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# Mechanisms of nuclear transport and interventions

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**Abstract**

One of the more overlooked aspects of drug action and delivery is the exploitation of nucleocytoplasmic shuttling. Eukaryotic cells regulate many biological processes by the compartmentation of specific proteins into designated areas. Drugs that have a direct effect on a single protein must be able to localize to the same site as the protein and interact with one or more of its domains. Alternatively, a drug that effectively blocks the target protein from reaching its proper organelle can also inhibit the protein's function. Exploiting the selective movement of macromolecules across the nuclear envelope represents an exciting new area of drug development. This review aims to explain the basic nuclear import/export pathways while focusing on the known drugs that alter the regulation of nucleocytoplasmic trafficking.


*Keywords:* Nuclear import; Nuclear export; Nuclear localization signal; Nuclear export signal; Inhibitors; Importin

**Contents**



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## 1. Mechanisms of nuclear transport

Evidence supporting the selective entry of proteins into the nucleus was first suggested by Bonner in 1975. His set of experiments showed that small proteins and nuclear specific proteins, such as histones, accumulate in the nucleus when microinjected into the cytoplasm of *Xenopus* oocytes. Interestingly, the histones were able to enter the nucleus at an equal or faster rate than some particles of smaller size suggesting the presence of an energy dependent nuclear import pathway. Conversely, very large or cytosolic proteins remain in the cytoplasm after the same microinjection procedure [1,2]. Since the nucleus is enclosed by a selectively-permeable envelope, the mechanism by which charged proteins were translocated across a lipid bilayer remained unknown until electron micrographs showed proteins and gold-labeled RNA moving through nuclear pore complexes (NPCs) [3,4] that were first described in 1962 [5].

### *1.1. The NPC*

The NPC is a large 125-MDa multiprotein structure that completely spans across the nuclear envelope (NE) and extends into cytoplasm and nucleoplasm [6,7]. The pore has a total diameter of 25 nm with a 9-nm aqueous channel in the center through which passive diffusion can occur [8,9]. Proteins under 30 kDa can readily diffuse through the NPC while proteins over 50 kDa and/or 6-nm diameter diffuse at an extremely slow rate. However, as initially seen by Bonner in the 1960s, even very small proteins such as histones that can diffuse through the NPC contain nuclear localization signals (NLSs) for their improved rate of nuclear import. Since proteins usually pass through the nuclear pore in a folded state, the NPC must be able to accommodate the import of large ribosomal subunits ($2.8 \times 10^6$ Da), and the export of even larger ribonucleoproteins (RNPs). These complexes are much too big to passively diffuse and are instead actively transported by an intricate network of import/export machinery discussed below.

### *1.2. Signal-mediated nuclear import*

The NPC physically allows for the signal-mediated passage of large, charged molecules across the nuclear envelope. Early on, two types of signals were hypothesized to account for selective nuclear localization of proteins, the NLS and the nuclear retention signal. Theoretically, proteins could traverse back and forth across the nuclear envelope but be specifically retained in the nucleus by a nuclear retention signal. Alternatively, trafficking across the nuclear envelope could be restricted to those proteins that carry an NLS to target them for active nuclear import. The first NLS identified was a basic stretch of amino acids (KKKRK) from the SV40 large T-antigen [10,11]. This sequence has been named the 'classical' NLS and variations are found in hundreds of nuclear proteins. A second class of NLS, the 'bipartite' signal, is characterized by the sequence from the *Xenopus* nucleoplasmin protein in which the classical NLS is broken into two halves by an intervening group of five to 20 amino acids (KRPAATKKAGQAKKKK) [12]. The hnRNP A1 protein contains yet a third class of NLS, termed the M9 sequence. Unlike the other NLSs, M9 is not rich in basic amino acids, but functions as both a nuclear import and nuclear export signal (NES) [13]. Apart from these sequences, many other potential NLSs have been identified, but to date, the mechanisms of transport of these other sequences have not been elucidated.

### *1.3. NLS receptors*

Active nuclear import of NLS-containing proteins is mediated by the importin family of transport molecules collectively known as karyopherins (Fig. 1). Using an in vitro assay that selectively permeablizes the high-cholesterol plasma membrane with digitonin while keeping the low-cholesterol NE intact has allowed researchers to remove all cytosolic contents and add back individual proteins to identify which are necessary for nuclear import [14]. This powerful assay led to the characterization of the importin $\alpha$ and $\beta$ family of proteins that serve as adaptor and receptor molecules used for nuclear import [15–19]. The SV40 large T-antigen 'classi-

