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*Expert Review*

# Controlling Protein Compartmentalization to Overcome Disease

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***Abstract.*** Over the past decade, considerable progress has been made to improve our understanding of the intracellular transport of proteins. Mechanisms of nuclear import and export involving classical receptors have been studied. Signal sequences required for directing a protein molecule to a specific cellular compartment have been defined. Knowledge of subcellular trafficking of proteins has also increased our understanding of diseases caused due to mislocalization of proteins. A specific protein on deviating from its native cellular compartment may result in disease due to loss of its normal functioning and aberrant activity in the "wrong" compartment. Mislocalization of proteins results in diseases that range from metabolic disorders to cancer. In this review we discuss some of the diseases caused due to mislocalization. We further focus on application of nucleocytoplasmic transport to drug delivery. Various rationales to treat diseases by exploiting intracellular transport machinery have been proposed. Although the pathways for intracellular movement of proteins have been defined, these have not been adequately utilized for management of diseases involving mislocalized proteins. This review stresses the need for designing drug delivery systems utilizing these mechanisms as this area is least exploited but offers great potential.

**KEY WORDS:** bi-directional protein switch; mislocalization of proteins; nucleocytoplasmic shuttling; signal sequences; targeting protein compartmentalization for therapy.

## INTRODUCTION

The discovery of the "Signal Hypothesis" led Gunter Blobel to win the 1999 Nobel Prize for Physiology or Medicine (1–4). Blobel discovered that protein "zip codes" exist for directing proteins to subcellular compartments such as the nucleus, cytoplasm, mitochondria, endoplasmic reticulum, lysosomes and endosomes, peroxisomes, golgi, and nucleolus (Table I). Proteins need to be directed to their proper cellular compartments in order to perform their necessary functions. For example, most transcription factors need to be in the nucleus to promote gene expression. Some proteins, such as the glucocorticoid receptor, may start in one compartment (cytoplasm), and move to another compartment (nucleus) in response to a stimulus (ligand).

## SPOTLIGHT ON NUCLEOCYTOPLASMIC SHUTTLING: FROM BASICS TO THERAPEUTIC POTENTIAL

### Regulation via Subcellular Compartmentation and the Nuclear Pore Complex

On the cellular level, macromolecular traffic between the interphase eukaryotic nucleus and cytoplasm of cells represents a highly sophisticated level of cellular regulation that requires effective and selective transport machinery. Protein activity can be regulated by selective import and export (20,21), and "compartmentation allows regulation of key cellular events" (20). Likewise, regulation of nuclear import provides a mechanism for control. Continuing with our example, transcription factors can be kept in the cytoplasm until a signal triggers their import into the nucleus where they can interact with DNA/genes (22). Import and export of proteins occurs through the nuclear pore complex (NPC) (Fig. 1) (20).

The approximately 125 MDa NPC perforates both lipid bilayers of the nuclear envelope (23), forming the exclusive site by which ions, small molecules, and macromolecules must pass. High resolution electron microscopic studies of *Saccharomyces cerevisiae*, *Xenopus laevis*, and rat liver cells have revealed an overall tripartite structure: cytoplasmic filaments, a central transporter, and a nuclear basket, all conferring 8-fold rotational symmetry in the plane normal to the membrane (24) [reviewed in (25–27)]. Eight long cytoplasmic filaments, connected proximally by a coaxial ring, radiate away from plane of the membrane, accounting for one-fourth of the total NPC mass. These projections contain so-called FG repeats, having amino acid repeats in the form of *FXFG*, *GLFG*, or *FG* (where *F* is phenylalanine, *G* is glycine, *L* is leucine, and *X* is any), which interact with karyopharins (family of transport receptors), and deflect non-shuttling proteins (28–31). The cylindrical intramembrane transporter is surrounded by eight spokes which anchor it to the membrane, collectively accounting for nearly half of the entire mass. Central pores allow the passive diffusion of

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**Table I.** Signal Sequences for Subcellular Compartments

| Targeted subcellular compartment | Signal(s) | References |
|---|---|---|
| Nucleus | Monopartite PKKKRKV | (4,5) |
| | Bipartite KRPAATKKAGQAKKKKLDK | (5) |
| Cytoplasm (export from nucleus) | $LX_{(1-3)}$ $LX_{(2-3)}$ LXJ<br>(L = Leu X = spacer J = Leu, Val or Ile) | (6–9) |
| Mitochondria | *M*LSL*R*QSI*R*FF*K*PAT*R*TL<br>(amphipathic α helix)<br>+ charged residues on one side (italics);<br>non-polar residues on the other side (bold) | (10,11) |
| Endoplasmic reticulum (return to ER) | KDEL at C-terminus | (12,13) |
| Lysosomes and endosomes | Tyrosine-based sorting signals: NPXY or YXX<br>Di-leucine-based [DE]XXXL[LI] and DXXLL<br>consensus motifs | (14) |
| Peroxisomes | SKL at C-terminus | (10,15) |
| Trans-golgi network | Di-leucine motif followed by two acidic clusters:<br>LL*EDDSDEEED* (acidic clusters italicized) | (16) |
| Nucleolus | Basic stretches of aa's such as<br>RRRANNRRR<br>KKKMKKHKNKSEAKKRKI | (17–19) |

Underlined residues are critical.

molecules less than 9 nm in diameter (~40 kDa) via an aqueous channel (32), while a gated, iris-like movement allows the selective active transport of macromolecules of up to 39 nm in diameter (40–60 MDa) (33,34). On the nucleoplasmic side, eight long filaments project away from the membrane and connect distally by a ring, forming a basket. The specific proteins that make up the NPC are called nucleoporins (nups). Due to the highly symmetric nature of the structure, only about 100 nups constitute the complex, but many are present in repeats of 8–16 (26,27). Nups and NPCs, however, are not as simple as a three-part structure only involved in nucleocytoplasmic shuttling. Several nups have been shown to shuttle within the cyto- and nucleoplasmic portions of the complex (35–38). Further, nups have been implicated in spindle and kinetochore assembly (39–41), and chromatin organization and transcriptional activation (42).

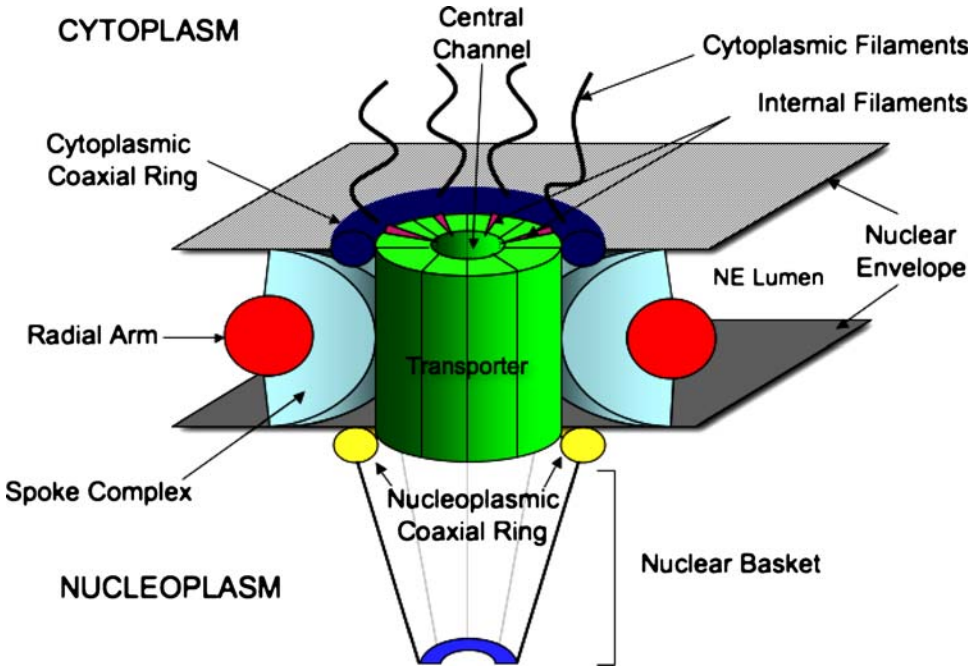


**Fig. 1.** The nuclear pore complex (NPC). Half of the complex is cut away, except with the central transporter which is shown complete. NPCs exhibit octagonal symmetry about the plane perpendicular to the nuclear envelope. Eight filaments stretch into the cytoplasm, directly interacting with the karyopharin family of transporters. Connecting the filaments is a coaxial ring. Within the double membrane, a central transporter with eight repeating proteins is surrounded by a spoke complex. Nucleoplasmic filaments project into the nucleoplasm and connect distally by a ring structure, forming a basket. The NPC is the sole location for translocation across the nucleus' double membrane.

