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Nuclear import of hnRNP A1 is mediated by a novel cellular cofactor related to karyopherin-β

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SUMMARY

Heterogeneous nuclear ribonucleoprotein A1 contains a sequence, termed M9, that functions as a potent nuclear localization signal (NLS) yet bears no similarity to the well-defined basic class of NLSs. Here, we report the identification of a novel human protein, termed MIP, that binds M9 specifically both in vivo and in vitro yet fails to interact with non-functional M9 point mutants. Of note, the 101 kDa MIP protein bears significant homology to human karyopherin/importin-β, a protein known to mediate the function of basic NLSs. The in vitro nuclear import of a protein substrate containing the M9 NLS was found to be dependent on provision of the MIP protein in *trans*. Cytoplasmic microinjection of a truncated form of MIP that retains the M9 binding site blocked the in vivo nuclear import of a substrate containing the M9 NLS yet failed to affect the import of a similar substrate bearing a basic NLS. These data indicate that nuclear import of hnRNP A1 is mediated by a novel cellular import pathway that is distinct from, yet evolutionarily related to, the pathway utilized by basic NLS sequences.

Key words: Nuclear transport, NLS, hnRNP A1

INTRODUCTION

The ongoing, active transport of appropriate proteins and RNAs between the cytoplasm and the nucleus of eukaryotic cells is essential for viability (reviewed by Sweet and Gerace, 1995; Görlich and Mattaj, 1996; Koepp and Silver, 1996). While relatively little is as yet known about how nuclear export is regulated, at least one pathway for nuclear protein import is now fairly well understood, i.e. the import pathway utilized by proteins containing basic NLSs similar to those identified in SV40 'T' antigen and in nucleoplasmin (Dingwall et al., 1982; Kalderon et al., 1984). In particular, it is now known that basic NLSs are specifically recognized by a cellular protein termed karyopherin-α or importin-α (Adam and Gerace, 1991; Görlich et al., 1994; Moroianu et al., 1995a; Weis et al., 1995). This complex in turn interacts with a second cellular cofactor, termed karyopherin-β or importin-β , which serves to dock the resultant trimeric complex at a nuclear pore (Chi et al., 1995; Görlich et al., 1995; Imamoto et al., 1995; Moroianu et al., 1995b; Radu et al., 1995). Subsequently, the NLS substrate/karyopherin-α complex is translocated into the nucleus in an active process that is dependent on the action of the Ran GTPase and a second factor termed p10 or nuclear transport factor 2 (Moore and Blobel, 1993; Görlich et al., 1995; Paschal and Gerace, 1995; Nehrbass and Blobel, 1996). This docking and translocation process is believed to require the sequential interaction of karyopherin-β with specific nucleoporins located at the cytoplasmic and nuclear faces of the nuclear pore and within the pore channel itself (Rexach and Blobel, 1995). Once the ternary complex has reached the inner face of the nuclear membrane, the karyopherin-α/β heterodimer dissociates, most probably as a result of a direct interaction between karyopherin-β and Ran-GTP (Görlich et al., 1996b; Moroianu et al., 1996), and the NLS substrate/karyopherin-α complex is released into the nucleoplasm.

Although the majority of nuclear proteins appear to use the basic NLS-specific nuclear import pathway, several apparent exceptions exist. In particular, heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1) and several related hnRNPs contain a large, ~38 amino acid (aa) glycine-rich NLS, termed M9, that has no evident similarity to basic NLS sequences (Michael et al., 1995; Siomi and Dreyfuss, 1995; Weighardt et al., 1995). In this report, we describe the identification and initial characterization of a novel cofactor for M9 NLS function that is related to, but distinct from, the karyopherin-β cofactor that has been shown to be essential for basic NLS function.

