

Differential Expression and Sequence-specific Interaction of Karyopherin α with Nuclear Localization Sequences*

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The process of nuclear protein transport requires the interaction of several different proteins, either directly or indirectly with nuclear localization or targeting sequences (NLS). Recently, a number of karyopherins α , or NLS-binding proteins, have been identified. We have found that the karyopherins hSRP1 and hSRP1 α are differentially expressed in various leukocyte cell lines and could be induced in normal human peripheral blood lymphocytes. We show that the two karyopherins bind with varied specificities in a sequence specific manner to different NLSs and that the sequence specificity is modulated by other cytosolic proteins. There was a correlation between binding of karyopherins α to different NLSs and their ability to be imported into the nucleus. Taken together, these data provide evidence for multiple levels of control of the nuclear import process.

Active nuclear transport of proteins with molecular weights greater than 40–60 kDa requires at least four different proteins, which act in a sequential manner with karyophilic proteins containing nuclear localization targeting sequences (NLS)¹ (1–4). There appear to be several discrete steps in the import process which involves: 1) binding of the NLS-binding protein, karyopherin α , to an NLS; 2) interaction of this complex with karyopherin β ; 3) targeting to nuclear pore proteins; and 4) the ATP/GTP-dependent translocation through the nuclear pore mediated by ran (1, 5, 6).

Recently, the proteins involved in NLS binding and transport have been identified. Those proteins that interact directly with the NLS have been termed karyopherins α (7–11). The *Xenopus* protein importin 60 was the first karyopherin α to be cloned, sequenced, and shown to be involved in nuclear protein import (7). Subsequently, a number of other karyopherins α have been identified, which suggests that there is a family of these NLS-binding proteins. The two major groups of karyopherins α include 1) the yeast protein SRP1 (12) and the human proteins hSRP1 and NPI-1 (8, 9), and 2) importin 60 (7) and the human proteins hSRP1 α (11) and Rch1 (10). In this report we have termed hSRP1 and hSRP1 α , K1 and K2, respectively. Each of these karyopherins α are capable of binding to

NLSs and facilitating nuclear import. Recently it was shown that there was tissue-specific expression of the mouse K1 (mSRP1) and K2 (mPendulin). The levels of K1 RNA appear higher in the brain and cerebellum, whereas K2 RNA was found mostly in the thymus and spleen (13).

Similar to karyopherin α , there are several homologs of karyopherin β , (also called importin 90 or p97) (11, 14). The function of karyopherin β appears to be the targeting of the karyophile-karyopherin α complex to the nuclear pore (11, 16). The interaction of karyopherin β with karyopherin α has been shown to enhance the latter protein's affinity for the NLS containing protein (1). Although the protein factors described above are sufficient to support nuclear protein transport, there are accessory factors which are also important for regulating nuclear transport. These factors include p10 (17–20), Rna1p (20), and the heat shock protein Hsc73 (21).

Perhaps most important for nuclear protein transport is the targeting sequence or NLS. Although there is a consensus for other organellar targeting sequences, there is little amino acid sequence homology among the large number of NLSs that have been identified (22). The most conserved feature of either the “simple” (5–7 amino acid sequences) or “bipartite” (two sets of positively charged amino acids separated by 10–11 amino acids) is the presence of two basic amino acids which constitutes the core of the NLS. Two mechanisms for regulating the activity of the NLS include protein phosphorylation and masking of the NLS to prevent its recognition by karyopherin α (23, 24).

In this report we show that there are multiple levels of control of nuclear import. These control points include the sequence specific binding of karyopherins α to various NLSs and modulation of this interaction by other cytoplasmic proteins. In addition, the differential and inducible expression of karyopherins α may play a role in regulating nuclear protein transport.

MATERIALS AND METHODS

Reagents—hSRP1 and hSRP1 α anti-peptide antibodies were prepared using the sequences: CMSTPGKENFRLKS and CMSTNENANTAAARLHR corresponding to the amino termini of hSRP1 and hSRP1 α , respectively (11). The peptides were coupled to keyhole limpet hemocyanin and injected into rabbits to raise polyclonal antibodies. Both antibodies were affinity purified over the corresponding peptide affinity column and shown not to be cross-reactive with one another (data not shown). BSA-FITC nuclear localization sequence conjugates were prepared by mixing 25 mg of BSA-FITC (Molecular Probes) with 10 mg of sulfo-SMCC (Pierce) for 2 h at room temperature. The complex was dialyzed into PBS and the NLS peptides added and coupled overnight at 4 °C. The FITC-BSA-NLS conjugate was finally dialyzed against transport buffer prior to use. Anti-Hsc73 antibodies were from StressGen Biotechnologies.