## Nuclear Import and Nuclear Localization Signals

Nuclear import and export utilize non-universal pathways that include many specific proteins. Discussed in this paper are only generalized cycles established for the classical import and export signals. For more detailed reviews, see Görlich *et al*. (20), Weiss (34), Pemberton *et al.* (43), and Macara (44). (Fig. 2) diagrams the generalized import pathway for classical nuclear localization signal-carrying proteins. Initiation of import begins in the cytoplasm, with the recognition of importin α to a nuclear localization signal (NLS). Importin α itself cannot interact with the NPC for translocation, so an adapter protein—importin β—is needed. This three-protein complex translocates across the nuclear envelope into the nucleoplasm. RanGTP binds to the amino-terminus of importin β, resulting in release of the cargo-importin α duplex. Subsequently, the cargo is available to function in the nucleoplasm, while importin α and β are recycled to the cytoplasm through separate RanGTP-dependent events. Back in the cytoplasm, RanGTP is hydrolyzed by RanGAP to RanGDP, releasing bound karyopharins, where another round of import may take place.

Nuclear localization signals (NLSs) were discovered decades ago as the means for active transport of larger macromolecules into the nucleus (45). Proteins containing classical nuclear localization sequences (NLSs) are imported into the nucleus by the importin α/β heterodimer (46). Importin α contains the NLS binding site, whereas importin β mediates the translocation through the nuclear pore (20,47). Ran, a small GTPase which converts from RanGDP to RanGTP in the nucleus, plays a critical role in both import and export. Its nucleotide state is determined by regulators which have opposite localization, and thus a gradient across the membrane can be formed (48). Importins respond to the RanGTP–GDP gradient, approximately 200-fold (49), which is a driving force for the transport of cargo proteins from the cytoplasm to the nucleus (20). Classical NLSs are either a single stretch (monopartite) of basic amino acids like the one from SV40 large T antigen (PKKKRKV; critical residues underlined) (45,50), or bipartite (two short sequences with spacer) basic amino acid stretches like the one from nucleoplasmin (KRPAATKKAGQAKKKKLDK) (5). With NLSs, flanking sequences often are important for localization as well (50). Additional tripartite signals have been identified in some steroid hormone receptors, encompassing a SV-40 monopartite signal within them (51). The human progesterone receptor (PR), like other steroid receptors, contains a constitutively active classical NLS (at position 637–645, RKFKKKFNK) and also contains a non-classical NLS that is ligand inducible ($NLS_i$) (52–54). This non-classical NLS has no apparent common motif compared to classical NLSs, although part of it encodes the second zinc finger of the DNA binding domain (DBD) of PR. PR's $NLS_i$ amino acid sequence is RAMEGQH NYLCAGRNDCIVDKIRRKNCPACRLRKCCQAG MVLGG (from position 593–636). Underlined sequences containing basic amino acids R and K likely interact with importin machinery (55). It has not been fully elucidated what import receptor(s) interact with $NLS_i$, but presumably they must still enter the nucleus via the NPC (54).

## Nuclear Export and Nuclear Export Signals

Importins and exportins both interact with RanGTP by an amino-terminal domain, but have opposite effects on

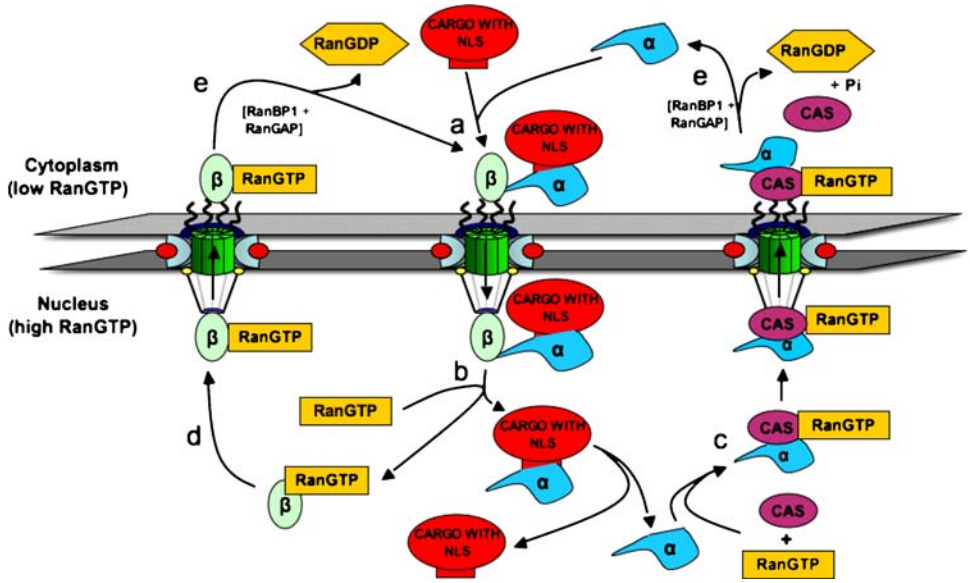


**Fig. 2.** Import of proteins carrying a classical NLS. (a) Initiation of import begins in the cytoplasm, with the recognition of importin α to a nuclear localization signal (NLS). Importin β is needed to translocate the complex across the envelope. (b) Once in the nucleoplasm, RanGTP binds to the amino-terminus of importin β, resulting in release of the cargo-importin α duplex. Subsequently, the cargo is available to function in the nucleoplasm, while importin α and β are recycled to the cytoplasm. (c) CAS and RanGTP are needed to shuttle importin α, (d) while only RanGTP is needed for importin β. Back in the cytoplasm, (e) each RanGTP is hydrolyzed by RanGAP to RanGDP, releasing bound karyopharins, where another round of import may take place. Therefore, two GTPs are needed for import of one protein cargo. Adapted from Görlich *et al.* (20).

protein trafficking. During import, proteins are released upon RanGTP binding in the nucleus. Conversely, in export, cargo proteins only bind to transporters in the presence of RanGTP (Fig. 3). Upon binding, the cargo/exporter/RanGTP complex translocates through to the cytoplasm where RanGAP catalyzes the hyrolysis of a phosphate bond. RanGDP no longer binds to the exporter, and the whole complex dissociates. RanGDP is imported to the nucleus by NTF2, preserving the Ran-nucleotide gradient. RCC1 in the nucleus exchanges GDP for GTP, and now the cycle is ready for another turn (20,34,43,44). CRM1 (exportin 1) is the classical export receptor for cargos proteins containing leucine rich cargos NESs (20,56). The formation of a CRM1-cargo complex requires RanGTP. Export by CRM1 is saturable (20,57,58). Another exporter is calreticulin (CRT) (59) which may function as an alternative exporter for proteins (such as steroid receptors) with leucine rich NESs (60–63). CRT interacts with cargo in a RanGTP dependent manner. However, Walther *et al.* have suggested that CRT can only export steroid receptors under stress conditions (64). Classical NESs are ~10 amino acid sequences with hydrophobic residues, including leucine. We have noted a common consensus NES is *LX*(1–3) *LX*(2–3) *LXJ* where *L* = Leu *X* = spacer *J* = Leu, Val or Ile (6), in agreement with others (7–9).