MATERIALS AND METHODS

Yeast two-hybrid interaction analysis

The yeast expression plasmids pGAL4/hnRNP A1 and pGAL4/M9 were made by inserting cDNA sequences encoding either full-length hnRNP A1 or hnRNP A1 aa 256-320 in frame with the GAL4 DNA binding domain present in pGBT9 (Clontech). The plasmids pGAL4/G274A and pGAL4/P275A were made by introducing the indicated missense mutations by recombinant polymerase chain reaction (PCR) into the parental plasmid pGAL4/M9. Two-hybrid interaction analysis and library screens were performed as described (Fields and Song, 1989; Bogerd et al., 1995) in the yeast strain Y190 (Harper et al., 1993). Partial MIP cDNA clones were isolated from

the two-hybrid screens using a HeLa cell cDNA two-hybrid library obtained from Clontech. A cDNA encoding the full-length MIP protein was isolated with 5′ rapid amplification of cDNA ends (RACE) using a Marathon Ready HeLa cell cDNA isolation kit (Clontech).

Recombinant protein expression

Fusion proteins consisting of full length (1-890) or truncated (541-890) forms of MIP linked to either glutathione-S-transferase (GST) (pGEX4T-1, Pharmacia) or the maltose binding protein (MBP) (pMAL-c2, New England Biolabs) were expressed in bacteria and purified as described (Fridell et al., 1996). The GST-MIP (541-890) fusion protein was used to immunize rabbits. The MBP-MIP (541-890) protein was coupled to an AffiGel 10 column (Bio-Rad) and used to affinity purify MIP specific antibodies from the serum of GST/MIP injected rabbits as described (Bogerd et al., 1995). The GST-M9 and GST-TNLS fusion proteins were expressed in bacteria using the pGEX4T-1 vector (Pharmacia) and consist of GST fused to the M9 NLS located between hnRNP A1 residues 264-308 (NH_2-GNYNNQNQSSNFGPMKGGNFGGRSSGPYGGGGQYFAKPRN-QGGYGGS-COOH) or GST fused to the basic NLS (NH_2-TPP-KKKRKVEDP-COOH) of the SV40 large 'T' antigen. These recombinant proteins were purified using standard procedures in the absence of detergent (Fridell et al., 1996). Prior to use in in vitro nuclear import assays, the GST-M9 and GST-G274A proteins were fluorescent-label conjugated with the FLOUS Fluorescein-derived dye at a ratio of 100:1 (Boehringer Mannheim).

Protein binding assays

A cDNA encoding full-length MIP was cloned into pGEM3zf(+) (Promega) and this plasmid was used to express [^{35}S]methionine labeled MIP in a rabbit reticulocyte lysate coupled transcription/translation system (Promega). This MIP programmed reticulocyte lysate was diluted 1:10 into affinity chromatography buffer (20 mM Hepes, pH 7.4, 10% glycerol, 0.1 mM dithiothreitol, 0.1 mM EDTA) containing 0.l M sodium chloride and samples passed over AffiGel 10 columns coupled to either GST, GST-M9 or GST-G274A. The columns were washed extensively with the loading buffer, eluted with affinity chromatography buffer containing 0.5 M sodium chloride and the eluate then analyzed by SDS polyacrylamide gel electrophoresis and autoradiography.

In vitro nuclear import reactions

HeLa cells were seeded onto poly-L-lysine coated coverslips at 2.0×10^6 cells/ml and grown for 18 hours. Fresh medium was added 3 hours prior to digitonin permeabilization. Cells were permeabilized with 40 μg/ml digitonin as described (Adam et al., 1990; Adam and Adam, 1994).

Import experiments were performed for 30 minutes at 30°C in a 40 μl volume overlay of the permeabilized HeLa cells. Import reactions contained 200 nmol FLOUS-labeled GST-M9 or GST-G274A, 400 nmol unlabeled GST, with or without 20 nmol MBP-MIP, 1 mM GTP, an ATP regeneration system (Adam et al., 1990) and transport buffer (Adam et al., 1990), consisting of 20 mM Hepes, pH 7.4, 110 mM potassium acetate, 2 mM magnesium acetate, 1 mM dithiothreitol, 0.2 mM EGTA and 1 μg/ml each aprotinin, leupeptin and pepstatin. After incubation, cover slips were washed once in transport buffer, blotted, and inverted onto 20 μl Fluoromount G (Southern Biotechnology) on a glass slide, allowed to set for one hour and the coverslip edges were then sealed with nail polish. Images were digitally captured on a Leica DMRB fluorescence microscope and converted to 8-bit gray scale with NIH Image 1.60 image analysis software.