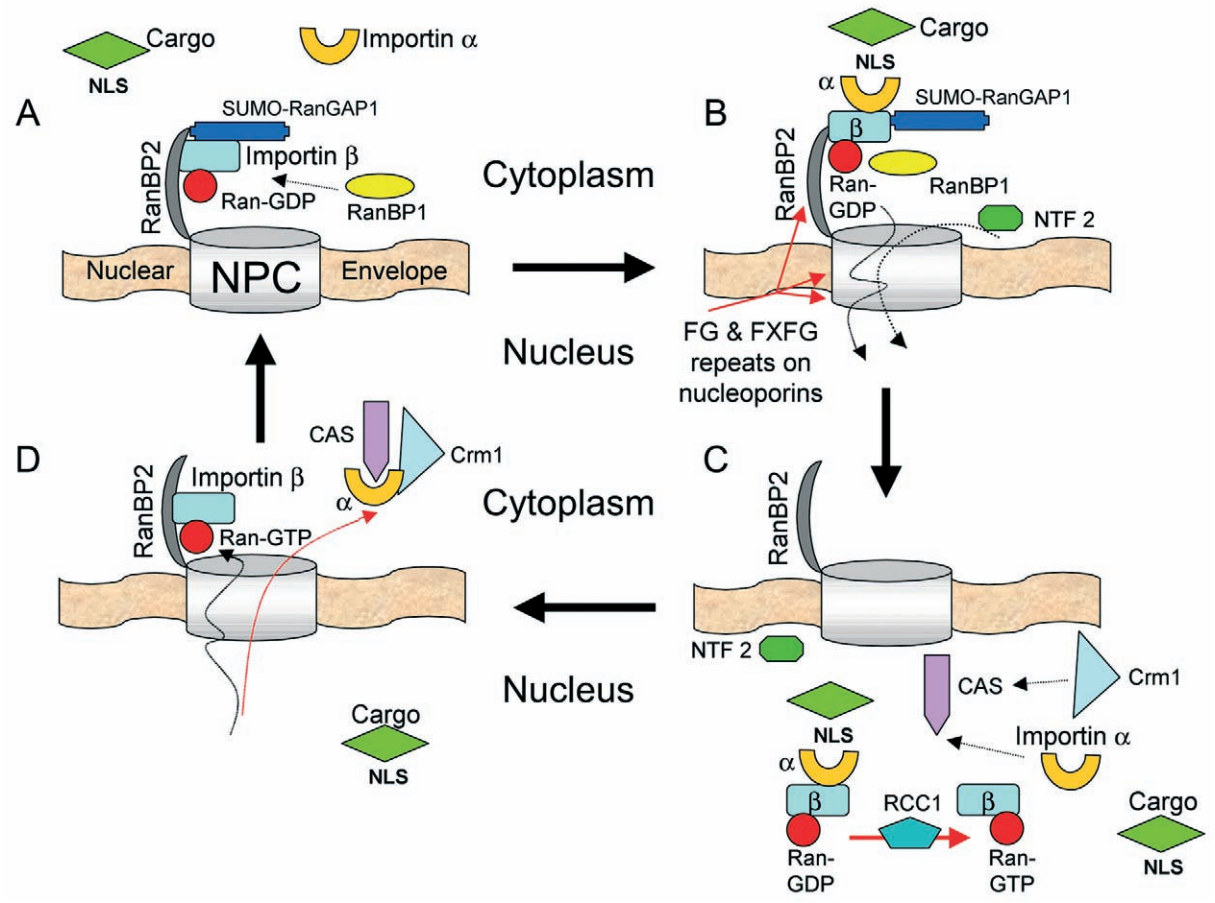


Fig. 1. Mechanism of signal-mediated protein nuclear import and import factor recycling.

cal' NLS was first used to identify these soluble receptors. Proteins with the 'classical' NLS present in the cytoplasm are bound by importin α, which recognizes the highly basic PKKKRKV string of amino acids [20]. Importin α acts as an adaptor molecule, binding both the NLS of the cargo protein and importin β. Nucleoplasmin and other proteins with a bipartite NLS use the same importin α/β import machinery [12]. It was later discovered that other β homologues can directly bind to various NLSs (including the classical NLS) independent of importin α, using similar NLS recognition motifs. These α/β/NLS or β/NLS multiprotein complexes localize to the NE as importin β binds to fiber-like proteins that protrude from the NPC into the cytoplasm [21,22]. Here the import substrate associates with another soluble protein involved in the import process, RanGDP, and is translocated through the pore. A third soluble protein, nuclear transport factor 2 (NTF2), assists in mediating translocation of Ran and the complex through the pore [23]. Once inside the nucleus, the guanine nucleotide exchange factor RCC1 replaces the Ran-bound GDP with GTP, causing the import cargo to dissociate from the importin/Ran complex [24]. When an importin α/β/cargo complex is dissociated after Ran guanine nucleotide exchange, importin β remains associated to RanGTP and is exported while importin α remains

in the nucleus. The nuclear export receptor CAS, another importin β homologue, has been shown to bind importin α and this CAS-importin α complex is exported with RanGTP [25,26]. The end result is that an NLS-containing protein is deposited in the nucleus where it can function and importin α and β are exported back to the cytoplasm where they can reinitiate the import cycle of another NLS-containing protein.