## Import, Export, and Other Signals: Application to Drug Delivery

Using signal sequences for precise drug delivery is attractive due to the potential for drugs to be targeted to specific cellular compartments. Peptide NESs have been attached to oligonucleotides for delivery to their site of action, the cytoplasm (65), while NLSs can be used to enhance non-viral gene transfer (66). Similarly, the M9 shuttling (import/export) signal sequence attached to cationic peptides have been used as a delivery system for plasmid DNA (67). An excellent review summarizes the nuclear import, export, and shuttling signals, and their application to drug delivery (68). In all of the examples mentioned here, signals have been used unidirectionally to target a drug—be it an oligonucleotide, polymer or plasmid—to a final compartment.

Known signal sequences are in part defined by their ability to confer localization to a particular compartment, even when taken out of context of the whole protein. Hodel *et al.* show that various NLSs linked to GFP can be used as a model system to test nuclear import (5), while we have shown the same for NESs to test nuclear export (6).

When proteins mislocalize to the wrong compartment, disease can occur (Table II). Many types of cancers arise from mislocalized proteins (69). For example, tumor suppressors that mislocalize to the cytoplasm in cancer cells include p53 (69,70) and INI1/hSNF5 (71,72). For p53, the tumor suppressor activity of this protein is suppressed due to its inability to localize in the nucleus. It has been reported that mutations of p53 exist in half of all human cancers, leading to inactivation (69,73). On the other end of the spectrum, the nuclear localization of oncoprotein Ras may be important in oncogenic activation as well (74).

Similarly, the mislocalization of cell cycle inhibitors can be detrimental. Relocalization of a cell cycle inhibitor to the "wrong" compartment can lead to tumor progression. This occurs with the cell cycle inhibitor $p21^{WAF-1}$ which normally localizes in the nucleus where it exerts its inhibitory action. Mislocalization of this protein to the cytoplasm leads to tumor progression (75).

G-protein coupled receptors (GPCRs) can also mislocalize and cause disease (76). Specific examples include mislocalized rhodopsin which can lead to retinitis pigmentosa, and mistargeted vasopressin $V_2$ receptor leading to nephrogenic diabetes insipidus. Finally, defects in trafficking or localization of the LDL receptor can cause familial hypercholesterolemia (77), and mislocalized CTFR leads to

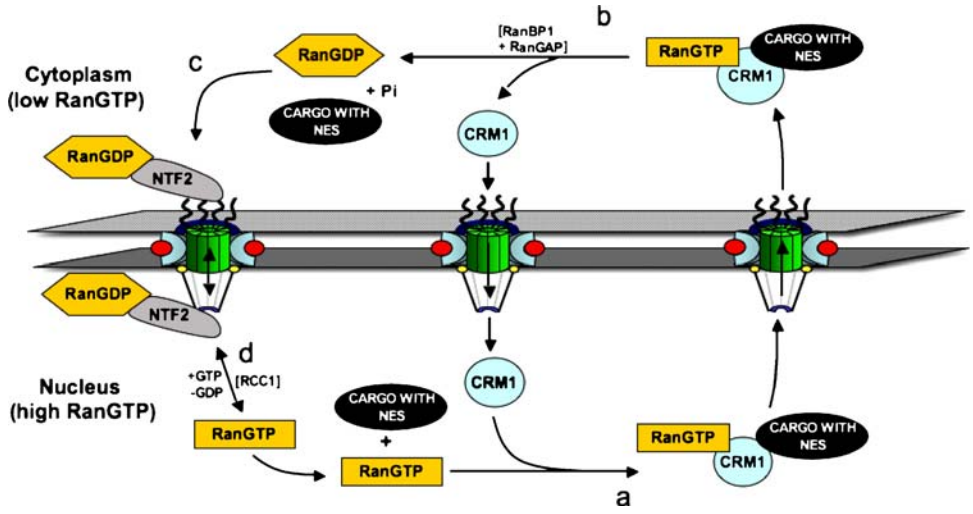


**Fig. 3.** Classical export of proteins from the nucleus. (a) Export beings with CRM1, the main export protein, binding a nuclear export signal (NES) on a protein cargo. CRM1 can only bind cargo in the presence of RanGTP. Once complexed, translocation to the cytoplasm takes place. (b) RanGAP and RanBP1 catalyze the hydrolysis of a phosphate bond, creating RanGDP and resulting in the dissociation of CRM1 with its cargo. (c) RanGDP is shuttled back to the nucleus via NTF2 and (d) GDP is exchanged for GTP by RCC1. Adapted from Görlich *et al.* (20).

**Table II.** Proteins whose Mislocalization Causes Disease

| Disease caused by mislocalization | Type of protein | Name of protein(s) | Normal localization | Mislocalization | References |
|---|---|---|---|---|---|
| Various types of cancers | Tumor suppressor | p53 | Nucleus | Cytoplasm | (70,71) |
| Various types of cancers | Member of the beta-galactoside binding protein family | Galectin-3 | Nucleus | Cytoplasmic when phosphorylated | (79) |
| Various types of cancers | Forkhead family of transcription factors | FOXO | Nucleus | Cytoplasm | (69,80) |
| Various types of cancers | Transcription factor | NF-κB | Cytoplasm | Nucleus | (69,81,82) |
| Breast cancer | Tumor suppressors | BARD1 and BRCA1 | Cytoplasm | Dimerization masks NES and causes nuclear accumulation | (83–85) |
| Malignant rhabdoid tumors | Tumor suppressor | INI1/hSNF5 | Nucleus | Cytoplasm | (69,71,72) |
| Cisplatin drug resistance | Multidrug resistant proteins | MRP1 | Recycled to plasma membrane | Cytoplasmic accumulation | (86) |
| Acute myelogenous leukemia (AML) | Cyclin-dependent kinase inhibitor | p27Kip1 | Nucleus | Cytoplasm | (87) |
| AML | Multifunctional nucleocytoplasmic shuttling protein | Nucleophosmin | Nucleolus | Cytoplasm | (88) |
| Colorectal cancer | Multifunctional protein; part of the Wnt signal transduction pathway | β-catenin | Cytoplasm | Nucleus | (69,89) |
| Chronic myelogenous leukemia (CML) | Bcr—a GTPase activating protein; Abl—a tyrosine kinase oncogene | Bcr-Abl | Bcr alone is cytoplasmic; Abl is nuclear and cytoplasmic | Cytoplasm | (90) |
| CML | Cell cycle inhibitor | $p21^{WAF1}$ | Nucleus | Cytoplasm | (75) |
| Retinitis pigmentosa (Class I deletion mutant) | G-protein coupled receptor (GPCR) | rhodopsin | Membrane sacs within the rod | Plasma membrane of photoreceptor cell body | (76,91) |
| Nephrogenic diabetes insipidus, X-linked (Class I and II) | GPCR | Vasopressin $V_2$ receptor | Cell surface | Majority of mutations lead to intracellular retention | (76,92) |
| Familial hypercholesterolemia (Class II and IV) | GPCR | LDL receptor | Cell surface and internalized | Class II—retained in ER; Class IV—defective internalization | (77) |
| Cystic fibrosis (some forms) | GPCR | CTFR | Cell surface | Endoplasmic reticulum | (76,78) |
| Schizophrenia | GPCR | Dopamine D3 receptor (mutant) | Plasma membrane | Intracellular compartment | (93) |
| Primary dystonia (DYT1) | *A*TPase associated with different cellular activities protein family | Torsin A | ER luminal protein | Nuclear envelope | (94) |
| Primary hyperoxaluria | Aminotransferase | Alanine:glyoxylate amino-transferase | Peroxisome | Mitochondria | (95) |
| Stargardt-like macular degeneration | Fatty acid elongase family | ELOVL4 | ER | Cytoplasm (5 bp deletion mutant) | (96) |

cystic fibrosis (78). See Table II for other examples of diseases caused by mislocalization.

