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COMMENTARY

Modulation of Nuclear Protein Import

A NOVEL MEANS OF REGULATING GENE EXPRESSION

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ABSTRACT. Eukaryotic cells depend upon the regulated exchange of proteins and RNA between the cytoplasm and the nucleus for survival. Various cytoplasmic and nuclear proteins play a fundamental role in this specific transport process. Over the last few years the components and stages of nuclear protein transport have been characterized in significant detail. Because many of the proteins that are transported into the nucleus are transcription factors, the import process is an interesting target for the manipulation of gene expression. Over time the eukaryotic cell has assembled a number of methods by which to regulate the nuclear localization of transcription factors. Within the last few years, there have been several reports of the pharmacologic manipulation of the localization of nuclear proteins as well. In addition, a recent study suggests that viruses are able to modulate host cell nuclear protein transport *in vivo*. This report will present an overview of nuclear protein import, describe the various *in vivo* mechanisms by which the cell regulates this process, and discuss recent attempts to manipulate the process with small molecule compounds. As nuclear import is a fundamental cellular process, potential opportunities for the future may arise from direct and specific ways to modulate this process and thereby treat diseases characterized by dysregulation of transcription factor activity. BIOCHEM PHARMACOL **56**;2:157–161, 1998. © 1998 Elsevier Science Inc.

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Over the last few years, the biochemical process of nuclear protein import has been studied extensively [for reviews, see 1–3]. Until the early 1990s, virtually all that was known about nuclear import was that those proteins larger than 40–60 kDa were actively transported into the nucleus and contained within their amino acid sequence a targeting domain that was basic in nature [4–8]. Mutational analyses and microinjection studies have defined NLSs† as the sequences responsible for the targeting of proteins into the nucleus [9–14]. While NLS function appears to be universal, the specific amino acid sequences vary for particular proteins [12, 14]. As more NLSs were identified, the lack of a true consensus sequence became apparent. A number of groups also directed their search toward the identification of proteins that mediate nuclear import. Several laboratories described complexes of proteins, distinguished only by molecular weight, that were assumed to be nuclear transport proteins [4–8]. It was not until an *in vitro* transport system was devised that these proteins were shown to participate directly in the nuclear import process and identified as binding proteins or cofactors [15].

There are two distinct steps in the nuclear transport process. In the first step, the NLS of the karyophilic protein interacts with the NLS-binding protein. The second step is a translocation of the karyophilic protein through the nuclear pore by an energy-dependent mechanism. In 1994, Görlich *et al.* [16], cloned and characterized the protein importin, which was determined to bind NLS-containing proteins and thereby facilitate their nuclear import. It now appears that there are several families of karyopherins α that may have very distinct functions [2]. Subsequent to the cloning of importin, several other proteins involved in the import process were identified quickly. There are now known to be at least five distinct proteins (and possibly more) that are required for nuclear transport: importin (also termed karyopherin α), karyopherin β, hsp70, Ran, and p10 [1–3]. Karyopherin α has since been shown to interact with NLS proteins in the cytoplasm in a sequence-specific manner [17]. The specificity of the karyopherin α–NLS interaction arises from differential binding activities of different karyopherins α as well as differential expression in various cell types, and these have been shown to correlate with efficiency of nuclear import of the NLS protein [17]. The karyopherin α–NLS protein complex interacts in the cytosol with karyopherin β, which, along with cytoplasmic factor hsp70, mediates docking to the nuclear pore [18]. The small GTPase Ran and its interacting protein p10/nuclear transfer factor 2 (NTF2) function in the active transport of the NLS protein and karyopherin α through

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†*Abbreviations:* DSG, 15-deoxyspergualin; FGF, fibroblast growth factor; HSV, herpes simplex virus; MA p17, viral matrix protein p17; NFAT, nuclear factor of activated T cells; NF-κB, nuclear factor kappa B; and NLS, nuclear localization sequence.

the channels of the nuclear pore complex, while karyopherin β remains at the nuclear pore complex [19].