Although the different classes of NLSs differ in structure (classic, bipartite, etc.), within a given class, the NLSs of many proteins are highly similar in amino acid sequence. However, not all NLSs within or between classes are functionally recognized by the same importin proteins. To date, over six importin α and 12 importin β family members have been identified in humans, many of which appear to mediate the nuclear localization of unique families of proteins [27]. For example, the importin α/importin β complex (α/β) binds the classic NLS from SV40 large T-antigen as well as certain bipartite sequences for nuclear import. By contrast, importin α3 recognizes NLSs present in a subgroup of ribosomal proteins [28], while importin α5 and α7 mediate import of another class of ribosomal proteins [29], and an importin α1–α7 heterodimer facilitates transport of histone H1 [30]. Table 1 shows a full list of the importins and their substrates. Thus, the ability of different importins to recognize subsets of nuclear proteins adds a level of specificity and regulation to the import/export process.

### 1.4. RanGTP/GDP gradient

The directional active nuclear transport is controlled by the different RanGDP and RanGTP concentration gradients within the cell. Ran is a small Ras-like protein with a GTP binding cassette. It has a very slow intrinsic GTP hydrolysis rate. However, the presence of Ran GTPase activating protein 1 (RanGAP1) in the cytoplasm in conjunction with

Table 1
List of importins and molecules they import or export

| Importin | Examples of interactions with class of molecules/function |
|---|---|
| α1 | Imports 'classical' NLS (PKKKRKV) containing proteins [20] |
| α2 | Imports transcription factors and many viral proteins [83,84] |
| α3 | Imports DNA binding proteins, recognizes classical NLS with additional flanking amino acids [85] |
| α5 | Imports activated STAT1 [86] |
| α7 | Expression strongly upregulated in diabetic rat kidney [87] |
| β1 | Imports importin α homologues with importin β binding domains [21,88] |
| Transportin (β2) | Imports/exports proteins containing the M9 sequence [35] |
| β3 (RanBP5, Pse1p) | Import of ribosomal proteins [89] |
| β4 (Kap123) | Import of ribosomal proteins [89] |
| Nmd5p (Kap119p) | Imports MAPK, TFIIS [90] |
| Snurportin 1 | m3G-cap receptor, import of snRNPs [91] |
| Kap114p | Imports TATA-binding protein [92] |
| Importin7 (RanBP7) | Forms heterodimers with β1, import of histone H1 [30] |
| Importin8 (RanBP8) | Signal recognition particle (SRP) import<br>Import of RNA binding proteins [93,94] |
| Mtr10p (Kap111p) | Polyadenylated RNA export, imports mRNA binding proteins [95] |
| Msn5p (Kap142p) | Import/export of cell cycle control proteins [93,96,97] |
| Crm1 | Export of proteins containing leucine based NES sequences |
| CAS | Exports importin αs [26] |
| Exportin-t (Los1p) | tRNA export [98,99] |

Ran binding protein 1 (RanBP1) on the cytoplasmic face of the NPC can speed up this GTPase activity. The result of this is a much higher concentration of RanGDP than RanGTP in the cytoplasm. Conversion of RanGDP to RanGTP occurs by exchanging the entire nucleotide and is catalyzed by the guanine nucleotide exchange factor RCC1 [24]. Since RCC1 is found almost exclusively in the nucleoplasm, the RanGTP/RanGDP ratio is high in the nucleus. This gradient of RanGTP/RanGDP across the nuclear envelope is used to drive protein trafficking back and forth through the NPC.

How this gradient drives nuclear import and export is believed to be at the level of protein–protein interactions. Importin β can associate with either the GTP or GDP state of Ran. Generally, nuclear import of importin β requires RanGDP while export requires RanGTP. It is possible that Ran nucleotide hydrolysis or exchange can occur anywhere in the cell, but is tightly controlled by several proteins in a microenvironment around the NPC. A 358-kDa multi-domain protein designated RanBP2 exists on the cytoplasmic face of the NPC and acts as a scaffold where import and export complexes can assemble and disassemble. RanBP2 binds several proteins, including importin β and RanGAP1 [31]. A current model of nuclear import begins with an importin β-RanGTP complex binding to RanBP2 on the cytoplasmic face of the NPC. Even though RanGAP1 also localizes to RanBP2, GTP hydrolysis of RanGTP is inhibited by the association of just importin β. Data suggest that RanBP1 and possibly an NLS-containing cargo bind to importin β on the scaffold, which activates RanGAP1 allowing GTP hydrolysis to occur to generate a RanGDP/importinα/importinβ/NLS complex [32]. The entire complex with RanGDP is then imported in a poorly understood fashion involving several nucleoporins and NTF2. Once inside the nucleus the high concentration of RCC1 exchanges the GDP with GTP causing the importin α and cargo molecules to dissociate leaving an importin β-RanGTP complex to translocate back through the NPC towards the cytoplasm where it will eventually bind to RanBP2 again. Nuclear import and export is a repeating cycle directed by the tandem shuttling of importin β with different Ran states.