Subcellular Fractionation—Human peripheral blood lymphocytes were purified by Ficoll gradient centrifugation. Cytosolic extracts from either peripheral blood lymphocyte or cell lines were prepared by resuspending PBS washed cells in buffer “A” (20 mM HEPES, pH 7.2, 20

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¹ The abbreviations used are: NLS, nuclear localization sequence; K1, hSRP1; K2, hSRP1 α ; BSA, bovine serum albumin; FITC, fluorescein isothiocyanate; ECL, electrochemiluminescent; PBS, phosphate-buffered saline; GST, glutathione S-transferase; PAGE, polyacrylamide gel electrophoresis.

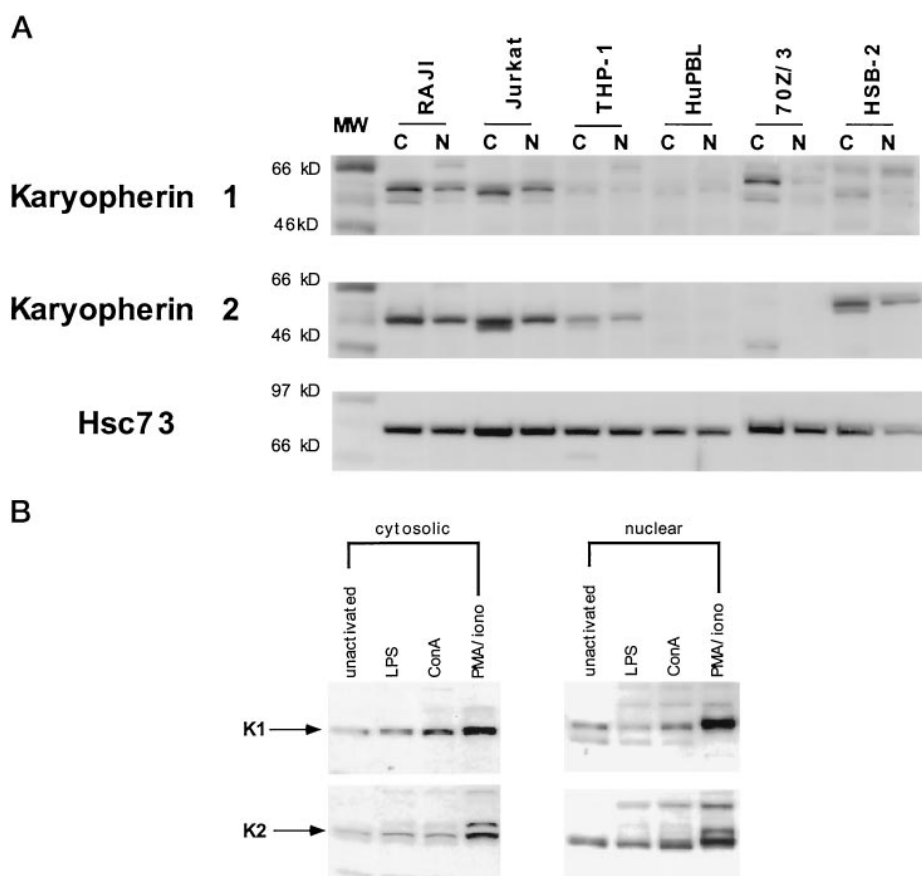


FIG. 1. Karyopherins are differentially expressed in leukocyte cell lines and can be induced in human peripheral blood lymphocytes. *A*, cytosolic or nuclear extracts were prepared as described under "Materials and Methods." Twenty-five μ g of protein were loaded per lane, separated by SDS-PAGE, and probed by Western blot and ECL detection for either K1, K2, or Hsc73. *B*, analysis of K1 and K2 in cytosolic and nuclear extracts from human peripheral blood lymphocytes. 2×10^7 cells each were activated for 2 h with either 100 ng/ml of lipopolysaccharide (LPS), 100 ng/ml concanavalin A (ConA), or 10 ng/ml phorbol myristic acid (PMA) + 1 μ M ionomycin. Twenty-five μ g of cytosolic protein was separated by SDS-PAGE and analyzed by Western blot for K1 and K2.