## SPECIFIC DISEASES INDUCED BY MISLOCALIZED PROTEINS

### Breast Cancer: BARD1 and BRCA1

The breast cancer-associated protein BARD1 (thought to be a tumor suppressor) in itself contains proapoptotic activity when in the cytoplasm. BARD1 contains a nuclear export signal that allows compartmentalization into the cytoplasm. However, the breast and ovarian cancer susceptibility protein 1 (BRCA1), a known tumor suppressor, can modulate BARD1 apoptotic activity by binding to BARD1 and blocking its export signal. This results in a marked reduction of BARD1 apoptotic activity. Interestingly, it was found that "BRCA1 and BARD1 regulate the subcellular localization of one another through the reciprocal masking of their respective nuclear export signals" (84). However, when BARD1 and BRCA1 dimerize, their resulting nuclear compartmentalization leads to events that promote cancer (DNA repair activity and prolonged cell survival) (83–85) instead of apoptosis. In this situation, the delicate balance between nuclear import and export dramatically alters the function of proteins, and this is controlled by the cell.

### Schizophrenia and the Dopamine D3 Receptor

In some forms of schizophrenia, a mutant dopamine D3 receptor has been implicated. This mutant dopamine receptor (D3nf) can physically interact with the normal version of the D3 receptor and causes mislocalization of D3 from the plasma membrane to an intracellular compartment, "a finding that may have significance in the etiology of schizophrenia" (93). In these studies, it was suggested that D3nf may act as a dominant-negative regulator of D3 receptor activity, forcing wild type D3 to mislocalize thereby resulting in disease.

### Leukemia and BCR-ABL

BCR-ABL protein can be converted from an oncogene to an apoptotic factor if it can be sent to the nucleus (90). BCR-ABL is an oncogene implicated in chronic myeloid leukemia and Philadelphia chromosome positive acute lymphoblastic leukemia. BCR-ABL proteins have abnormal tyrosine kinase activity that leads to oncogenesis (97). BCR-ABL proteins are oncogenic in the cytoplasmic compartment only, and must multimerize in order to be active. BCR-ABL, when directed to the nucleus, indeed becomes apoptotic (90), so this is a possible new way to treat CML. These same authors found that inhibiting export of BCR-ABL in cell culture using leptomycin B (LMB), a general export inhibitor (causing nuclear accumulation of BCR-ABL), resulted in cancer cell apoptosis. However, LMB cannot be used clinically due to neuronal toxicity in phase I clinical trials (90).

### Colorectal Cancer and β-Catenin

In most colorectal cancers the adenomatous polypsis coli (APC) protein, a tumor suppressor, is mutated (69,98). In normal cells this protein shuttles between the nucleus and the cytoplasm; however, in colorectal cancer APC is mostly nuclear due to truncations resulting in loss of functional NES. The nuclear export of APC is very critical for its normal functioning in destabilizing and reducing the transcriptional activity of a signal transduction protein β-catenin. Any level of nuclear import of APC reduces the transcriptional activity of β-catenin. The mutations in APC lead to loss of its nuclear export function and inactivation. This is indicated as an early event in tumorigenesis. Loss of APC causes an increase in nuclear β-catenin and its transcriptional activity, which leads to tumor progression (89).

## TARGETING PROTEIN COMPARTMENTALIZATION FOR THERAPY

Targeting nucleocytoplasmic shuttling represents an under-explored area for drug delivery, drug targeting, and therapeutics (99,100). As the mechanisms of mislocalization are being elucidated, there exist more opportunities for drug therapy besides the standard direct inhibition (or activation) of the protein target. There are several ways in which overcoming aberrant localization of protein can be achieved and are outlined as follows.

### Blocking General Nuclear Import or Export Machinery

If a disease is caused by mislocalization of a protein to the nucleus (or cytoplasm), inhibitors of general import (or export) could be utilized for treatment. To date, there are no small molecule inhibitors of nuclear import; however, there are several small molecule inhibitors of nuclear export. These are inhibitors of CRM1, the general export receptor. Leptomycin A and B (101), Ratjodone A (102,103), and PFK050-637 (104) are all small molecule inhibitors of CRM1 that bind to a critical cysteine residue in CRM1 to abrogate nuclear export. Another possible way to inhibit import or export would be to supply peptides encoding a NLS or NES, and competitively inhibit import/export of other cargo containing the NLS/NES signals. Hawiger and coworkers have designed cyclic peptides containing a cell-penetrating motif and a cyclized form of a NLS from NF-kB. This peptide inhibits import of NF-kB (105). The main disadvantages with blocking general nuclear import or export would be toxicity due to general halting of transport of all proteins going into/out of the nucleus, like Leptomycin B (90,106).

### Enhancing Import or Export of Proteins

Proteins given therapeutically may be modified so that import (or export) is enhanced. NLSs and NESs have routinely been added onto proteins to enhance or modify their cellular destination; for an excellent review see Jans *et al*. (68). The main disadvantage with adding proteins with NLSs/NESs is that the patient still expresses the mislocalized protein, and in some cases, the mislocalized protein exhibits a dominant effect over the non-mislocalized protein (93).

Another way to enhance import/export is to increase the interactions of proteins with the components of the NPC. Molecular proteomics methods are being used to determine

interactions of proteins with nucleoporins (107). This information could be used to enhance or block import (or export).

### Alteration of Post-Translational Modifications

It is known that protein modifications including phosphorylation, methylation, and ubiquitinylation can lead to altered compartmentalization of proteins in cells (69). Galectin-3 (Gal-3), a novel regulator of apoptosis, translocates from the nucleus to the cytoplasm under apoptotic stimuli. Phosphorylation enhances export of Gal-3 to the cytoplasm. Interestingly, an increase in the cytoplasmic levels of Gal-3 (and loss from the nuclei) correlates with tumor progression (79).

### Blocking Import/Export Partner

Many proteins are imported/exported via piggyback mechanisms with other proteins (and not via direct interactions with the actual import/export receptors). Piggybacking of proteins may occur with heat shock protein 90 (Hsp90). Hsp90, a molecular chaperone, is known to retain steroid hormone receptors (and other proteins) in the cytoplasm and may be involved in nuclear import of other proteins (108). In another case mitogen activated protein kinase (MAPK) may be involved in regulating nuclear association of the human progesterone receptor (109). The import/export partner itself (Hsp90 or MAPK) likely still interacts with the general import/export machinery.

### Controlled Localization using a Protein Switch

Ligand inducible nuclear import signals and export signals (a bi-directional on/off switch) can be used for the controlled targeting of therapeutic proteins to subcellular compartments. Our work has shown that a model protein (EGFP in this case) can be directed from the cytoplasm to the nucleus using ligand, in a dose-dependent manner (110). The protein is genetically engineered to constitute a nuclear export signal, a nuclear import signal, and a ligand binding domain (LBD) from a steroid hormone receptor. When the plasmid encoding this protein is transfected into mammalian cells, the protein is expressed mostly in the cytoplasm. When ligand is added, the protein translocates to the nucleus. The rate and extend of nuclear import depends on the dose of ligand and the incubation time. When ligand is removed from the system, the protein exports back to the cytoplasm. The protein can be re-imported again by addition of ligand (110). Since our initial studies, we have optimized the protein switch so that its localization is more cytoplasmic in the absence of ligand, and more of the protein translocates to the nucleus in the presence of ligand (unpublished data). Optimization involves striking the desired balance between nuclear import and export signals of different strengths, and utilizing different steroid hormone receptor LBDs. Such a protein switch could be used in gene therapy, for controlled localization (and function) of a protein such as a transcription factor, or any other type of protein that is active only in the nucleus of the cell. Our first application of the protein switch is for breast cancer, where the nuclear corepressor NCoR will be engineered into the protein switch. NCoR is known to repress both progesterone receptor and estrogen receptor mediated transcriptional activity.