### *1.5. Nuclear export*

Up to now, only the nuclear import of proteins containing a NLS sequence has been discussed. On the opposite end of that spectrum, certain proteins have sequences that mark them for nuclear export. Many of these nuclear export signals (NESs) are characterized by a leucine rich string of amino acids that are recognized by the exportin protein Crm1, or simply, ‘exportin’. The HIV-1 Rev protein and a polypeptide cAMP-dependent protein kinase inhibitor (PKI) were the first two proteins in which NESs were identified. Rev is an early viral gene synthesized from full length viral RNA. Later in the viral life cycle Rev functions to carry spliced viral RNA messages out of the nucleus into the cytosol for translation. Rev facilitates this shuttling by binding to the shorter viral mRNAs and using its NES sequence (LPPLERLTLD) to bind to Crm1 [33]. Together with RanGTP the entire complex is exported out of the nucleus. Unlike the situation with importin β, RanGTP appears to promote the interaction between exportin (Crm1) and NES-containing cargo proteins. Using a similar Crm1-mediated export mechanism in which Crm1 binds PKI’s NES (LALKLAGLDI), PKI can block a signal transduction cascade by exporting protein kinase A from the nucleus where it is active [34]. These two proteins (Rev and PKI) both use Crm1 as a transport protein, but more NES sequences are being discovered every year, each with a different set of adaptor and receptor proteins that mediate their nuclear export (see Table 1 for a list).

Some proteins have sequences that double as import and export signals. The first protein identified with such a sequence was hnRNP A1 [13]. hnRNP A1 is involved in the nuclear export of mRNA, but it has the capability to be quickly imported back into the nucleus for another round of mRNA shuttling. It has a stretch of 38 amino acids, termed the M9 sequence that is necessary for import and export. However, hnRNP A1 uses different transport machinery than the large T-antigen. Instead of using an adapter molecule like importin α to connect the NLS to importin β, it uses a single shuttling protein called transportin, which has about 25% amino acid sequence homology with importin β [35]. As with the

importin α/β complexes, transportin together with RanGDP and RanGTP are the necessary soluble factors for both import and export of hnRNP.

## 2. Cellular modulation of nuclear transport

### *2.1. The NPC as a modulator of nuclear transport*

Apart from acting simply as an architectural structure through which nuclear transport occurs, the NPC may also play a more dynamic role in regulating transport. In addition to the different substrates recognized by the importins, import and export specificity may also be achieved by altering the nucleoporin expression, thus allowing different interactions between the NPC and karyopherins. The total number of NPCs within the nuclear envelope depends on the cell type and its metabolic activity. A proliferating human cell is estimated to have 3000–5000 NPCs, each composed of multiple copies of 50–100 different proteins. By comparison, yeast have less than 200 NPCs per cell [36], each composed of multiple copes of about 30 different proteins. Nucleoporins interact with importins through their FG or FXFG repeats, however, different nucleoporins may have different affinities for individual karyopherins. The unicellular organism *Tetrahymena thermophilia* has a macronucleus and a micronucleus within the same plasma membrane [37]. One study separately injected histones H1 and H4 into the cytosol of *T. thermophilia*. Over time H1 was imported into the macronucleus but not the micronucleus [38]. Interestingly, histone H4 was imported into the macronucleus of interphase cells after injection, but when the cell was dividing the same histone targeted to the micronucleus instead [38].

Another study created the fusion of a dividing and non-dividing mammalian cell of the same species for microinjection of gold particles. The hybrid cell shared the same cytoplasm but had distinct mitotic and quiescent nuclei. When large gold particles were injected, they accumulated in the dividing nucleus more rapidly [39]. In both of these experiments the soluble transport factors present in the cytoplasm remained constant, only the nuclei and their associated proteins changed. Therefore, the data suggest that the NPC composition and conformation may change depending on the cell's current demands. A cell undergoing division must import and export more than just the usual 'housekeeping' proteins and RNPs, so the NPCs may change their specificity for sizes of imported complexes and karyopherins. Thus, the NPC itself may one day become a drug target to selectively attack dividing (cancerous) versus non-dividing (normal) cells.

### *2.2. Post-translational modifications influencing import*

Modulation of nuclear import and export based on the presence of certain proteins or differences in gene expression have been discussed above. However, the most common influence on nucleocytoplasmic movement stems from post-translational modifications of the cargo proteins themselves (Fig. 2). These can include protein–protein interactions to mask or expose a protein's NLS mediated by phosphorylation or dephosphorylation, acetylation, ubiquitination or sumoylation. Examples of each of these mechanisms follow.

