHARDS, AND J. MILNER. Correlation between the conformational phenotype of p53 and its subcellular location. *Oncogene* 7: 1371–1381, 1992.
397. ZHANG, Q., J. A. DIDONATO, M. KARIN, AND T. W. McKEITHAN. BCL3 encodes a nuclear protein which can alter the subcellular location of NF- κ B proteins. *Mol. Cell. Biol.* 14: 3915–3926, 1994.
398. ZHAO, L.-J., AND R. PADMANABHAN. Nuclear transport of adenovirus DNA polymerase is facilitated by interaction with preterminal protein. *Cell* 55: 1005–1015, 1988.
399. ZHONG, Z., Z. WEN, AND J. E. DARNELL. Stat3: a STAT family member activated by tyrosine phosphorylation in response to epidermal growth factor and interleukin-6. *Science Wash. DC* 264: 95–98, 1994.
400. ZÜRCHER, T., J. PAVLOVIC, AND P. STAHELI. Nuclear localization of mouse Mx1 protein is necessary for inhibition of influenza virus. *J. Virol.* 66: 5059–5066, 1992.