IN VIVO REGULATION OF NUCLEAR PROTEIN IMPORT

The complexity of the nuclear protein import process yields many potential points of control. Regulation of transcription factor nuclear translocation is especially critical because these proteins are targeted to the nucleus only under specific conditions, such as the activation state of the cell. Control of nuclear protein import, therefore, allows the cell a means of regulating gene expression and signal transduction events. As NLSs are essential for nuclear protein localization and this function appears to be conserved across eukaryotes, numerous cellular mechanisms controlling nuclear protein import have targeted NLS activity. Not surprisingly, many of these cellular mechanisms are common to diverse eukaryotes, and several examples will be discussed below.

One of the transcription factors that has been characterized most extensively with regard to its nuclear import is NF-κB. Important for the regulation of various immunological processes, the active form of NF-κB usually exists as a homo- or heterodimer composed of 50-kDa and 65-kDa subunits that share homology with the products of the c-*rel* oncogene and the *Drosophila dorsal* gene [20, 21]. Presynthesized NF-κB is stored in the cytoplasm associated with the specific inhibitor IκB, which functions as a cytoplasmic anchor for NF-κB. A number of other nuclear targeted proteins are also kept predominantly cytoplasmic through an interaction with a retention factor protein. In *Drosophila*, the nuclear localization of *period* protein fluctuates with a circadian rhythm [22]. Mutations in *timeless* have been shown to result in the specific block of nuclear localization of *period*, resulting in arrhythmic behavior and suppression of the circadian oscillation of *period* RNA. Thus, a protein encoded by *timeless* is thought to itself interact with *period* or perhaps to regulate factors responsible for the cytoplasmic retention of *period*. Similarly, the *D. dorsal* gene becomes nuclear only in cells undergoing dorsalization. Nuclear localization of *dorsal* is controlled by the *cactus* gene product, which has an analogous function to IκB and also shows sequence homology to IκB-like proteins [23].

One efficient means of modulating NLS accessibility to effect nuclear transport activity is through phosphorylation. Intermolecular masking of the NF-κB p65 NLS by IκB is the mechanism responsible for the cytoplasmic retention of this transcription factor subunit. When the inhibitor protein is bound to the p65 subunit, the p65 NLS is unavailable for recognition by karyopherin α. Unmasking of the NF-κB NLS is regulated by phosphorylation of IκB [24, 25]. Phosphorylation of IκB by a large multisubunit complex induces its degradation, resulting in the cytoplasmic release of NF-κB and allowing it to bind karyopherin α and localize to the nucleus. In *Drosophila*, phosphorylation of *dorsal* itself results in its release from *cactus* and subsequent nuclear translocation. Phosphorylation also regulates the transcription factor NFAT, as dephosphorylation of NFAT by calcineurin in the cytoplasm results in nuclear localization of this transcription factor.

In addition to dephosphorylation, proteolysis also plays a role in the control of nuclear transport. Like p65, the NF-κB p50 subunit is subject to specific masking of the NLS. The p50 subunit is produced as a 105-kDa precursor that is not able to bind DNA [26]. The p105 C terminus contains ankyrin repeats, is homologous to IκB-like proteins, and has been demonstrated to possess the IκB-like activity of maintaining the associated protein in the cytoplasm by masking the NLS *in cis*. Intramolecular unmasking of the p50 subunit NLS by the C-terminus of the p105 precursor is achieved through proteolytic processing. When the p105 C-terminus is cleaved, the p50 NLS then becomes available for binding to karyopherin α.

Finally, the nuclear translocation of some proteins can be regulated throughout the cell cycle, as in the case of the transcription factor v-Jun [27]. The nuclear import of v-Jun is blocked by the cell cycle-dependent phosphorylation of a serine residue adjacent to the NLS. In another example, the *Saccharomyces cerevisiae* transcription factor SWI5 is phosphorylated by the cdk CDC28 at a site within the spacer of its bipartite NLS [28]. During anaphase, when CDC28 activity decreases, SWI5 is dephosphorylated and can then become localized to the nucleus. These varied cellular methods of nuclear transport control illustrate the importance of regulating this key biological event.