The NF-κB pathway is a complex signal transduction pathway that results in the stimulation of apoptotic and immune response genes. Inactive NF-κB is found bound to inhibitor of κB (IκB) in the cytosol. The bound IκB masks the NLS located on NF-κB. When a TNF-α signal is transduced through a cell surface receptor, IκB is phosphorylated and the IκB–NF-κB complex dissociates unmasking the NF-κB NLS while the phosphorylated IκB is subsequently ubiquinated and degraded [40–42]. Once the NF-κB is free of IκB, with the assistance of importin α and β it can translocate to the nucleus where it activates apoptotic and pro-inflammatory genes. One of the genes NF-κB stimulates is IκB, which results in an accumulation of IκB in the nucleus. In addition to the IκB NLS responsible for its nuclear import, IκB has an NES that is recognized by Crm1. Nuclear IκB binds to activated NF-κB and shuttles it out of the nucleus, effectively inhibiting its transcriptional activity in a negative feedback loop. However, Chen et al. have shown that nuclear NF-κB that is acetylated remains in the nucleus because it interacts weakly with IκB. The acetyl group can be removed by HDAC3, followed by subsequent binding of IκB and nuclear export of the entire complex

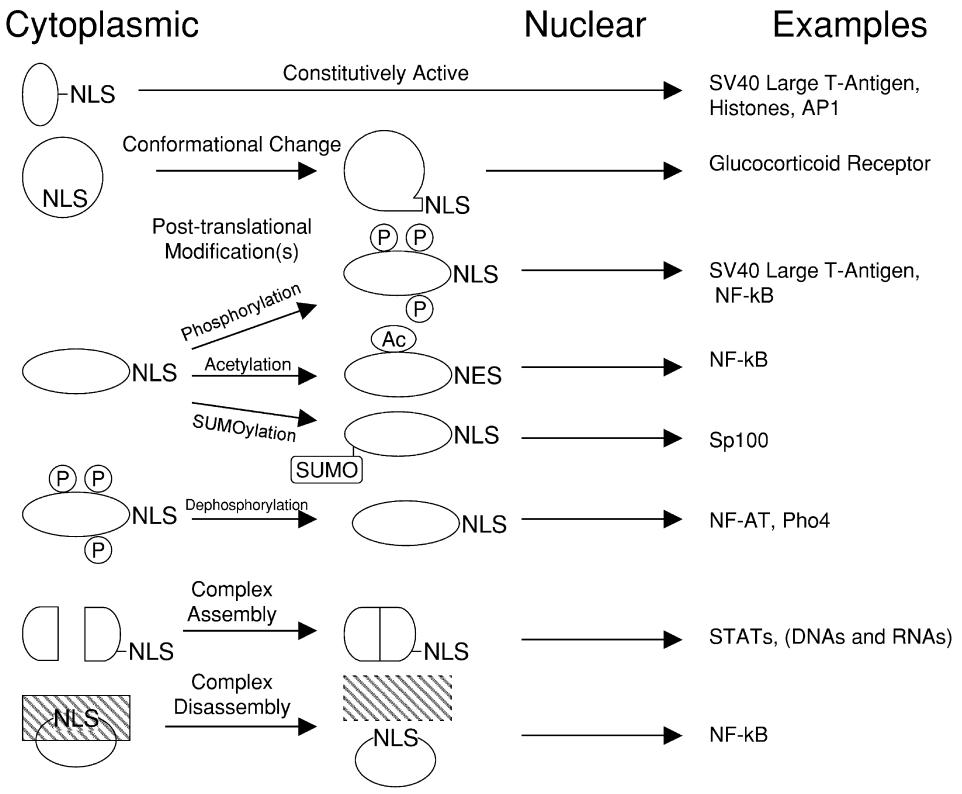


Fig. 2. Regulatory mechanisms for nuclear localization of proteins.

[43]. Thus, in addition to regulation by phosphorylation, ubiquitination, and NLS masking/unmasking, nuclear localization of NF-κB is also regulated by acetylation.

Another well-studied signal transduction pathway that utilizes nucleocytoplasmic localization as a mechanism of regulation centers on the transcription factor NF-AT. Before activation, NF-AT is localized to the cytoplasm in a highly phosphorylated state. An influx of $Ca^{2+}$ activates the phosphatase calcineurin, which then binds to the N-terminal region of NF-AT. Calcineurin dephosporylates NF-AT revealing NLS sequences allowing the transcription factor to translocate into the nucleus where it can activate cytokine genes such as IL-2 and IL-4 [44]. Once the $Ca^{2+}$ stimulus has subsided, calcineurin is deactivated and dissociates from NF-AT exposing its NES. The transcriptional activation mediated by NF-AT is attenuated via the Crm1-dependent nuclear export of the protein to the cytoplasm where it is rephosphorylated by a host of kinases such as GSK-3, JNK, and CK-1α to help maintain its cytosolic localization.