Mammalian cell assays

The localization of endogenous MIP was determined by indirect immunofluorescence as described (Bogerd et al., 1995) using a 1:1,000 dilution of the affinity purified anti-MIP antiserum and a 1:200 dilution of a rhodamine-conjugated goat anti-rabbit antibody (Boehringer Mannheim). Cells were visualized with a Leica DMRB fluorescence microscope. Immunoprecipitation of [^{35}S]methionine labeled MIP from human 293T cells was performed as described (Cullen, 1986).

A plasmid expressing an influenza hemagglutinin (HA) tagged form of the full-length MIP protein was constructed in the context of the pBC12/CMV expression plasmid (Cullen, 1986). The MIP cDNA was amplified by PCR using primers that inserted a unique *Nco*I site coincident with the MIP translation initiation codon and a unique *Xho*I site just 3′ to the coding region. This PCR product was then cleaved with *Nco*I and *Xho*I and inserted into the pBC12/CMV plasmid. A DNA fragment encoding 3 tandem copies of the HA epitope tag (NH_2-YPYDVPDYA-COOH) was then introduced at the *Nco*I site, thus inserting the tag at the MIP amino terminus. COS cells were transfected with the HA-MIP expression plasmid and the localization of the tagged protein determined by indirect immunofluorescence, as previously described (Fridell et al., 1996).

Prior to microinjection, GST fusion proteins were diluted to 2 mg/ml in phosphate buffered saline (PBS) and each preparation was supplemented with 1 mg/ml rabbit immunoglobulin G (IgG) (Jackson ImmunoResearch). The MBP-MIP (541-890) protein was used at 4 mg/ml in PBS. Two days prior to injection, 2×10^5 HeLa cells were plated onto glass coverslips placed in 35 mm dishes. After injection, cells were returned to 37°C for 30 minutes and then fixed with 3% paraformaldehyde. The cellular localization of the injected proteins was determined by double label indirect immunofluorescence at a magnification of ×100 (Fridell et al., 1996). GST-fusion proteins were detected with an anti-GST monoclonal antibody (Santa Cruz Biotechnology) followed by a rhodamine-conjugated goat anti-mouse antibody (Cappel). IgG was directly visualized with a fluorescein-conjugated donkey anti-rabbit antibody (Jackson ImmunoResearch).

RESULTS

Identification of an M9 interacting protein

The M9 NLS found in hnRNP A1 has been mapped to an ~38 aa glycine-rich sequence located between residues 268 and 305 of the 320 aa hnRNP A1 protein (Michael et al., 1995; Siomi and Dreyfuss, 1995; Weighardt et al., 1995). Mutants of M9 bearing substitutions of alanine for glycine 274 (G274A) or proline 275 (P275A) have been shown to entirely lack NLS function (Michael et al., 1995). We therefore performed a yeast

Table 1. MIP specifically binds to the M9 NLS found in hnRNP A1

	β-Gal activity	
	+VP16/MIP	+VP16
GAL4	<0.01	<0.01
GAL4/hnRNP A1	0.94	<0.01
GAL4/M9	2.52	<0.01
GAL4/G274A	0.01	<0.01
GAL4/P275A	0.03	<0.01

Human hnRNP A1, as well as wild-type and mutant forms of the M9 NLS present in hnRNP A1 (Michael et al., 1995), were expressed in the Y190 yeast indicator strain as fusions with the GAL4 DNA binding domain (Fields and Song, 1989; Harper et al., 1993; Bogerd et al., 1995). Coexpression of a fusion protein consisting of the human MIP protein (here residues 541 to 890) linked to the VP16 activation domain resulted in the activation of an integrated *lacZ* indicator gene linked to GAL4 DNA binding sites, but only if the M9 NLS was expressed in a functional form. All GAL4 fusion proteins were expressed at similar levels, as determined by western analysis using a GAL4 specific antiserum (data not shown).

two-hybrid screen (Fields and Song, 1989; Bogerd et al., 1995) to identify human proteins able to bind full-length hnRNP A1 and the M9 NLS but not the G274A or P275A mutants. While seven clones were identified that satisfied these criteria (Table 1), these were all found to encode overlapping segments of the same gene product, which we initially named M9 interacting protein (MIP).