mm NaCl, 2.5 mM $MgCl_2$, 0.1% Nonidet P-40), 5 min, 4 $^{\circ}C$. Nuclei were pelleted at $2000 \times g$ for 4 min at 4 $^{\circ}C$ and vortexing. The cytosolic extract was gently removed by pipette and diluted 1:1 with cold PBS. Nuclear proteins were extracted using buffer A plus 0.42 M NaCl. A BCA protein assay (Pierce) was used to determine protein concentration.

Preparation of NLS-BSA-Sepharose Resins—BSA-Sepharose was prepared using activated CH-Sepharose as described by the manufacturer (Pharmacia Biotech Inc.). In order to couple the NLS peptides to the BSA-Sepharose, sulfo-succinimidyl 4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate was added to a 3-fold weight excess to the BSA-Sepharose and mixed at room temperature for 2 h. The resin was washed five times in PBS followed by incubation with the NLS peptides overnight at 4 $^{\circ}C$. Peptides were synthesized with a cysteine at either the C or N terminus for coupling to the affinity resin. Equal molar amounts of each of the different peptides was coupled to the BSA resin. There was approximately 1.0 peptide bound per BSA molecule. The amount of peptide bound to BSA-Sepharose was determined by measuring the amount of free cysteine (derived from the peptide) using Ellmans reagent before and after coupling. This procedure agrees well with amino acid analysis to measure coupling efficiency. The sequences of the NLSs are as follows: SV40 large T antigen, CGGGPKKKRKV; SV40 mutant, CGGGPKKAAAV; ICP8, CHRIEKKRKRTYETFKSI; HIV1422, CGGGKKKKYKLG; HIV 1423, CGGGKSKKKAQ (15); NF- κ B P50, CYPEIKDKKEEVQRKQKL; NF- κ B P50 mutant, CYPEIKDKKEEVQRAAQKL; Myc, CGGGPAKRVLKLD. Peptides were synthesized on a Gilson multiple peptide synthesizer using Fmoc (*N*-(9-fluorenyl)methoxycarbonyl) amino acids. All of the peptides were analyzed by mass spectrometry and yielded the correct molecular weight. Similar results were obtained with three different preparations of affinity resins.

Precipitation of NLS-binding Proteins—Raji or Jurkat cell extracts were prepared as described above. The cytosolic extracts were pre-cleared for 2 h at 4 $^{\circ}C$ with the BSA-Sepharose resin with no NLS attached. After pre-clearing, 25 μ l of Sepharose-BSA-NLS resin was incubated with cytosolic extract from 5×10^6 cells with mixing at 4 $^{\circ}C$ for 1 h. Samples were then washed four times with ice-cold PBS (1 ml each wash). 100 μ l of SDS-gel sample buffer were then added to each sample.

For the experiment using purified K2-GST fusion protein, a cytosolic

extract from 1×10^7 Jurkat cells was mixed with 2 μ g of K2-GST fusion protein for 2 h at 4 $^{\circ}C$ prior to incubating with the NLS affinity resins. The purified protein alone (2 μ g) was incubated in the presence of 1 mg/ml BSA in a 1:1 mixture of buffer A and PBS with the affinity resins. After incubation, the samples were washed four times with cold PBS and analyzed by Western blot.