A more recently discovered post-translational modification that can regulate the subcellular localization of proteins is the conjugation of small proteins to different members of nuclear import machinery and/or the transcription factors that they shuttle. The most studied member of this category is the 7-kDa protein, ubiquitin. While ubiquitin does not seem to have a direct effect on the nuclear localization of a protein, it is known to tag membrane proteins for internalization (in the case of monoubiquitination) and target other proteins for degradation in the cytoplasm (commonly by polyubiquitination). p53 is a well-known tumor suppressor gene expressed in response to DNA damage that is also a ubiquitin target. p53 is imported into the nucleus as a monomer by importin α and β via its NLS [45]. Once nuclear, p53 forms homotetramers to activate many

genes resulting in cell cycle arrest and apoptosis. Mdm2 is a p53-regulated gene that acts as an ubiquitin E3 ligase with an NLS and NES within its sequence and possesses an affinity for p53 [46]. Active nuclear p53 can be sequestered by mdm2 and exported out of the nucleus in a Crm1 dependent fashion [47]. In the presence of free cytosolic ubiquitin, mdm2 polyubiquitinates p53, which results in p53 degradation by the 26S proteosome [48]. Ubiquitin moieties do not directly influence the cellular localization of p53, but instead prevent p53 from returning to the nucleus effectively terminating the DNA damage response.

Another small conjugatible protein that may play a role in nuclear transport is the small ubiquitin-like modifier 1 (SUMO1). SUMO1 has less than 20% amino acid sequence homology to ubiquitin, but shares a very similar three-dimensional shape as well as the C-terminal Gly–Gly motif where isopeptide conjugation occurs [49,50]. SUMO1 is conjugated to transcription factors, structural proteins, and NPC associated proteins by a ubiquitin-like E1 (activating enzyme), E2 (conjugating enzyme), and E3 (ligating enzyme) pathway. Exact effector functions of SUMO1 linkages are still poorly understood but evidence suggests that the addition of a SUMO1 moiety may prevent ubiquitin mediated proteosome degradation. Sumoylated IκB is resistant to ubiquitin dependent degradation, which prevents nuclear translocation of activated NFκB [51].

A recent study has identified RanBP2, an NPC cytoplasmic fibril protein, as a SUMO1 E3 ligase [52]. Pichler et al. showed that RanBP2 can selectively sumoylate the nuclear structural protein Sp100 [52]. Other studies have also related sumoylated Sp100 with the stability of nuclear dots [53,54] and the disappearance of these dots correlate with the onset of certain leukemias [55,56]. Free activated SUMO molecules also appear to localize along the outer nuclear rim in permeabilized cells in the absence of cytoplasm [52]. The fact that the sumoylated state of Sp100 seems to control its nuclear localization and free SUMO1 targets to NPCs on the outer layer of the NE where RanBP2 can act as an E3 ligase for Sp100 leads one to speculate that sumoylation and nuclear import may be coupled events. Indeed, many sumoylation target proteins require a functional NLS for the conjugation to occur [53,57]. Other evidence that couples sumoylation to nuclear import requires RanGAP1 to be sumoylated before it can bind to the RanBP2 scaffold to help create the RanGTP/RanGDP gradient [31]. RanBP2 itself can become hypersumoylated, which may effect its E3 ligase activity or change its scaffold properties. Sumoylation of proteins at the NPC could also create different binding associations between proteins, ultimately assisting complexes through the NPC by mediating a series of binding/unbinding interactions. However, there is no direct evidence that supports this speculation, and further research is needed to test these hypotheses.

## 3. Pharmacological modulation of nuclear import and export

An important aspect of drug action that is often overlooked is that a drug need not directly inhibit, or stimulate, a target's activity to be effective. Rather, the redistribution or mis-targeting of a drug target, be it an enzyme, structural protein, transport, etc., may have just as profound an effect. The remainder of this review will focus on examples demonstrating this in terms of nuclear localization.

### *3.1. Drugs that target the NPC*

The most obvious target to use when attempting to inhibit nuclear import or export is at the place where it occurs, namely the NPC. One of the most common characteristics of nucleoporins is the presence of conserved FG or FXFG repeats that bind to the importin family members [18,58,59]. The actual physiological movement of proteins through the NPC is not mechanistically understood, but is thought to be mediated by sequential binding of the importins to FG repeats along the length of the nucleoporins associated with the pore. Translocation through the NPC can be blocked by monoclonal antibodies (including mAb414 and RL2) against the FG and FXFG epitopes of the nucleoporins [60]. Consequently, several nucleoporin proteins were identified by their reactivity with the anti-FG antibodies. The majority of these FG repeat proteins exist as the cytoplasmic fibrils or protrusions on the nuclear side

of the NPC. The monoclonal antibodies prevent cargo from ever associating around the rim of an NPC so it cannot traverse to the other side of the membrane.

Another common property of several nucleoporins in higher organisms is the covalent addition of a single O-linked *N*-acetylglucosamine to serine and threonine residues. The exact function of the sugar moiety is poorly understood but it probably mediates some of the interactions with importins. Wheat germ aggulatinin (WGA) is a lectin that binds to *N*-acetylglucosamine. When added to cells, WGA can inhibit nuclear import by associating with the sugar modified nucleoporins and block the channel [61]. It will non-specifically block proteins that are normally imported through the NPC, thus acting as a useful diagnostic test for a protein potentially targeted to the nucleus.