We next used the rapid amplification of cDNA ends procedure to obtain an apparently full-length human MIP cDNA. This cDNA sequence predicts an 890 aa gene product with a calculated molecular mass of 101 kDa (Fig. 1). Based on this sequence, the largest MIP clone obtained in the two-hybrid screen encoded residues 351 to 890 of MIP while the smallest encoded residues 620 to 890. This latter MIP sequence appears to be close to the minimal limit for functional interaction with the M9 NLS, in that VP16 activation domain fusion proteins containing residues 660 to 890 of MIP, or containing residues 620 to 843, both failed to detectably interact with the M9 NLS in the yeast two-hybrid assay (data not shown).

To examine the pattern of MIP protein expression in vivo, we prepared a MIP-specific antiserum by injecting rabbits with a fusion protein containing GST linked to residues 541 to 890 of MIP. Antibodies reactive with MIP were then affinity purified (Bogerd et al., 1995), using a fusion protein consisting of MBP fused to the identical MIP sequence, and used to immunoprecipitate MIP from [^{35}S]methionine labeled human 293T cells (Cullen, 1986). As can be seen in Fig. 2A, a single band of ~100 kDa representing endogenously expressed MIP protein was observed. In vitro translation of an RNA transcript co-linear with the MIP cDNA sequence shown in Fig. 1 gave rise to a single protein of ~100 kDa that precisely co-migrated with the endogenous MIP protein (Fig. 2A). We therefore conclude that this MIP cDNA sequence is full-length.

Radiolabeled, full-length MIP protein obtained by in vitro translation was next used to ask whether MIP would bind the M9 NLS specifically in vitro. As shown in Fig. 2B, radiolabeled MIP protein was bound specifically by a wild-type GST-M9 fusion protein but did not interact with either GST itself or with GST fused to the G274A mutant of M9. While these data demonstrate that MIP binds the M9 NLS specifically in vitro, they do not prove a direct interaction in that an intervening protein derived from the reticulocyte lysate could in theory be involved. However, a soluble recombinant fusion-protein consisting of MBP fused to residues 541 to 890 of MIP, which are fully sufficient for M9 binding in the yeast two-hybrid system (Table 1), also proved able to specifically bind to the GST-M9 protein in vitro, but not to the GST-G274A mutant or to GST (data not shown). We therefore conclude that the MIP/M9 interaction is indeed direct.

MIP is related to human karyopherin-β

A search of computer data banks for proteins similar to MIP identified human karyopherin/importin-β as the mammalian gene product with the greatest homology to MIP (Fig. 1). As noted above, karyopherin-β has been shown to play a key role in the nuclear import of proteins bearing basic NLSs similar to the NLS first defined in SV40 'T' antigen (Chi et al., 1995; Görlich et al., 1995; Imamoto et al., 1995; Moroianu et al., 1995b; Radu et al., 1995). Although relatively little is known about the functional domain organization of karyopherin-β, an acidic sequence located between residues 333 and 341 has been