## FUTURE DIRECTIONS FOR CONTROLLING INTRACELLULAR LOCALIZATION OF PROTEINS

As we have outlined in this paper, malfunctioning of nucleocytoplasmic transport leading to mislocalization of proteins causes disease. Thus, manipulation of transport pathways could be used to treat disease. Exploiting the intracellular transport pathways provides an exciting area for treatment of such diseases. Specific signal sequences can be utilized in drug delivery systems to improve the efficacy of a drug by increasing the amount of drug reaching its active therapeutic compartment, or to increase compartment accumulation. Based on the knowledge of intracellular transport, new modalities can be designed targeting specific components of the cellular transport machinery. However, for regulating localization of proteins to treat diseases, further investigation of transport pathways that have not yet been clearly understood is warranted.

The classical pathways of nucleocytoplasmic trafficking have been well defined. Consensus sequences have been proposed for nuclear import and export signals, and their strengths have been characterized. Nevertheless, it has been studied that certain proteins despite having an import (or export) signal are localized to a different compartment. In some cases this occurs because various proteins mutually regulate their localization by masking and de-masking the localization signal(s). For example, breast-cancer associated protein BRCA1 is involved in masking the export signal of BARD1 via the dimerization domain and keeps it in the nucleus (83,84,111). Another such example involves proteins c-Jun and ATF2, involved in cellular transformation, stress response and regulating organ development. Even though ATF2 possesses a nuclear export signal, dimerization with c-Jun in the nucleus prevents its export (112). In another example, the nuclear export signal of BCR-ABL protein has been suggested to be inactive due to its location in the hydrophobic core of the protein (113). The area of intracellular transport involved with mutual regulation of protein localization by masking or unmasking the localization signal has been the least explored for therapeutic purposes. Designing delivery systems and drugs (small molecules as well as peptides) to mask and unmask the localization signals is a promising avenue for controlling intracellular movement of proteins.

Besides the classical subcellular pathways of transport, there are other mechanisms of protein import/export that have not been completely delineated yet. CRT has been studied to be involved in nuclear export of steroid hormone receptors, independent of the classical CRM1 pathway (60–62). However, some researchers have claimed that CRT acts as an export receptor only under stress conditions (64), and steroid hormone receptors such as glucocorticoid receptor (GR) are indeed exported via a CRM1 dependent transport (114). Another group has proposed that a β helix structure in androgen receptor ligand binding domain acts as a NES (115) though the mechanism for its activity has yet to be delineated. BCR-ABL, a cytoplasmic protein, still localizes to the cytoplasm on removal of its NES (90) which is likely due to interactions between its F-actin binding domain

and the cellular cytoskeleton (113). Similar to nuclear export, it appears that multiple pathways exist for nuclear import as well. In our recent study we have shown that the progesterone receptor (PR) translocates to the nucleus on ligand induction even after mutating its constitutive NLS, albeit at a much slower rate (116). GR has also been shown to constitute two NLSs with one acting via the classical importin α pathway, while the other through an agonist specific pathway independent of importin α (114).

Some proteins are transported between two compartments by a facilitator protein or a "piggy-back" mechanism. Localization of various proteins involved in cellular functioning such as p53 (69,70,117,118), β-catenin (69,89,98) are regulated by other interacting proteins. Heat shock protein (Hsp90), one of the most abundant proteins in cells, is touted to be a chaperone protein involved in intracellular movement of various other proteins (119–121). However, the mechanism of transport of Hsp90 is still not defined. Ligand binding domains of steroid receptors are known to affect change in localization of these molecules but no single mechanism explaining change in their localization has been proposed as of yet. Thus, there are mechanisms of subcellular trafficking that are still needed to be elucidated.

Exploring mechanisms of intracellular transport holds the key to devise a means to treat a large number of diseases ranging from metabolic disorders to cancer. Studying cellular pathways of macromolecule movement would also aid in a more comprehensive understanding of certain known diseases, resulting in management. Harnessing signal sequences to change localization of proteins and hence their activity, is a novel way of finding cure for many diseases. Designing new delivery systems and drug modalities based on the current knowledge of signal sequences and transport pathways, for treatment and management of diseases, is an exciting new area of molecular pharmaceutics that offers great potential.

## ACKNOWLEDGMENTS

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## REFERENCES

1. A Nobel Prize for cell biology. *Nat. Cell Biol.* **1**:E169 (1999).
2. M. Hagmen. Protein ZIP codes make Nobel journey. *Science* **286**:666 (1999).
3. M. T. Heemels. Medicine Nobel goes to pioneer of protein guidance mechanisms. *Nature* **401**:625 (1999).
4. D. Shields. Gunter Blobel—still passionate after all these years. *Trends Cell Biol.* **11**:349–350 (2001).
5. M. R. Hodel, A. H. Corbett, and A. E. Hodel. Dissection of a nuclear localization signal. *J. Biol. Chem.* **276**:1317–1325 (2001).
6. C. Kanwal, H. Li, and C. S. Lim. Model system to study classical nuclear export signals. *AAPS PharmSci*. **4** (2002).
7. H. P. Bogerd, R. A. Fridell, R. E. Benson, J. Hua, and B. R. Cullen. Protein sequence requirements for function of the human T-cell leukemia virus type 1 Rex nuclear export signal delineated by a novel *in vivo* randomization–selection assay. *Mol. Cell Biol.* **16**:4207–4214 (1996).
8. B. R. Henderson and A. Eleftheriou. A comparison of the activity, sequence specificity, and CRM1-dependence of different nuclear export signals. *Exp. Cell Res.* **256**:213–224 (2000).
9. T. Ikuta, H. Eguchi, T. Tachibana, Y. Yoneda, and K. Kawajiri. Nuclear localization and export signals of the human aryl hydrocarbon receptor. *J. Biol. Chem.* **273**:2895–2904 (1998).
10. B. Alberts, A. Johnson, J. Lewis, M. Raff, K. Roberts, and P. Walter. *Molecular Biology of the Cell*, Garland Science (Taylor and Francis Group), 2002.
11. H. K. Anandatheerthavarada, G. Biswas, M. A. Robin, and N. G. Avadhani. Mitochondrial targeting and a novel transmembrane arrest of Alzheimer's amyloid precursor protein impairs mitochondrial function in neuronal cells. *J. Cell Biol.* **161**:41–54 (2003).
12. S. Munro and H. R. Pelham. A C-terminal signal prevents secretion of luminal ER proteins. *Cell* **48**:899–907 (1987).
13. D. A. Andres, I. M. Dickerson, and J. E. Dixon. Variants of the carboxyl-terminal KDEL sequence direct intracellular retention. *J. Biol. Chem.* **265**:5952–5955 (1990).
14. J. S. Bonifacino and L. M. Traub. Signals for sorting of transmembrane proteins to endosomes and lysosomes. *Annu. Rev. Biochem.* **6**:6 (2003).
15. S. J. Gould, G. A. Keller, N. Hosken, J. Wilkinson, and S. Subramani. A conserved tripeptide sorts proteins to peroxisomes. *J. Cell Biol.* **108**:1657–1664 (1989).
16. Q. Zeng, H. T. Tran, H. X. Tan, and W. Hong. The cytoplasmic domain of Vamp4 and Vamp5 is responsible for their correct subcellular targeting: the N-terminal extension of Vamp4 contains a dominant autonomous targeting signal for the trans-Golgi network. *J. Biol. Chem.* **6**:6 (2003).
17. Y. X. Guo, K. Dallmann, and J. Kwang. Identification of nucleolus localization signal of betanodavirus GGNNV protein alpha. *Virology* **306**:225–235 (2003).
18. J. Liu, X. Du, and Y. Ke. Mapping nucleolar localization sequences of 1A6/DRIM. *FEBS Lett.* **580**:1405–1410 (2006).
19. T. Nakamura, H. Imai, N. Tsunashima, and Y. Nakagawa. Molecular cloning and functional expression of nucleolar phospholipid hydroperoxide glutathione peroxidase in mammalian cells. *Biochem. Biophys. Res. Commun.* **311**:139–148 (2003).
20. D. Gorlich and U. Kutay. Transport between the cell nucleus and the cytoplasm. *Annu. Rev. Cell Dev. Biol.* **15**:607–660 (1999).
21. P. Turpin, B. Ossareh-Nazari, and C. Dargemont. Nuclear transport and transcriptional regulation. *FEBS Lett.* **452**:82–86 (1999).
22. J. K. Hood and P. A. Silver. In or out? Regulating nuclear transport. *Curr. Opin. Cell Biol.* **11**:241–247 (1999).
23. R. Reichelt, A. Holzenburg, E. L. Buhle Jr., M. Jarnik, A. Engel, and U. Aebi. Correlation between structure and mass distribution of the nuclear pore complex and of distinct pore complex components. *J. Cell Biol.* **110**:883–894 (1990).
24. C. W. Akey and M. Radermacher. Architecture of the Xenopus nuclear pore complex revealed by three-dimensional cryo-electron microscopy. *J. Cell Biol.* **122**:1–19 (1993).
25. M. Suntharalingam and S. R. Wente. Peering through the pore: nuclear pore complex structure, assembly, and function. *Dev. Cell* **4**:775–789 (2003).
26. T. D. Allen, J. M. Cronshaw, S. Bagley, E. Kiseleva, and M. W. Goldberg. The nuclear pore complex: mediator of translocation between nucleus and cytoplasm. *J. Cell Sci.* **113**:1651–1659 (2000).
27. D. Stoffler, B. Fahrenkrog, and U. Aebi. The nuclear pore complex: from molecular architecture to functional dynamics. *Curr. Opin. Cell Biol.* **11**:391–401 (1999).
28. M. Seedorf, M. Damelin, J. Kahana, T. Taura, and P. A. Silver. Interactions between a nuclear transporter and a subset of nuclear pore complex proteins depend on ran GTPase. *Mol. Cell. Biol.* **19**:1547–1557 (1999).
29. S. Shah, S. Tugendreich, and D. Forbes. Major binding sites for the nuclear import receptor are the internal nucleoporin Nup153 and the adjacent nuclear filament protein Tpr. *J. Cell Biol.* **141**:31–49 (1998).