PHARMACOLOGIC AND VIRAL MANIPULATION OF NUCLEAR PROTEIN IMPORT

Studies on the mechanism of action of several pharmacologic agents have revealed that although the nuclear protein import machinery was not the intended molecular target, these compounds act by modulating the nuclear localization of transcription factors. Probably the most widely studied examples of drugs that inhibit nuclear import are the immunosuppressants cyclosporin and FK506. These molecules block the nuclear import of a T-lymphocyte specific transcription factor termed NFAT [29, 30] (see Fig. 1). The transport of NFAT is dependent upon its phosphorylation state; only the dephosphorylated form is transported to the nucleus. Although cyclosporin and FK506 are known to bind to the prolyl isomerases cyclophilin and FKBP, respectively, inhibition of isomerase activity is not responsible for the immunosuppression. Rather, the binding of these drugs to their targets promotes the formation of a ternary complex that includes the phosphatase calcineurin. This ternary complex leads to inactivation of calcineurin, which, in turn, inhibits the dephosphorylation of NFAT and thereby prevents its import into the nucleus. Another example of a class of drugs that regulate the nuclear import of transcription factors is the glucocorticoids. In addition to influencing steroid

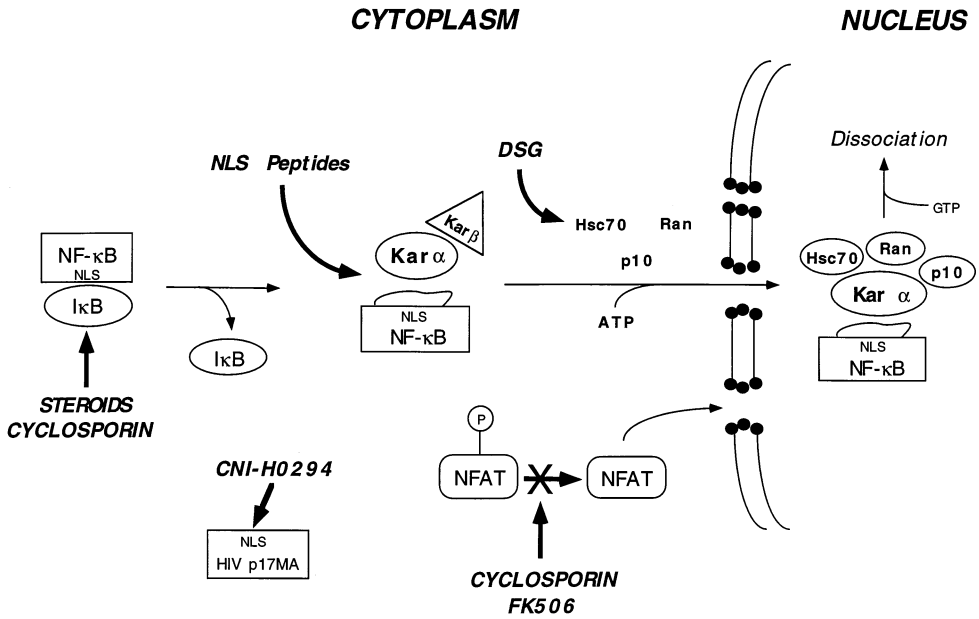

FIG. 1. Inhibition of the nuclear protein transport pathway by various pharmacological agents. Immunosuppressive drugs such as cyclosporin, FK506, and DSG exert their effects at different stages in the nuclear transport pathway. NLS peptides and CNI-H0294 disrupt transport by interfering with specific NLS activity.

receptor activity, glucocorticoids also inhibit the nuclear transport of NF-κB. This blockage is due to a steroid-induced increase in the synthesis of the NF-κB inhibitor IκB, which traps this transcription factor in the cytoplasm [31]. It is likely that regulation of transcription factor nuclear import will prove to be a common mechanism of action for other drugs that affect transcriptional responses.