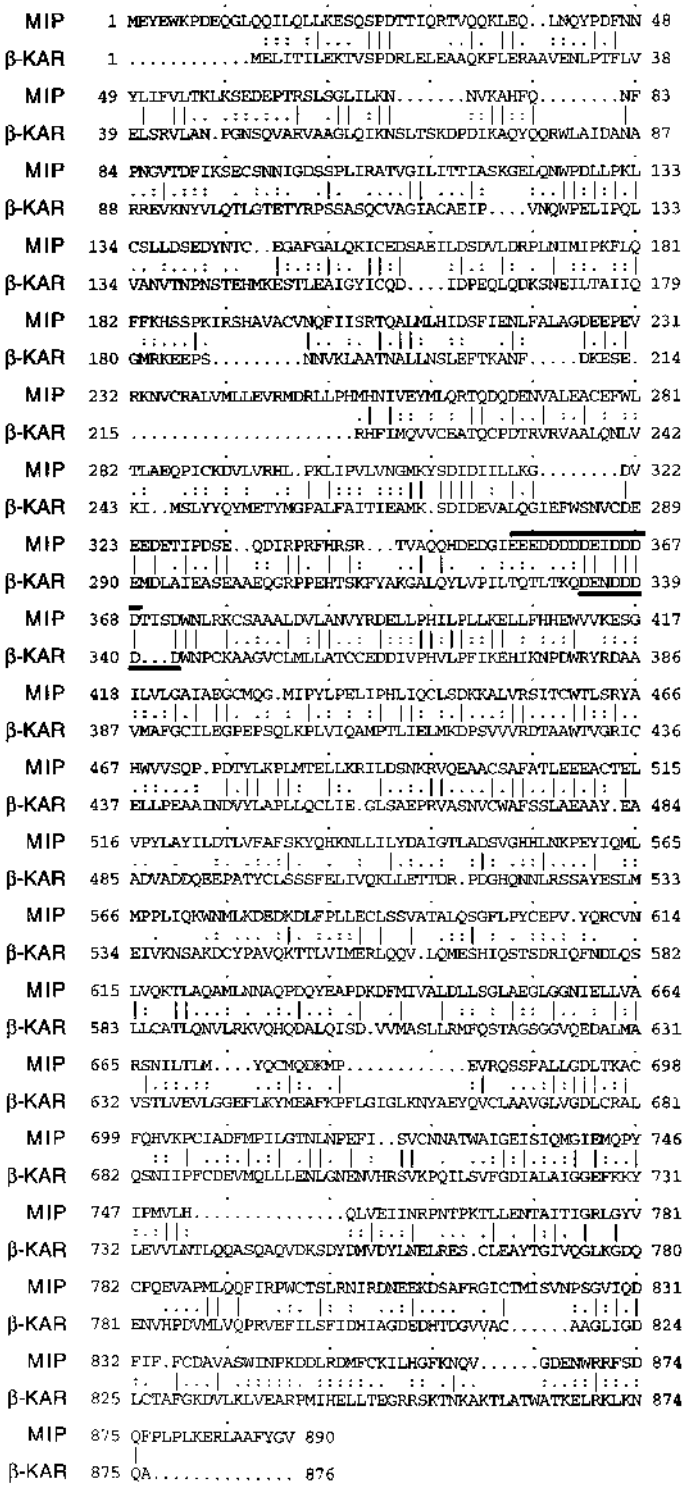

Fig. 1. Predicted primary sequence of the human MIP protein and alignment with the sequence of human karyopherin/importin-β. The solid line indicates the presence of a highly acidic protein motif within karyopherin-β that is critical for binding to Ran-GTP and that is conserved in MIP.

implicated in binding to both karyopherin-α and the Ran cofactor (Moroianu et al., 1996). While this acidic sequence is conserved in MIP (Fig. 1), we have as yet been unable to