Nuclear Import Assay—*In vitro* nuclear import was measured using a modification of previously published procedures (25). Cytosol extracts were prepared from either Jurkat, HSB2, or 70Z/3 cells as follows. Cells were suspended in 1.5 volumes of lysis buffer (5 mM HEPES-KOH, pH 7.35, 110 mM potassium acetate, 5 mM sodium acetate, 2 mM magnesium acetate, 0.1 mM EGTA, 2 mM dithiothreitol, and protease inhibitor mixture containing 0.2 mM phenylmethylsulfonyl fluoride, 1 μ g/ml pepstatin, 1 μ g/ml leupeptin, and 1 μ g/ml tosylphenylalanyl chloromethyl ketone). The cells were allowed to swell for 10 min on ice and then homogenized with a motorized Teflon pestle for 15 strokes. The cytosol was dialyzed against transport buffer (20 mM HEPES-KOH, pH 7.35, 110 mM potassium acetate, 5 mM sodium acetate, 2 mM magnesium acetate, 0.1 mM EGTA, 2 mM dithiothreitol) overnight. Protein concentration of the extract was between 8 and 16 mg/ml. For the import assay, cells were diluted into carbonate buffer and attached to glass slides using Cell-Tak (Collaborative Research). To permeabilize cells, digitonin was diluted from a frozen 20 mg/ml stock in Me_2SO to 40 μ g/ml in transport buffer. Cells were rinsed twice with cold transport buffer after permeabilization.

The final transport reaction consists of 1 mM ATP, 10 mM creatine phosphate, 0.5 unit/ml creatine phosphokinase, varying concentrations of NLS-FITC-BSA, and 50% cytosolic extract in a 25- μ l aliquot. This mixture was placed on the digitonin-permeabilized cells, and coverslips were incubated for 1 h in a 37 $^{\circ}C$ incubator. Cells were rinsed twice with cold PBS and fixed in 2% paraformaldehyde, PBS for 15 min, protected from light. Cells were again rinsed twice and aspirated to near dryness, and 7 μ l of Slo-Fade (Molecular Probes) were added per circle. Samples were analyzed by fluorescent confocal microscopy (Bio-Rad).

Production of K2 Fusion Protein—The cDNA for the Rch1 (K2) protein was amplified from a phorbol myristic acid-activated human T cell library by polymerase chain reaction and inserted into pCDM8. The GST-K2 fusion protein was produced by amplifying from this construct, inserting the polymerase chain reaction fragment into the *Bam*HI-*Eco*RI sites of pGEX-2T (Pharmacia) and transforming into *Escherichia*

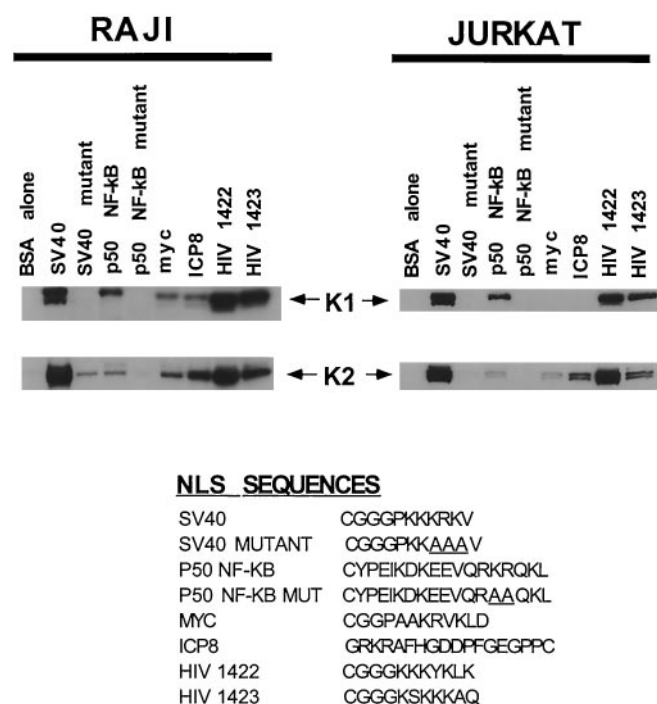


FIG. 2. **Differential interaction of karyopherin α with NLS affinity resins.** NLS binding proteins were precipitated from cytosolic extracts prepared from 5×10^6 Raji or Jurkat cells as described under "Materials and Methods." Bound protein was separated by 12% SDS-PAGE and analyzed by Western blot and ECL analysis. The bottom portion shows the sequences of the various NLSs used for the precipitation study. Similar results were obtained using three different batches of affinity resins.

coli (DH5 α , Life Technologies, Inc.). Protein was purified on a glutathione-agarose affinity column (Sigma).