30. M. K. Iovine and S. R. Wente. A nuclear export signal in Kap95p is required for both recycling the import factor and interaction with the nucleoporin GLFG repeat regions of Nup116p and Nup100p. *J. Cell Biol.* **137**:797–811 (1997).
31. M. K. Iovine, J. L. Watkins, and S. R. Wente. The GLFG repetitive region of the nucleoporin Nup116p interacts with Kap95p, an essential yeast nuclear import factor. *J. Cell Biol.* **131**:1699–1713 (1995).
32. P. L. Paine, L. C. Moore, and S. B. Horowitz. Nuclear envelope permeability. *Nature* **254**:109–114 (1975).
33. C. M. Feldherr and D. Akin. The location of the transport gate in the nuclear pore complex. *J. Cell Sci.* **110**:3065–3070 (1997).
34. K. Weis. Regulating access to the genome: nucleocytoplasmic transport throughout the cell cycle. *Cell* **112**:441–451 (2003).
35. M. E. Lindsay, K. Plafker, A. E. Smith, B. E. Clurman, and G. Macara. Npap60/Nup50 is a Tri-S switch that stimulates importin-[alpha]:[beta]-mediated nuclear protein import. *Cell* **110**:349–360 (2002).
36. E. R. Griffis, N. Altan, J. Lippincott-Schwartz, and M. A. Powers. Nup98 is a mobile nucleoporin with transcription-dependent dynamics. *Mol. Biol. Cell* **13**:1282–1297 (2002).
37. C. E. J. Pritchard, M. Fornerod, L. H. Kasper, and J. M. A. Van Deursen. RAE1 is a shuttling mRNA export factor that binds to a GLEBS-like NUP98 motif at the nuclear pore complex through multiple domains. *J. Cell Biol.* **145**:237–253 (1999).
38. S. Nakielny, S. Shaikh, B. Burke, and G. Dreyfuss. Nup153 is an M9-containing mobile nucleoporin with a novel Ran-binding domain. *EMBO J.* **18**:1982–1995 (1999).
39. M. D. Blower, M. Nachury, R. Heald, and K. Weis. A Rae1-containing ribonucleoprotein complex is required for mitotic spindle assembly. *Cell* **121**:223–234 (2005).
40. N. Belgareh, G. Rabut, S. W. Bai, M. Van Overbeek, J. Beaudouin, N. Daigle, O. V. Zatsepina, F. Pasteau, V. Labas, M. Fromont-Racine, J. Ellenberg, and V. Doye. An evolutionarily conserved NPC subcomplex, which redistributes in part to kinetochores in mammalian cells. *J. Cell Biol.* **154**:1147–1160 (2001).
41. M. Winey, M. A. Hoyt, C. Chan, L. Goetsch, D. Botstein, and B. Byers. NDC1: a nuclear periphery component required for yeast spindle pole body duplication. *J. Cell Biol.* **122**:743–751 (1993).
42. S. Karniely and O. Pines. Single translation–dual destination: mechanisms of dual protein targeting in eukaryotes. *EMBO Rep.* **6**:420–425 (2005).
43. L. F. Pemberton and B. M. Paschal. Mechanisms of receptor-mediated nuclear import and nuclear export. *Traffic* **6**:187–198 (2005).
44. I. G. Macara. Transport into and out of the nucleus. *Microbiol. Mol. Biol. Rev.* **65**:570–594 (2001).
45. D. Kalderon, 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).
46. D. S. Goldfarb, A. H. Corbett, D. A. Mason, M. T. Harreman, and S. A. Adam. Importin [alpha]: a multipurpose nuclear-transport receptor. *Trends Cell Biol.* **14**:505–514 (2004).
47. I. W. Mattaj and L. Englmeier. Nucleocytoplasmic transport: the soluble phase. *Annu. Rev. Biochem.* **67**:265–306 (1998).
48. E. Izaurralde, U. Kutay, C. vonKobbe, I. W. Mattaj, and D. Gorlich. The asymmetric distribution of the constituents of the Ran system is essential for transport into and out of the nucleus. *EMBO J.* **16**:6535–6547 (1997).
49. P. Kalab, K. Weis, and R. Heald. Visualization of a Ran-GTP gradient in interphase and mitotic Xenopus egg extracts. *Science* **295**:2452–2456 (2002).
50. A. Efthymiadis, H. Shao, S. Hubner, and D. A. Jans. Kinetic characterization of the human retinoblastoma protein bipartite nuclear localization sequence (NLS) *in vivo* and *in vitro*. A comparison with the SV40 large T-antigen NLS. *J. Biol. Chem.* **272**:22134–22139 (1997).
51. T. Ylikomi, 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).
52. A. Guiochon-Mantel, H. Loosfelt, P. Lescop, S. Sar, M. Atger, M. Perrot-Applanat, and E. Milgrom. Mechanisms of nuclear localization of the progesterone receptor: evidence for interaction between monomers. *Cell* **57**:1147–1154 (1989).
53. A. Guiochon-Mantel, 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).
54. R. K. Tyagi, L. Amazit, P. Lescop, E. Milgrom, and A. Guiochon-Mantel. Mechanisms of progesterone receptor export from nuclei: role of nuclear localization signal, nuclear export signal, and ran guanosine triphosphate. *Mol. Endocrinol.* **12**:1684–1695 (1998).
55. M. Kohler, C. Speck, M. Christiansen, F. R. Bischoff, S. Prehn, H. Haller, D. Gorlich, and E. Hartmann. Evidence for distinct substrate specificities of importin alpha family members in nuclear protein import. *Mol. Cell Biol.* **19**:7782–7791 (1999).
56. M. Fornerod, M. Ohno, M. Yoshida, and I. W. Mattaj. CRM1 is an export receptor for leucine-rich nuclear export signals. *Cell* **90**:1051–1060 (1997).
57. M. E. Lindsay, J. M. Holaska, K. Welch, B. M. Paschal, and I. G. Macara. Ran-binding protein 3 is a cofactor for Crm1-mediated nuclear protein export. *J. Cell Biol.* **153**:1391–1402 (2001).
58. B. E. Black, J. M. Holaska, L. Levesque, B. Ossareh-Nazari, C. Gwizdek, C. Dargemont, and B. M. Paschal. NXT1 is necessary for the terminal step of Crm1-mediated nuclear export. *J. Cell Biol.* **152**:141–155 (2001).
59. K. H. Krause and M. Michalak. Calreticulin. *Cell* **88**:439–443 (1997).
60. J. M. Holaska, B. E. Black, D. C. Love, J. A. Hanover, J. Leszyk, and B. M. Paschal. Calreticulin is a receptor for nuclear export. *J. Cell Biol.* **152**:127–140 (2001).
61. J. M. Holaska, B. E. Black, F. Rastinejad, and B. M. Paschal. $Ca^{2+}$-dependent nuclear export mediated by calreticulin. *Mol. Cell. Biol.* **22**:6286–6297 (2002).
62. B. E. Black, J. M. Holaska, F. Rastinejad, and B. M. Paschal. DNA binding domains in diverse nuclear receptors function as nuclear export signals. *Curr. Biol.* **11**:1749–1758 (2001).
63. D. B. DeFranco. Nuclear export: DNA-binding domains find a surprising partner. *Curr. Biol.* **11**:R1036–R1037 (2001).
64. R. F. Walther, C. Lamprecht, A. Ridsdale, I. Groulx, S. Lee, Y. A. Lefebvre, and R. J Hache. Nuclear export of the glucocorticoid receptor is accelerated by cell fusion-dependent release of calreticulin. *J. Biol. Chem.* **278**:37858–37864 (2003).
65. L. Meunier, R. Mayer, M. Monsigny, and A. C. Roche. The nuclear export signal-dependent localization of oligonucleopeptides enhances the inhibition of the protein expression from a gene transcribed in cytosol. *Nucleic Acids Res.* **27**:2730–2736 (1999).
66. C. K. Chan and D. A. Jans. Using nuclear targeting signals to enhance non-viral gene transfer. *Immunol. Cell Biol.* **80**: 119–130 (2002).
67. A. Subramanian, P. Ranganathan, and S. L. Diamond. Nuclear targeting peptide scaffolds for lipofection of nondividing mammalian cells. *Nat. Biotechnol.* **17**:873–877 (1999).
68. D. A. Jans, C. K. Chan, and S. Huebner. Signals mediating nuclear targeting and their regulation: application in drug delivery. *Med. Res. Rev.* **18**:189–223 (1998).
69. T. R. Kau, J. C. Way, and P. A. Silver. Nuclear transport and cancer: from mechanism to intervention. *Nat. Rev. Cancer* **4**:106–117 (2004).
70. U. M. Moll, G. Riou, and A. J. Levine. Two distinct mechanisms alter p53 in breast cancer: mutation and nuclear exclusion. *Proc. Natl. Acad. Sci. USA* **89**:7262–7266 (1992).
71. M. Fabbro and B. R. Henderson. Regulation of tumor suppressors by nuclear-cytoplasmic shuttling. *Exp. Cell Res.* **282**:59–69 (2003).
72. E. Craig, Z. K. Zhang, K. P. Davies, and G. V. Kalpana. A masked NES in INI1/hSNF5 mediates hCRM1-dependent nuclear export: implications for tumorigenesis. *EMBO J.* **21**:31–42 (2002).
73. C. C. Harris and M. Hollstein. Clinical implications of the p53 tumor-suppressor gene. *N. Engl. J. Med.* **329**:1318–1327 (1993).
74. G. Wurzer, W. Mosgoeller, M. Chabicovsky, C. Cerni, and J. Wesierska-Gadek. Nuclear Ras: unexpected subcellular distribution of oncogenic forms. *J. Cell Biochem.* **81**:1–11 (2001).