The inhibitors of nuclear transport described above refer to drugs that act in an indirect manner; that is, they bind to proteins other than those directly involved in the import process. More recently, approaches have been taken to specifically inhibit the nuclear import of proteins either through modification of the NLS or interference with binding to karyopherin α. One of the more direct approaches was taken by Dubrovsky, *et al.* [32], who designed arylene bis(methyl ketone) compounds, which interact with the MA p17 of HIV and prevent its transport into the nucleus. The MA p17 protein is required for the efficient nuclear transport of the HIV preintegration complex. By modifying the basic amino acids of the MA p17 NLS, these compounds directly (by virtue of their ability to mask the NLS) blocked the nuclear localization of this complex. It was subsequently demonstrated that the activity of these NLS-modifying compounds was dependent upon the presence of the enzyme reverse transcriptase [33]. It appears that the interaction between the drug and the reverse transcriptase is also the determining factor in the specificity of the modification of the HIV MA p17 NLS.

Another method of blocking nuclear transport which several groups have used is to treat cells with nuclear localization sequence peptides of 12 amino acids in length [34]. Once these peptides become internalized by the cell, they are predicted to bind to the karyopherins α in the cytoplasm, where they function as competitive inhibitors of nuclear protein transport. However, this technique has not proven very effective, as high micromolar concentrations of peptides are necessary to inhibit transport. This requirement is not surprising, in light of reports that most peptides are generally not efficiently transferred across cell membranes [35].

A similar but more effective approach toward inhibiting nuclear transport was taken by Lin *et al.* [36]. Rather than treating cells with simple NLS peptides, this group used a peptide containing an amino acid sequence derived from FGF, which has the ability to translocate through cell membranes. This sequence corresponds to the h-region of Kaposi's sarcoma FGF and has been shown to target proteins and peptides into the cytoplasm of cells. The sequence of this peptide, though not related to typical leader sequences, allows an associated protein to be both imported into and exported out of the cell. The ability to translocate through cell membranes in the absence of a typical leader sequence is not unique to FGF. At least three other proteins—the Antennapedia homeodomain protein, HIV tat, and HSV VP22—have been shown to translocate through cell membranes [37]. Specific amino acid sequences of between 16 and 34 residues in length have been identified in each of these proteins as the regions responsible for cellular uptake. The mechanisms of membrane translocation at the biophysical level are unknown but appear to be complex. Some of these sequences can be imported at 4° as well as at 37°, suggesting that the mechanism is ATP independent and does not involve the classical endocytic pathway. Translocation has also been shown to occur in multiple cell types. The amino acid sequence from FGF appears to be very efficiently trans-

ported intracellularly. We have shown that within 1 hr transport of this peptide is maximal and reaches intracellular concentrations of approximately 100 μM (unpublished data).

Lin *et al.* [36] have reported that a peptide that consists of the nuclear localization sequence from the p50 subunit of NF-κB in conjunction with the FGF translocation sequence effectively (at approximately 10–30 μM) inhibited the nuclear import of NF-κB in response to cell activation. This peptide was more effective than the nuclear localization sequence alone, demonstrating the enhanced potency of this intracellular delivery approach. Interestingly, the substitution in this peptide of the p50 NLS with the NLS from SV40 large T antigen also resulted in inhibition of the nuclear protein import of NF-κB. This apparent lack of specificity would suggest that the nuclear import of NF-κB may be more susceptible to inhibition than that of other nuclear proteins. This observation is consistent with those we made during studies on the mechanism of action of the immunosuppressant DSG. We have shown that DSG, which has some structural similarity to an NLS, is able to inhibit NF-κB nuclear localization [38]. DSG interacts in the cytoplasm with the heat shock protein hsc73, which has been reported to play a role in nuclear import of some proteins [39]. We predict that hsc73 is specifically involved in the nuclear transport of NF-κB.