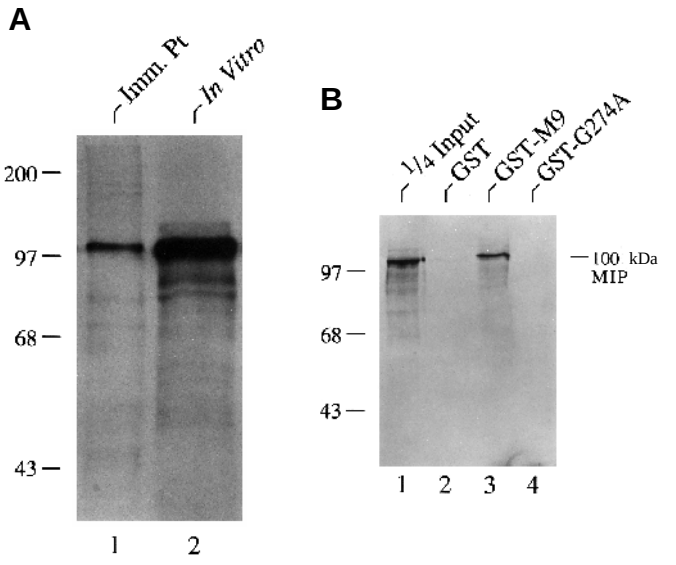

Fig. 2. Initial characterization of the MIP protein. (A) SDS-polyacrylamide gel showing that endogenous MIP protein immunoprecipitated (Imm. Pt) from [^{35}S]methionine labeled 293T cells co-migrates with MIP protein obtained by in vitro translation of the MIP cDNA shown in Fig. 1. (B) In vitro binding assay showing a specific interaction between full-length MIP protein and a wild-type GST-M9 fusion protein. Neither GST itself, nor GST linked to the G274A mutant of M9, proved able to interact.

demonstrate a specific in vitro interaction between MIP and Ran bound to either GTP or GDP (data not shown). However, we have observed, using the yeast two-hybrid system, that MIP can specifically interact with a nucleoporin, Nup153, that also binds to karyopherin-β under the same experimental conditions (data not shown) (Sukegawa and Blobel, 1993).

While the hnRNP A1 M9 sequence is clearly an active NLS, it has also been proposed that M9 may act as a nuclear export signal that mediates the nuclear-cytoplasmic shuttling that is characteristic of hnRNP A1 (Michael et al., 1995). If the MIP protein induces both the nuclear import and export of M9, it should be present in both the nucleus and cytoplasm of cells. In contrast, karyopherin-β, which is thought to mediate protein import exclusively (Sweet and Gerace, 1995; Görlich and Mattaj, 1996), has been reported to localize to the cell cytoplasm and nuclear membrane but to be excluded from the nucleoplasm (Chi et al., 1995; Görlich et al., 1995). As shown in Fig. 3A, immunofluorescence analysis in human cells using the affinity purified rabbit anti-MIP antiserum shows that endogenously expressed MIP is concentrated in the cell nucleus, although cytoplasmic staining is also observed. An essentially identical pattern of MIP localization was also observed using transfected COS cells expressing epitope tagged MIP (Fig. 3B). The nuclear concentration of MIP shown in Fig. 3, when contrasted with the nuclear exclusion of karyopherin-β reported by others (Chi et al., 1995; Görlich et al., 1995), could be consistent with the hypothesis that MIP may also play a role in the nuclear export of proteins, such as hnRNP A1, that contain MIP binding sites.

MIP mediates the nuclear import of proteins containing the M9 NLS

To examine whether MIP actually mediates the nuclear import of proteins bearing the M9 NLS, we used the in vitro nuclear import assay, based on the use of digitonin-permeabilized cells, first described by Adam et al. (1990). As shown in Fig. 4A, a recombinant GST-M9 fusion protein is normally unable to enter the nucleus of permeabilized cells in this assay. However, addition of a fusion protein consisting of MBP linked to full-length MIP results in efficient nuclear uptake (Fig. 4B). This import is dependent on the integrity of the M9 NLS in that the GST-G274A fusion protein, bearing a defective M9 NLS, is not imported into the nucleus in the presence of the MBP-MIP (1-890) fusion protein (Fig. 4C). These data therefore demonstrate that MIP can function as a cofactor for the nuclear import of M9 NLS containing substrates.