NLS:K2-GST Competitive Solution Binding Assay—A 96-well Immulon plate was coated overnight with 20 ng of a p50 NF- κ B-ovalbumin conjugate. Varying concentrations of unconjugated soluble inhibitor peptides were then added and incubated for 15 min, followed by the addition of 100 ng of biotinylated K2-GST. The plate was incubated for 1 h at 22 °C followed by four washes with PBS containing 0.01% Tween. The biotinylated K2-GST was detected using streptavidin-horseradish peroxidase. All reactions were performed in PBS plus 0.01% Tween. The IC₅₀ was determined to be the concentration of soluble peptide which yielded 50% inhibition of binding of K2-GST to the NLS conjugate bound to the plate.

RESULTS

K1 and K2 Are Differentially and Inducibly Expressed in Human Lymphocytes and Leukocyte Cell Lines—In order to study the expression of karyopherin α , cytosolic and nuclear extracts from various lymphocyte cell lines were prepared and analyzed by Western blot for levels of K1 and K2. Although equal amounts of protein were loaded for each sample, there were clear differences in the levels and cytoplasmic:nuclear distribution of K1 and K2 in the various cell lines as detected by ECL analysis (Fig. 1A). All of the cell lines except for the murine 70Z/3 cells are of human origin. Raji (human B) and Jurkat (human T) cells express the highest levels of the karyopherins and have slightly higher cytoplasmic:nuclear ratios for K1 than for K2. We found that karyopherin α is present in both the cytoplasm as well as the nucleus in different nuclear:cytoplasmic ratios, depending on the cell type. THP-1 (human monocytic), 70Z/3 (mouse pre-B), and HSB-2 (human pre-T) cells express significantly lower levels of K1 and K2 and appear to express one predominant form per cell type. That is, THP-1 and HSB-2 cells express mostly K2, whereas the 70Z/3 cells express mostly K1. Although the 70Z/3 cells are of mouse origin, there are only two amino acid differences between the

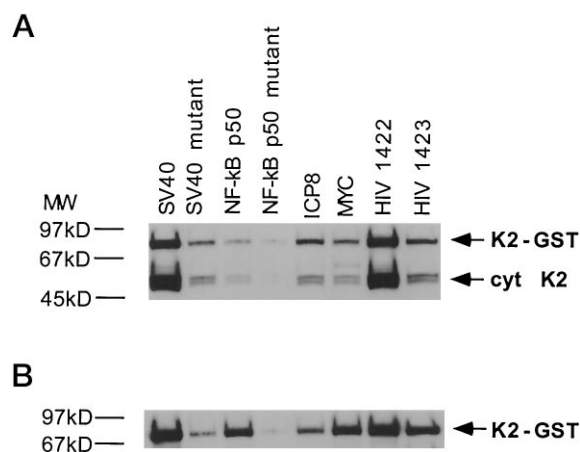


FIG. 3. **Effect of cytosolic proteins on the interaction of K2 with the NLS affinity resins.** The K2-GST fusion protein was incubated with the different NLS affinity resins in either the presence (panel A) or absence (panel B) of a cytosolic extract from Jurkat cells. After washing as described under "Materials and Methods," the samples were analyzed by Western blotting using an anti-K2 antibody and ECL analysis.

mouse pendulin (K2) and human hSRP1 α (K2) in the sequence we used for raising antibodies (11). Hence, we believe that K2 is not expressed in this mouse cell line. As a control, levels of the heat shock protein Hsc73 were similar in the different cell lines and appeared equally distributed between the cytoplasm and nucleus (Fig. 1A).

In comparison to the cell lines, the levels of K1 and K2 were much lower, on a per protein basis, in normal human peripheral blood lymphocytes. Although there was variation in the basal levels of K1 and K2 depending on the donor, in each case the levels were lower than in the cell lines. Since the levels were quite low we explored whether the levels could be increased upon cell activation. As seen in Fig. 1B, activation of peripheral blood lymphocyte with lipopolysaccharide (LPS), concanavalin A (ConA), or phorbol myristic acid (PMA) + ionomycin, causes significant increases in the level of expression of K1 and K2 in the cytoplasm and nucleus. In cell lines already expressing K1 and K2, there was no significant increase after cell activation (data not shown).