Our laboratory has extended and improved upon the studies using the targeted NLS peptides reported by Lin *et al.* [36]. Since karyopherin α contains approximately 5–6 potential binding sites for NLSs, we predicted that a molecule having more than one NLS would bind to karyopherin α with greater avidity than a molecule with only a single NLS, due to cooperative binding effects. Therefore, we designed an NLS intracellular targeted peptide composed of two NLSs adjacent to the FGF translocation sequence. We found that such a peptide was approximately 35-fold more potent at inhibiting NF-κB nuclear transport than a single NLS attached to the translocation sequence (Fujihara *et al.*, manuscript in preparation). We also found, in agreement with Lin *et al.* [36], that NLSs other than the NF-κB NLS are able to inhibit the nuclear translocation of this transcription factor. We hypothesize that the NLS peptides act as competitive inhibitors of the interaction between karyopherin α and the NLS on the karyophilic protein, in this case NF-κB. To support this hypothesis, we have demonstrated that there is a differential interaction between various NLSs and two forms of karyopherin α, termed K1 and K2 [17]. There are also differences in the abilities of the NLSs to target proteins into the nucleus. Hence, based on these data we predict that the interaction between the NLSs on NF-κB subunits and karyopherin α is weaker than the interaction of other nuclear proteins with karyopherin α. Therefore, this NLS peptide approach would allow only proteins with a relatively low affinity for karyopherin α to be competed by the intracellularly targeted NLS peptides. A more specific approach will be to target the NLSs themselves, as described above in the case of the inhibitor of the HIV matrix NLS. Since there exists no true consensus for NLSs, it may be possible to target individual NLSs with small molecules and thereby, in a very precise manner, inhibit the nuclear import of particular transcription factors.

A final example of nuclear transport regulation is described in the study by Her *et al.* [40], which reported that the matrix protein of vesicular stomatitis virus can inhibit Ran GTP-dependent nuclear transport. It was suggested that this inhibition would lead to decreases in RNA and protein synthesis in the infected cell. Although the matrix protein targets a fundamental component of nuclear import, there appears to be very specific effects on gene regulation. Therefore, it appears that viruses, whose activity can often provide us with clues for new ways to manipulate biological processes, may have evolved a mechanism for modulation of host cell nuclear import. Interestingly, of the NLSs that we have studied thus far, those NLSs derived from viral proteins appear to interact with greatest affinity for the karyopherins α and are most efficient at targeting proteins to the nucleus. It is tempting to speculate that certain viral proteins may have been selected to maintain potent NLSs in order to competitively inhibit nuclear import of host proteins, down-regulate immune responses, thereby enabling the virus to avoid immunological surveillance.

CONCLUDING REMARKS

The complex process of nuclear protein import is subject to multiple levels of control. These include *in vivo* mechanisms such as phosphorylation/dephosphorylation, proteolysis, variable expression of karyopherins, and specificity of NLSs. We have reviewed recent evidence for the use of various pharmacological methods as another approach toward the control of gene expression. Drugs such as cyclosporin, steroids, peptides, and NLS-modifying reagents have given us invaluable insight into the mechanisms involved in the regulated movement of transcription factors into the nucleus. While there are many other established methods of influencing gene expression (e.g. antisense technology, gene therapy, and drugs that bind to DNA), direct modulation of the nuclear protein transport process will provide a more potent means of regulating the transcription of genes. The two examples cited above—the HIV MA p17 transport inhibitor CNI-H0294 and the peptide inhibitors of nuclear transport—represent the first of a new class of tools that directly target specific NLSs, paving the way for future studies and applications in modulating gene activity.

References

1. Görlich D, Nuclear protein import. *Curr Opin Cell Biol* **9:** 412–419, 1997.
2. Panté N and Aebi U, Toward the molecular dissection of