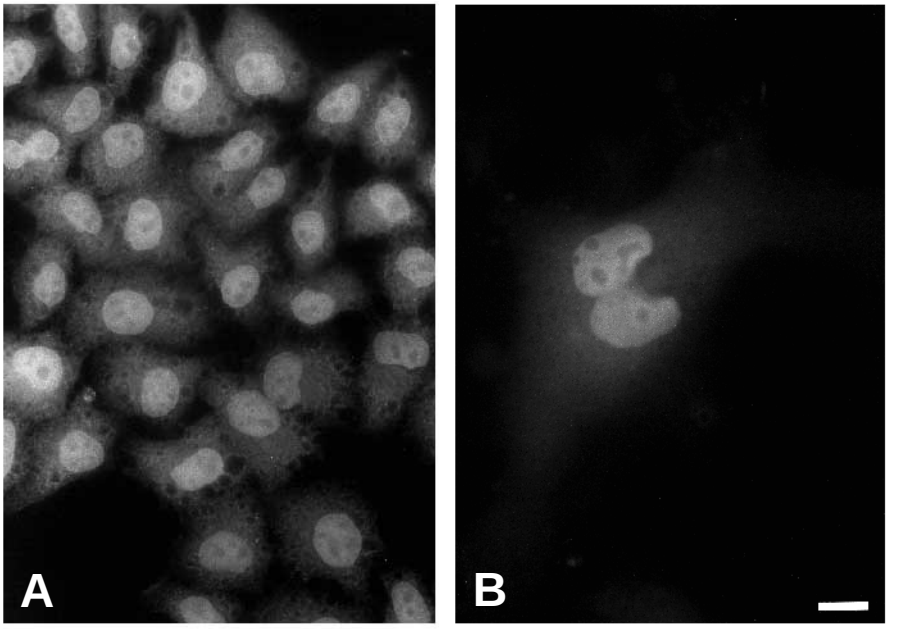

Fig. 3. Subcellular localization of MIP. (A) The subcellular localization of endogenously expressed MIP protein in HeLa cells was determined using an affinity purified rabbit anti-MIP antiserum. (B) The predominantly nuclear localization of MIP was confirmed in transfected COS cells expressing an HA tagged form of MIP, using the anti-HA monoclonal antibody 12C5, as described (Fridell et al., 1996). Cells were visualized using a Leica DMRB fluorescence microscope with a ×100 objective. Bar, 20 μm.

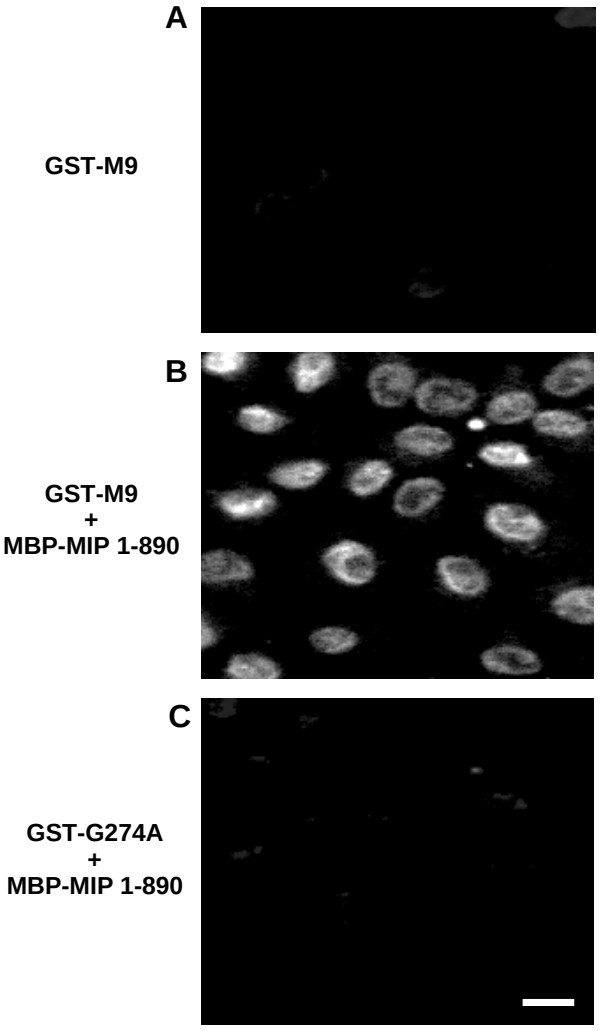

Fig. 4. MIP can mediate the in vitro nuclear import of M9-containing proteins. Digitonin permeabilized HeLa cells were incubated with the indicated fluorescein-labeled import substrates in the presence or absence of a fusion protein consisting of MBP linked to the full-length MIP protein (aa 1-890). All import assays included 1 mM GTP and an ATP regeneration system (Adam et al., 1990). Bar, 20 μm.