K1 and K2 Bind Differently to Various NLSs—Since there are many different NLSs (22), we investigated whether K1 and K2 bind with similar or different specificities to a variety of NLS peptides. To study this interaction, Sepharose-BSA-NLS affinity resins were incubated with cytosolic extracts from either Raji or Jurkat cells. As seen in Fig. 2, using either Raji or Jurkat lysates, there were clear differences in the ability of either K1 or K2 to interact with the different NLS resins. K1 and K2 appeared to interact with greatest affinity for the SV40 T antigen NLS, but there was very little binding to the SV40 mutant resin which had 3 amino acid substitutions. The HIV1422 NLS appeared to interact similarly to the SV40 NLS, while K1 and K2 had a lower affinity for the other NLSs. To support the affinity chromatography studies, we also quantitated the binding of soluble free NLS peptides to purified K2-GST using a competition binding assay as described under "Materials and Methods." Based on the chromatography data we analyzed low (p50 NF- κ B) and high (SV40) affinity NLS:K2 interactions in the solution binding assay. We found that the SV40 and p50 NF- κ B NLSs had IC₅₀ values of 0.1 and 12.5 μ M, respectively. This suggests that there is an approximately two orders of magnitude difference in the affinity of these NLSs for K2. These data support the results shown in Figs. 2 and 3 and confirm our findings that the NLSs interact with differential affinities to karyopherin α .

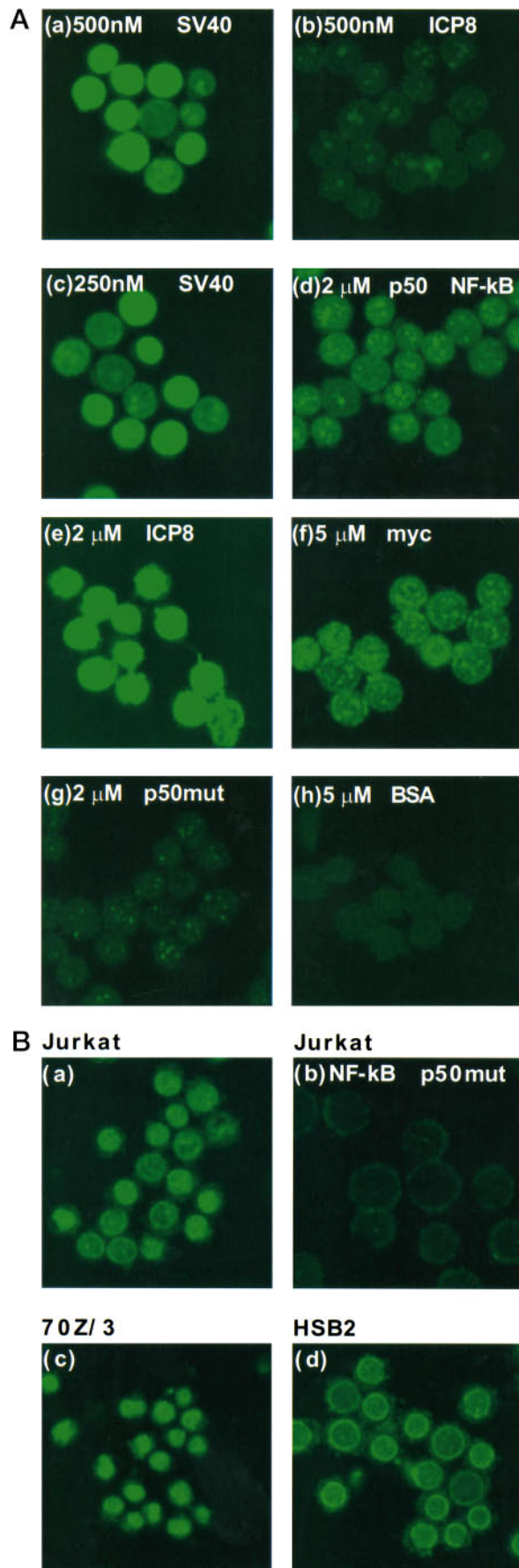


FIG. 4. Nuclear transport of BSA-FITC-NLS conjugates. Nuclear transport assays were performed as described under "Materials and Methods." A, nuclear transport of different NLS conjugates using

The differential interaction of K1 and K2 with the various NLSs appeared to be dependent on the cell type used for affinity chromatography (Fig. 2). For example, whereas K1 binds to the ICP8 and Myc NLS in the Raji cells, there was no binding of these NLSs in the Jurkat cells. In addition, K2 bound to the Myc, ICP8, and HIV1423 NLS to a greater extent in the Raji cell lysate, as opposed to the Jurkat lysate.