protein import into nuclei. *Curr Opin Cell Biol* **8:** 397–406, 1996.
3. Görlich D and Mattaj IW, Nucleocytoplasmic transport. *Science* **271:** 1513–1518, 1996.
4. Pandey S and Parnaik VK, Identification and characterization of nuclear location signal-binding proteins in nuclear envelopes. *Biochim Biophys Acta* **1063:** 81–89, 1991.
5. Adam SA, Lobl TJ, Mitchell MA and Gerace L, Identification of specific binding proteins for a nuclear location sequence. *Nature* **337:** 276–279, 1989.
6. Stochaj U, Osborne M, Kurihara T and Silver P, A yeast protein that binds nuclear localization signals: Purification localization, and antibody inhibition of binding activity. *J Cell Biol* **113:** 1243–1254, 1991.
7. Yamasaki L, Kanda P and Lanford RE, Identification of four nuclear transport signal-binding proteins that interact with diverse transport signals. *Mol Cell Biol* **9:** 3028–3036, 1989.
8. Radu A, Blobel G and Moore MS, Identification of a protein complex that is required for nuclear protein import and mediates docking of import substrate to distinct nucleoporins. *Proc Natl Acad Sci USA* **92:** 1769–1773, 1995.
9. Chelsky D, Ralph R and Jonak G, Sequence requirements for synthetic peptide-mediated translocation to the nucleus. *Mol Cell Biol* **9:** 2487–2492, 1989.
10. Lanford RE, Kanda P and Kennedy RC, Induction of nuclear transport with a synthetic peptide homologous to the SV40 T antigen transport signal. *Cell* **46:** 575–582, 1986.
11. Michaud N and Goldfarb DS, Most nuclear proteins are imported by a single pathway. *Exp Cell Res* **208:** 128–136, 1993.
12. Lanford RE, Feldherr CM, White RG, Dunham RG and Kanda P, Comparison of diverse transport signals in synthetic peptide-induced nuclear transport. *Exp Cell Res* **186:** 32–38, 1990.
13. Goldfarb DS, Gariépy J, Schoolnik G and Kornberg RD, Synthetic peptides as nuclear localization signals. *Nature* **322:** 641–644, 1986.
14. Boulikas T, Nuclear localization signals (NLS). *Crit Rev Eukaryot Gene Exp* **3:** 193–227, 1993.
15. Adam SA, Sterne-Marr R and Gerace L, Nuclear protein import in permeabilized mammalian cells requires soluble cytoplasmic factors. *J Cell Biol* **111:** 807–816, 1990.
16. Görlich D, Prehn S, Laskey RA and Hartmann E, Isolation of a protein that is essential for the first step of nuclear protein import. *Cell* **79:** 767–778, 1994.
17. Nadler SG, Tritschler D, Haffar OK, Blake J, Bruce AG and Cleaveland JS, Differential expression and sequence-specific interaction of karyopherin α with nuclear localization sequences. *J Biol Chem* **272:** 4310–4315, 1997.
18. Shulga N, Roberts P, Gu Z, Spitz L, Tabb MM, Nomura M and Goldfarb DS, *In vivo* nuclear transport kinetics in *Saccharomyces cerevisiae*: A role for heat shock protein 70 during targeting and translocation. *J Cell Biol* **135:** 329–339, 1996.
19. Koepp DM and Silver PA, A GTPase controlling nuclear trafficking: Running the right way or walking RANdomly? *Cell* **87:** 1–4, 1996.
20. Barnes PJ and Karin M, Nuclear factor-κB—A pivotal transcription factor in chronic inflammatory diseases. *N Engl J Med* **336:** 1066–1071, 1997.
21. Grilli M, Chiu JJ-S and Lenardo MJ, NF-κB and Rel: Participants in a multiform transcriptional regulatory system. *Int Rev Cytol* **143:** 1–62, 1993.
22. Vosshall LB, Price JL, Sehgal A, Saez L and Young MW, Block in nuclear localization of *period* protein by a second clock mutation, *timeless*. *Science* **263:** 1606–1609, 1994.