### *3.2. Inhibiting the nuclear export of NF-κB*

As previously mentioned, the acetylation of NF-κB in the nucleus prevents it from associating with IκB and being exported out of the nucleus in a Crm1 dependent fashion. The acetyl group is specifically removed by the nuclear protein histone deacetylase 3 (HDAC3) [43]. The entire class of HDACs is known to remove the acetyl groups from different histones, thus compacting the structure of chromatin. HDACs can also deacetylate transcription factors and other chromatin structural proteins. Trichostatin A (TSA) is a Streptomyces microbial product that was originally characterized as a cell cycle inhibitor [62], but more recently, it has been shown to act as a histone deacetylase inhibitor [63]. Only small amounts of TSA are needed to curtail the hydrolysis of acetyl groups in vitro meaning it behaves as a non-competitive inhibitor. Researchers have begun to use TSA as an anti-cancer drug thinking that inhibition of HDACs may keep histones acetylated, maintaining the transcription of anti-cancer genes. A recent study showed that the addition of TSA increased the duration of NF-κB in the nucleus after a TNF-α stimulus [43]. The anti-cancer affect of TSA could then be attributed to maintaining NF-κB's nuclear localization (by prevention of nuclear export) and prolonged expression of pro-apoptotic genes. Indeed, it was later discovered that addition of TSA blocks proliferation and activates apoptosis in hepatoma cells, which are at least partially resistant to chemotherapy making TSA a potential clinical drug for future cancer treatment [64]. Synthetic derivatives of TSA and similar drugs are now being tested for their anti-tumor properties, with several of them, such as 4(dimethylamino)-*N*-[7-(hydroxyamino)-7-oxoheptyl]-benzamide showing promise against multiple cancers in early studies [65].

As TSA blocks NF-κB from leaving the nucleus and has been exploited for its anti-tumor capabilities, fumaric acid esters like dimethylfumarate (DMF) have been used to treat psoriasis by sequestering NF-κB in the cytoplasm thus limiting expression of certain genes involved in the pathophysiology of the disease. Although the addition of DMF does not inhibit the dissociation of the cytoplasmic NF-κB/IκB complex initiated by TNF-α, only a small amount of active NF-κB is found in the nuclei of these cells, based on protein localization and DNA binding activity [66]. These data suggest that DMF blocks NF-κB from ever getting into the nucleus but the exact mechanism by which this is accomplished is still unknown.

### *3.3. Inhibiting nuclear localization of NF-AT*

Two clinical drugs are available to physicians that act as immunosuppressors following graft and transplant procedures. Cyclosporin A binds to cyclophilin while FK506 acts by associating with FKBP12. Both of these drug/target complexes interact with calcineurin to regulate its activity [67]. Calcineurin, as described above, is activated by increased calcium internalization allowing the binding of hyper-phosphorylated NF-AT. Calcineurin dephosphorylates NF-AT causing its nuclear entry and subsequent transcriptional activation of key immune genes. Cyclosporin A and FK506 prevent calcineurin from dephosphorylating NF-AT, which keeps the NF-AT NLS hidden from soluble shuttling proteins and blocks its nuclear import [68–70]. Unfortunately, the problem with these drugs is the side effects they generate because of their inhibition of calcineurin activity, which can normally regulate several other signaling cascades as well.

More recently, a group has developed a set of low molecular weight synthetic pyrazole compounds

(3,5-bistrifluoromethyl pyrazoles) that disrupt cytokine expression [71]. Further investigation has revealed that they do not directly inhibit calcineurin activation and binding, but still result in the cytoplasmic accumulation of NF-AT even after a cytokine response [71]. Therefore, these new compounds probably either act as an activator of an NF-AT kinase (GSK3, MKKK1, c-JNK2) or an inhibitor of NF-AT desphorylation. The result could be powerful immunosuppressive drugs that can be delivered at low doses without the same side effects as more general drugs that work through calcineurin. However, the exact mechanisms by which these compounds prevent nuclear import of NF-AT must be elucidated to determine other potential targets that may be affected by these drugs (Table 2).