In order to address whether other cytoplasmic proteins could affect the ability of K2 to interact with NLSs we cloned and expressed K2 as a GST fusion protein. We then analyzed the interaction of the fusion protein alone *versus* the fusion protein incubated with a cytoplasmic extract. As seen in Fig. 3B, the K2-GST fusion protein had the highest relative affinity for the SV40 and HIV1422 NLS. Binding among the NF- κ B p50, Myc, and HIV1423 NLSs to K2-GST was equivalent, whereas the amount of binding to ICP8 and the mutant NLSs was much less. When the fusion protein was mixed with cytosolic extracts the relative binding specificities were quite different compared to using the purified protein alone (Fig. 3A). There were some slight differences in binding between the native K2 and the fusion protein which migrates at a higher molecular weight; however, the most significant differences are between Fig. 3, A and B. The purified K2-GST binds well to the NF- κ B p50 NLS, whereas it does not bind when present in the cytosolic mix of protein. The binding of K2-GST to the Myc and HIV1423 NLSs was also diminished when mixed with the cytoplasmic proteins, whereas binding to SV40, HIV1423, and ICP8 was unchanged. These data clearly show that cytoplasmic proteins can alter the binding specificity of K2 to different NLSs.

Nuclear Transport of NLS-BSA Conjugates—As described above, we showed that there was differential expression and interaction of karyopherins with NLSs depending on the cell type. Therefore, using an *in vitro* nuclear transport assay (25) we examined whether there was a correlation between nuclear import and either the expression or interaction of karyopherins with NLSs. Fig. 4A shows that the SV40 NLS-BSA conjugate is transported into the nucleus much more efficiently than the ICP8 NLS conjugate at equimolar concentrations (*panel a versus b*). There was also a good correlation between the nuclear transport of the SV40, p50 NF- κ B, ICP8, and Myc NLS conjugates and their ability to bind to the NLS affinity resins (Fig. 4A, *panels c–f*, compared to Fig. 2). We predicted that there might be differences in the ability of different cell extracts to reconstitute nuclear transport based on the differential expression of the karyopherins α . As seen in Fig. 4B, there were subtle differences in the ability of different cell extracts to reconstitute import of the p50 NF- κ B NLS BSA conjugate. It appears that the 70Z/3 extract was most efficient at supporting nuclear import followed by the Jurkat and the HSB2 extracts. Although there are no dramatic differences in the ability of the cell extracts to reconstitute import, there is a correlation with expression of K1 and K2 and their ability to bind to the p50 NF- κ B NLS conjugate. That is, 70Z/3 cells express K1 which binds best to the p50 NF- κ B NLS compared to the HSB2 cells which express K2 which binds weaker to this particular NLS. We have reproducibly seen that in the HSB2 cells, the import substrate is mostly concentrated at the nuclear rim. This may be due to limited expression of other components of the import

Jurkat cell extracts. In *panels c–f*, different concentrations of the conjugates were used to attain similar levels of nuclear transport. *Panels g and h* show that there is minimal transport of the p50 NF- κ B mutant-BSA-FITC conjugate or BSA-FITC alone. B, nuclear transport using cytosolic extracts from either HSB2, Jurkat, or 70Z/3 cells. 1.0 μ M of the p50 NF- κ B BSA-FITC conjugate was used with equivalent amounts of cytosolic protein from each of the different cell types. *Panels a and b*, Jurkat cell extracts; *panel c*, HSB2 extract; *panel d*, 70Z/3 extract.

machinery such as ran which is required for the energy dependent translocation through the nuclear pore, among other possibilities. Based on the data presented above it appears that nuclear transport correlates best with the ability of karyopherins α to bind to NLSs, as opposed to the expression of the karyopherins α in the cell.

DISCUSSION

Many of the recent studies on nuclear protein transport have focused on the mechanics of the import process in nonhematopoietic cells such as HeLa and *Xenopus* oocytes, as well as in reconstituted *in vitro* systems (1, 4). In contrast to these studies, we have focused on the transport machinery in lymphocytes and leukocyte cell lines. In the studies presented here, we have analyzed the expression and interactions of human karyopherins α with different NLSs.