75. K. Keeshan, T. G. Cotter, and S. L. McKenna. Bcr-Abl upregulates cytosolic p21WAF-1/CIP-1 by a phosphoinositide-3-kinase (PI3K)-independent pathway. *Br. J. Haematol.* **123**:34–44 (2003).
76. S. W. Edwards, C. M. Tan, and L. E. Limbird. Localization of G-protein-coupled receptors in health and disease. *Trends Pharmacol. Sci.* **21**:304–308 (2000).
77. H. H. Hobbs, D. W. Russell, M. S. Brown, and J. L. Goldstein. The LDL receptor locus in familial hypercholesterolemia: mutational analysis of a membrane protein. *Annu. Rev. Genet.* **24**:133–170 (1990).
78. M. J. Welsh and A. E. Smith. Molecular mechanisms of CFTR chloride channel dysfunction in cystic fibrosis. *Cell* **73**: 1251–1254 (1993).
79. Y. Takenaka, T. Fukumori, T. Yoshii, N. Oka, H. Inohara, H. R. Kim, R. S. Bresalier, and A. Raz. Nuclear export of phosphorylated galectin-3 regulates its antiapoptotic activity in response to chemotherapeutic drugs. *Mol. Cell Biol.* **24**:4395–4406 (2004).
80. N. Nakamura, S. Ramaswamy, F. Vazquez, S. Signoretti, M. Loda, and W.R. Sellers. Forkhead transcription factors are critical effectors of cell death and cell cycle arrest downstream of PTEN. *Mol. Cell Biol.* **20**:8969–8982 (2000).
81. M. Karin, Y. Cao, F. R. Greten, and Z. W. Li. NF-kappaB in cancer: from innocent bystander to major culprit. *Nat. Rev. Cancer* **2**:301–310 (2002).
82. T. Henkel, U. Zabel, K. van Zee, J. M. Muller, E. Fanning, and P. A. Baeuerle. Intramolecular masking of the nuclear location signal and dimerization domain in the precursor for the p50 NF-kappa B subunit. *Cell* **68**:1121–1133 (1992).
83. M. Fabbro, J. A. Rodriguez, R. Baer, and B. R. Henderson. BARD1 induces BRCA1 intranuclear foci formation by increasing RING-dependent BRCA1 nuclear import and inhibiting BRCA1 nuclear export. *J. Biol. Chem.* **277**: 21315–21324 (2002).
84. J. A. Rodriguez, S. Schuchner, W. W. Au, M. Fabbro, and B. R. Henderson. Nuclear-cytoplasmic shuttling of BARD1 contributes to its proapoptotic activity and is regulated by dimerization with BRCA1. *Oncogene* **23**:1809–1820 (2004).
85. J. A. Rodriguez and B. R. Henderson. Identification of a functional nuclear export sequence in BRCA1. *J. Biol. Chem.* **275**:38589–38596 (2000).
86. X. J. Liang, D. W. Shen, S. Garfield, and M. M. Gottesman. Mislocalization of membrane proteins associated with multidrug resistance in cisplatin-resistant cancer cell lines. *Cancer Res.* **63**:5909–5916 (2003).
87. Y. H. Min, J. W. Cheong, J. Y. Kim, J. I. Eom, S. T. Lee, J. S. Hahn, Y. W. Ko, and M. H. Lee. Cytoplasmic mislocalization of p27Kip1 protein is associated with constitutive phosphorylation of Akt or protein kinase B and poor prognosis in acute myelogenous leukemia. *Cancer Res.* **64**:5225–5231 (2004).
88. E. Colombo, P. Martinelli, R. Zamponi, D. C. Shing, P. Bonetti, L. Luzi, S. Volorio, L. Bernard, G. Pruneri, M. Alcalay, and P. G. Pelicci. Delocalization and destabilization of the Arf tumor suppressor by the leukemia-associated NPM mutant. *Cancer Res.* **66**:3044–3050 (2006).
89. R. Rosin-Arbesfeld, A. Cliffe, T. Brabletz, and M. Bienz. Nuclear export of the APC tumour suppressor controls beta-catenin function in transcription. *EMBO J.* **22**:1101–1113 (2003).
90. P. Vigneri and J. Y. Wang. Induction of apoptosis in chronic myelogenous leukemia cells through nuclear entrapment of BCR-ABL tyrosine kinase. *Nat. Med.* **7**:228–234 (2001).
91. T. P. Dryja and T. Li. Molecular genetics of retinitis pigmentosa. *Hum. Mol. Genet.* **4 Spec No**:1739–1743 (1995).
92. H. Tsukaguchi, H. Matsubara, S. Taketani, Y. Mori, T. Seido, and M. Inada. Binding-, intracellular transport-, and biosynthesis-defective mutants of vasopressin type 2 receptor in patients with X-linked nephrogenic diabetes insipidus. *J. Clin. Invest.* **96**:2043–2050 (1995).
93. K. D. Karpa, R. Lin, N. Kabbani, and R. Levenson. The dopamine D3 receptor interacts with itself and the truncated D3 splice variant d3nf: D3–D3nf interaction causes mislocalization of D3 receptors. *Mol. Pharmacol.* **58**:677–683 (2000).
94. R. E. Goodchild and W. T. Dauer. Mislocalization to the nuclear envelope: an effect of the dystonia-causing torsinA mutation. *Proc. Natl. Acad. Sci. USA* **101**:847–852 (2004).
95. A. Motley, M. J. Lumb, P. B. Oatey, P. R. Jennings, P. A. De Zoysa, R. J. Wanders, H. F. Tabak, and C. J. Danpure. Mammalian alanine/glyoxylate aminotransferase 1 is imported into peroxisomes via the PTS1 translocation pathway. Increased degeneracy and context specificity of the mammalian PTS1 motif and implications for the peroxisome-to-mitochondrion mistargeting of AGT in primary hyperoxaluria type 1. *J. Cell Biol.* **131**:95–109 (1995).
96. G. Karan, Z. Yang, and K. Zhang. Expression of wild type and mutant ELOVL4 in cell culture: subcellular localization and cell viability. *Mol. Vis.* **10**:248–253 (2004).
97. K. M. Kirschner and K. Baltensperger. Erythropoietin promotes resistance against the Abl tyrosine kinase inhibitor imatinib (STI571) in K562 human leukemia cells. *Mol. Cancer Res.* **1**:970–980 (2003).
98. R. Rosin-Arbesfeld, F. Townsley, and M. Bienz. The APC tumour suppressor has a nuclear export function. *Nature* **406**:1009–1012 (2000).
99. D. A. Dean. Nuclear transport: an emerging opportunity for drug targeting. *Adv. Drug Deliv. Rev.* **55**:699–702 (2003).
100. J. Z. Gasiorowski and D. A. Dean. Mechanisms of nuclear transport and interventions. *Adv. Drug Deliv. Rev.* **55**:703–716 (2003).
101. N. Kudo, B. Wolff, T. Sekimoto, E. P. Schreiner, Y. Yoneda, M. Yanagida, S. Horinouchi, and M. Yoshida. Leptomycin B inhibition of signal-mediated nuclear export by direct binding to CRM1. *Exp. Cell Res.* **242**:540–547 (1998).
102. T. Meissner, E. Krause, and U. Vinkemeier. Ratjadone and leptomycin B block CRM1-dependent nuclear export by identical mechanisms. *FEBS Lett.* **576**:27–30 (2004).
103. M. Koster, S. Lykke-Andersen, Y. A. Elnakady, K. Gerth, P. Washausen, G. Hofle, F. Sasse, J. Kjems, and H. Hauser. Ratjadones inhibit nuclear export by blocking CRM1/exportin 1. *Exp. Cell Res.* **286**:321–331 (2003).
104. D. Daelemans, E. Afonina, J. Nilsson, G. Werner, J. Kjems, E. De Clercq, G. N. Pavlakis, and A. M. Vandamme. A synthetic HIV-1 Rev inhibitor interfering with the CRM1-mediated nuclear export. *Proc. Natl. Acad. Sci. USA* **99**:14440–14445 (2002).
105. X. Yan Liu, D. Robinson, R. A. Veach, D. Liu, S. Timmons, R. D. Collins, and J. Hawiger. Peptide-directed suppression of a pro-inflammatory cytokine response. *J. Biol. Chem.* **275**: 16774–16778 (2000).
106. E. S. Newlands, G. J. Rustin, and M. H. Brampton. Phase I trial of elactocin. *Br. J. Cancer* **74**:648–649 (1996).
107. N. P. Allen, S. S. Patel, L. Huang, R. J. Chalkley, A. Burlingame, M. Lutzmann, E. C. Hurt, and M. Rexach. Deciphering networks of protein interactions at the nuclear pore complex. *Mol. Cell Proteomics.* **1**:930–946 (2002).
108. P. Csermely, T. Schnaider, C. Soti, Z. Prohaszka, and G. Nardai. The 90-kDa molecular chaperone family: structure, function, and clinical applications. A comprehensive review. *Pharmacol. Ther.* **79**:129–168 (1998).
109. M. Qiu, A. Olsen, E. Faivre, K. B. Horwitz, and C. A. Lange. Mitogen activated protein kinase regulates nuclear association of human progesterone receptors. *Mol. Endocrinol.* **9**:9 (2003).
110. C. Kanwal, S. Mu, S. E. Kern, and C. S. Lim. Bidirectional on/off switch for controlled targeting of proteins to subcellular compartments. *J. Control. Release* **98**:379–393 (2004).
111. M. Fabbro, S. Schuechner, W. W. Au, and B. R. Henderson. BARD1 regulates BRCA1 apoptotic function by a mechanism involving nuclear retention. *Exp. Cell Res.* **298**:661–673 (2004).
112. H. Liu, X. Deng, Y. J. Shyu, J. J. Li, E. J. Taparowsky, and C. D. Hu. Mutual regulation of c-Jun and ATF2 by transcriptional activation and subcellular localization. *EMBO J.* **25**:1058–1069 (2006).
113. O. Hantschel, S. Wiesner, T. Guttler, C. D. Mackereth, L. L. Rix, Z. Mikes, J. Dehne, D. Gorlich, M. Sattler, and G. Superti-Furga. Structural basis for the cytoskeletal association of Bcr-Abl/c-Abl. *Mol. Cell* **19**:461–473 (2005).
114. J. G. Savory, B. Hsu, I. R. Laquian, W. Giffin, T. Reich, R. J. Hache, and Y. A. Lefebvre. Discrimination between NL1- and NL2-mediated nuclear localization of the glucocorticoid receptor. *Mol. Cell Biol.* **19**:1025–1037 (1999).