### *3.4. Inhibiting nucleocytoplasmic movement of HIV-1*

Currently, most anti-HIV therapy has focused on developing drugs that inhibit viral entry, viral genome replication, and virus specific proteolysis. However, the nature of HIV-1 reverse transcriptase replication allows it to mutate rapidly in an environment with heavy selective pressures created by the presence of antiviral drugs. This makes the continuous development of new drugs necessary if AIDS is to be effectively treated. As a by-product, HIV drug research has also provided insights to the mechanisms of cellular nuclear import and export. While searching for ways to prevent productive HIV-1 infections, Wolff et al. discovered the first low molecular weight inhibitor of nuclear export [72]. It had long been known that the Streptomyces product leptomycin B (LMB) induced yeast cell cycle arrest, improper gene expression, and formation of abnormal nuclei, but its molecular mode of action was not known until yeast genetic screens produced mutants of crm1 that were resistant to LMB [73]. As previously mentioned, crm1 acts as an exportin, a class of molecules that binds the NES of cargo proteins and transports them out of the nucleus. Not surprisingly, LMB can also be a potent inhibitor of HIV-1 infections. Once the HIV-1 genome is transcribed from its integrated position within host cell chromatin, mRNA is shuttled out of the nucleus by the viral protein Rev, which has an NES that crm1 recognizes. Indeed, LMB is an effective inhibitor of Rev-Crm1 mRNA dependent nuclear export [72]. Unfortunately, LMB cannot be used therapeutically because LMB non-specifically inhibits all Crm1 mediated export, which could result in lethal side effects. Nonetheless, it still serves as a useful tool for studying nucleocytoplasmic movement.

In contrast to LMB, compounds are being developed to inhibit HIV-1 nuclear import, which would prevent viral integration and transcription from ever occurring. The arylene bis (methylketone) small molecular weight compounds show therapeutic

Table 2
Drugs that affect nuclear import

| Drug/agent | Target | Nuclear import/export effect |
|---|---|---|
| Wheat germ aggulatinin | *N*-Acetylglucosamine moieties on nucleoporins | Binds to sugar and blocks import of proteins through the NPC |
| Anti-FG repeat antibodies | Nucleoporins | Blocks importins from docking to NPC |
| Dimethylfumarate | NF-κB | Retains NF-κB in cytoplasm even after TNF-α signal |
| Cyclosporin A | Cyclophilin | Cyclophilin–cyclosporin complex binds calcinuerin and inhibits dephosphorylation of NFAT/maintains cytosolic localization of NFAT/immunosuppressant |
| FK506 | FKBP12 | FKBP12–FK506 complex binds calcinuerin to inhibit dephosphorylation of NFAT/immunosupressant |
| 3,5-Bistrifluoromethyl pyrazoles | NF-AT pathway | Blocks NF-AT nuclear import without inhibiting calcinuerin activity |
| Leptomycin B | Crm1 | Non-specifically inhibits all Crm1 mediated nuclear export |
| Arylene bis(methylketone) compounds | HIV-1 reverse transcriptase | Associates with RT component of PIC, blocks nuclear import of viral genome |
| HIV-1 monoclonal antibodies | HIV-1 envelope proteins | Bind virus before cellular entry, block nuclear import of genome |
| Transfected importin β binding domain (IBB) | Importin β | Conserved domain from importin alpha family acts as a dominant negative competitive inhibitor of importin α/β-mediated import |

promise by inhibiting PIC nuclear import [74]. Studies have shown that the arylene bis agent, CNI-H1194, can suppress HIV-1 replication in primary macrophages and peripheral blood mononuclear cell cultures in vitro [75,76]. CNI-H1194 can also inhibit productive infections in an ex vivo model using tonsil primary lymphoid tissue [77]. It appears to interact with RT without inhibiting its polymerase activity. Instead, CNI-H1194 possibly disrupts formation of the PIC, which could lead to inefficient nuclear import [76]. However, CNI-H1194 does not interfere with other agents that inhibit RT activity, such as AZT, and has a very low level of cytotoxicity [77]. Therefore, arylene bis compounds possess the desired anti-HIV qualities that could be used in combination with other HIV-1 drugs creating an effective cocktail therapy.

Finally, SIV infection in macaques mimics the HIV-like disease that produces AIDS in humans making it a good model for study [78,79]. Antisera from SIV infected animals is unable to block cellular entry of the virus but still prevents replication in macrophages [80,81]. Apparently, the antibodies produced in these animals prevent nuclear import of the PIC instead. Anti-envelope monoclonal antibodies pre-incubated with $SIV_{mac}$ blocked nuclear import of the viral genome in macrophages in vitro, which means the antibodies may have been internalized with the virion to block nuclear import while in the cytoplasm. However, it is interesting to note that macrophages harboring the viral genome in the cytoplasm could still pass on the infection to $CD4^+$ T cells via cell-to-cell contact regardless of the presence of antibodies [82]. These studies hold important implications for understanding the role of the host immune system and possible passive immunity treatments during HIV-1 infection.

## 4. Conclusion

In this review, we have discussed the basic machinery the cell uses for regulating nucleocytoplasmic trafficking as well as some of the chemical compounds and agents that modulate it. Drug action and delivery can take advantage of cellular compartmentation instead of simply blocking enzymatic active sites. Hopefully this review has demonstrated the often-overlooked effectiveness of preventing a molecule from reaching its normal subcellular location. No doubt the development of agents that alter nuclear import and export will be instrumental in treating disease in the future.

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