Lymphocyte activation induces the expression of a wide variety of genes, which are dependent upon the translocation of specific transcription factors to the nucleus (26, 27). A key protein in this translocation process is karyopherin α , the NLS-binding protein (7). We found that, in general, the less differentiated cells (THP-1, 70Z/3, HSB-2) have lower levels of karyopherin α and tend to express only one predominant form (Fig. 1). In contrast, the more mature Raji and Jurkat cell lines express much higher levels of both K1 and K2. Differences were also seen in the cytoplasmic:nuclear ratios of the karyopherins. The differences in both overall expression and intracellular distribution of karyopherins may reflect the transcriptional activity of the particular cell line and the requirement for transport of particular proteins into the nucleus. Unexpectedly, the levels of both K1 and K2 were very low in resting human peripheral blood lymphocytes in comparison to the cell lines. This suggested that other karyopherins α may be expressed in these cells, or that the levels are low due to the "resting" state of the cells which require a low level of transcription. We found that commonly used stimuli of peripheral blood lymphocytes were able to induce the expression of K1 and K2. Although it has been shown that cell activation leads to increased numbers of nuclear pores to facilitate protein nuclear transport (28), the increased expression of the proteins involved in the transport process may be an additional mechanism for enhancing nuclear transport rates.

Another potential mechanism for regulation of nuclear transport could be at the level of the NLS itself. Previous studies have shown that different NLSs have varying abilities to target proteins to the nucleus (29, 30). In order to determine whether NLSs vary in their ability to interact with the NLS-binding proteins K1 and K2, we precipitated intracellular proteins using NLS affinity resins and found that there were differences in the ability of K1 and K2 to interact with NLSs (Fig. 2). These results were confirmed using a solution binding assay. These data suggest that the different classes of karyopherins have both different and overlapping specificities for the various NLSs.

The specificity for the NLSs was also dependent upon the cell type. For example there was no binding of K1 to the Myc and ICP8 NLS in the Jurkat cell, whereas there was binding to these NLSs in the Raji cell extract. These data suggest that other cytosolic proteins may influence the way in which K1 or K2 interact with NLSs. To address this possibility we analyzed the ability of a K2 fusion protein to interact with NLSs in the presence or absence of other cytosolic proteins. As seen in Fig. 3, the binding specificity of K2 for the NLS is clearly altered in the presence of other cytosolic proteins. This modulation of binding could be explained by a number of possibilities. One possibility is that karyopherin β , which has previously been shown to increase the affinity of karyopherin α for NLSs, may

also alter its binding specificity. The heat shock protein Hsc73, which has been shown to play a role in nuclear transport (21), may modulate NLS:karyopherin α interactions. In fact, Hsc73 does bind to NLSs and appears to modulate the association and dissociation reaction of karyopherins.² A third possibility is that endogenous NLS-containing proteins can compete for binding of karyopherins α to the NLS affinity resins. These possibilities are now being explored.

Finally, we analyzed the ability of different NLS substrates to be targeted to the nucleus. The ability of NLS-BSA conjugates to be imported into the nucleus appeared to be determined by the strength of the interaction between the NLS and karyopherin α . Although the Jurkat, HSB-2, and 70Z/3 cells expressed different levels and ratios of the two karyopherins α , there were only subtle differences in the ability of extracts from these cells to facilitate nuclear import. This would suggest that there may be other karyopherins expressed that are not detected by our antibodies or that other cytoplasmic proteins can modulate the ability of the existing karyopherins to import proteins to the nucleus. It appears that the ability of proteins to be imported into the nucleus is predominantly determined by the interaction of the NLS with karyopherins and to a limited extent by the differential expression of the karyopherins.

The process of nuclear protein import plays a key role in gene regulation based on its ability to modulate transcription factor nuclear localization. The results described above suggest that there are multiple levels of control of nuclear import in lymphocytes and leukocyte cell lines. Studies are in progress to determine whether the activation and induction of karyopherins in resting, G_0 , peripheral blood lymphocytes has an impact on nuclear transport. Taken together, our data suggest there are complex mechanisms for the regulation of nuclear import in different leukocyte cell lines that are mainly governed by the NLS and its sequence specific interaction with karyopherin α .

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