23. Norris JL and Manley JL, Selective nuclear transport of the *Drosophila* morphogen *dorsal* can be established by a signaling pathway involving the transmembrane protein *Toll* and protein kinase A. *Genes Dev* **6:** 1654–1667, 1992.
24. Mercurio F, Zhu H, Murray BW, Shevchenko A, Bennett BL, Li JW, Young DB, Barbosa M, Mann M, Manning A and Rao A, IKK-1 and IKK-2: Cytokine-activated IκB kinases essential for NF-κB activation. *Science* **278:** 860–866, 1997.
25. Woronicz JD, Gao X, Cao Z, Rothe M and Goeddel DV, IκB kinase-β: NF-κB activation and complex formation with IκB kinase-α and NIK. *Science* **278:** 866–869, 1997.
26. Palombella VJ, Rando OJ, Goldberg AL and Maniatis T, The ubiquitin-proteasome pathway is required for processing the NF-κB1 precursor protein and the activation of NF-κB. *Cell* **78:** 773–785, 1994.
27. Tagawa T, Kuroki T, Vogt PK and Chida K, The cell cycle-dependent nuclear import of v-Jun is regulated by phosphorylation of a serine adjacent to the nuclear localization signal. *J Cell Biol* **130:** 255–263, 1995.
28. Moll T, Tebb G, Surana U, Robirsch H and Nasmyth K, The role of phosphorylation and the CDC28 protein kinase in cell cycle-regulated nuclear import of the *S. cerevisiae* transcription factor SWI5, *Cell* **66:** 743–758, 1991.
29. Rao A, NF-ATp: A transcription factor required for the co-ordinate induction of several cytokine genes. *Immunol Today* **15:** 274–281, 1994.
30. Schreiber SL, Chemistry and biology of the immunophilins and their immunosuppressive ligands. *Science* **251:** 283–287, 1991.
31. Scheinman RI, Cogswell PC, Lofquist AK and Baldwin AS Jr, Role of transcriptional activation of IκBα in mediation of immunosuppression by glucocorticoids. *Science* **270:** 283–290, 1995.
32. Dubrovsky L, Ulrich P, Nuovo GJ, Manogue KR, Cerami A and Bukrinsky M, Nuclear localization signal of HIV-1 as a novel target for therapeutic intervention. *Mol Med* **1:** 217–230, 1995.
33. Popov S, Dubrovsky L, Lee MA, Pennathur S, Haffar O, aL-Abed Y, Tonge P, Ulrich P, Rexach M, Blobel G, Cerami A and Bukrinsky M, Critical role of reverse transcriptase in the inhibitory mechanism of CNI-H0294 on HIV-1 nuclear translocation. *Proc Natl Acad Sci USA* **93:** 11859–11864, 1996.
34. Gulizia J, Dempsey MP, Sharova N, Bukrinsky MI, Spitz L, Goldfarb D and Stevenson M, Reduced nuclear import of human immunodeficiency virus type 1 preintegration complexes in the presence of a prototypic nuclear targeting signal. *J Virol* **68:** 2021–2025, 1994.
35. Burton PS, Conradi RA, Ho NFH, Hilgers AR and Borchard RT, How structural features influence the biomembrane permeability of peptides. *J Pharm Sci* **85:** 1336–1340, 1996.
36. Lin Y-Z, Yao S, Veach RA, Torgeson TR and Hawiger J, Inhibition of nuclear translocation of transcription factor NF-κB by a synthetic peptide containing a cell membrane-permeable motif and nuclear localization sequence. *J Biol Chem* **270:** 14255–14258, 1995.
37. Cleves AE, Protein transport: The nonclassical ins and outs. *Curr Biol* **7:** R318–R320, 1997.
38. Tepper MA, Nadler SG, Esselstyn JM and Sterbenz KG, Deoxyspergualin inhibits κ light chain expression in 70Z/3 pre-B cells by blocking lipopolysaccharide-induced NF-κB activation. *J Immunol* **155:** 2427–2436, 1995.
39. Yang J and DeFranco DB, Differential roles of heat shock protein 70 in the *in vitro* nuclear import of glucocorticoid receptor and simian virus 40 large tumor antigen. *Mol Cell Biol* **14:** 5088–5098, 1994.
40. Her L-S, Lund E and Dahlberg JE, Inhibition of Ran guanosine triphosphatase-dependent nuclear transport by the matrix protein of vesicular stomatitis virus. *Science* **276:** 1845–1848, 1997.