115. A. J. Saporita, Q. Zhang, N. Navai, Z. Dincer, J. Hahn, X. Cai, and Z. Wang. Identification and characterization of a ligand-regulated nuclear export signal in androgen receptor. *J. Biol. Chem.* **278**:41998–42005 (2003).
116. H. Li, M. L. Fidler, and C. S. Lim. Effect of initial subcellular localization of progesterone receptor on import kinetics and transcriptional activity. *Mol. Pharm.* **2**:509–518 (2005).
117. D. A. Freedman, C. B. Epstein, J. C. Roth, and A. J. Levine. A genetic approach to mapping the p53 binding site in the MDM2 protein. *Mol. Med.* **3**:248–259 (1997).
118. G. Shaulsky, N. Goldfinger, A. Ben-Ze'ev, and V. Rotter. Nuclear accumulation of p53 protein is mediated by several nuclear localization signals and plays a role in tumorigenesis. *Mol. Cell Biol.* **10**:6565–6577 (1990).
119. W. B. Pratt, U. Gehring, and D. O. Toft. Molecular chaperoning of steroid hormone receptors. *EXS* **77**:79–95 (1996).
120. W. B. Pratt and D. O. Toft. Steroid receptor interactions with heat shock protein and immunophilin chaperones. *Endocr. Rev.* **18**:306–360 (1997).
121. W. B. Pratt and D. O. Toft. Regulation of signaling protein function and trafficking by the hsp90/hsp70-based chaperone machinery. *Exp. Biol. Medicine (Maywood)* **228**:111–133 (2003).