We next wished to address whether MIP plays a role in mediating M9 nuclear import in vivo. As shown in Fig. 5A, cytoplasmic microinjection into HeLa cells of a recombinant fusion protein consisting of GST linked to the 38 aa M9 NLS results in the efficient nuclear translocation of the GST-M9 protein. As previously shown (Michael et al., 1995), introduction of the G274A or P275A mutation into M9 blocks this activity and results in cytoplasmic co-localization of the GST fusion protein with the rabbit immunoglobulin G (IgG) internal control, which is used here to confirm that the microinjected proteins were indeed introduced exclusively into the cytoplasm.

Analysis of the MIP/M9 interaction demonstrated that the carboxy-terminal third of MIP (residues 541 to 890) was fully sufficient to bind the M9 NLS efficiently (Table 1). If MIP binding is critical for M9 NLS function, and assuming that the amino-terminal two-thirds of MIP are essential for MIP function, we reasoned that overexpression of the M9 binding domain of MIP should block M9 NLS function. To test this hypothesis directly, we purified a recombinant fusion protein consisting of MBP linked to MIP residues 541 to 890 and microinjected this MBP-ΔMIP fusion, along with the GST-M9 fusion protein and the rabbit IgG internal control, into the cytoplasm of HeLa cells. As shown in Fig. 5C, the truncated MBP-ΔMIP fusion protein entirely blocked the NLS activity of the hnRNP A1 M9 sequence. To confirm that this inhibition was specific to the M9 NLS, and not due to nonspecific toxicity, we asked whether the MBP-ΔMIP fusion would also inhibit the ability of the SV40 'T' antigen basic NLS (TNLS) to direct a substrate to the nucleus, by cytoplasmic co-injection with a GST-TNLS fusion protein. As shown in Fig. 5E, the MBP-ΔMIP fusion failed to exert any inhibitory effect on SV40 T antigen NLS function, thus demonstrating that MIP function is specifically required for M9 NLS activity.

DISCUSSION

The data presented in this manuscript strongly support the hypothesis that function of the unusual M9 NLS found in hnRNP A1 is mediated by a novel human protein that is related to, but distinct from, the karyopherin-β cofactor for basic NLS function. This finding suggests that basic NLSs and M9-like NLSs utilize nuclear import pathways that are evolutionarily related yet, at least in part, distinct. Of interest, although MIP binds the M9 NLS directly, karyopherin-β does not bind to basic NLSs directly, a function which is instead performed by the karyopherin-α adaptor protein (Sweet and Gerace, 1995; Görlich and Mattaj, 1996). However, a 41 aa domain located at the amino terminus of karyopherin-α can confer efficient nuclear translocation on heterologous proteins in a process dependent on karyopherin-β , as well as Ran and p10/NTF2, but independent of karyopherin-α (Görlich et al., 1996a; Weis et al., 1996). It has been suggested that this simpler nuclear translocation pathway might be an evolutionary precursor to the karyopherin-α dependent process utilized by basic NLS sequences (Görlich et al., 1996a; Weis et al., 1996). It will therefore be of interest to test whether nuclear localization conferred by the M9/MIP interaction is mechanistically similar to this alternate, karyopherin-β mediated transport mechanism and, in particular, whether MIP and karyopherin-β share a requirement for the Ran and p10/NTF2 cofactors. Surprisingly, the in vitro nuclear import of a protein substrate containing the M9 NLS was found to proceed efficiently, in our hands, upon addition of only the MIP cofactor (Fig. 3). However, it clearly remains possible that other factors potentially required for M9 nuclear import were not fully depleted in these permeabilized cell preparations, so that their contribution to this process was not detectable. This question is currently under active investigation.

Immediately prior to submission of this manuscript, two papers appeared which directly bear on the identity of the cellular cofactor for nuclear import of hnRNPs. In particular, Aitchison et al. (1996) have recently reported the identification of a set of three novel karyopherin-β homologs in yeast and have presented data showing that one of these, termed Kap104p, is directly involved in mediating the nuclear import of two yeast hnRNP-like proteins. Of interest, a computer search of the yeast database shows that Kap104p is the yeast

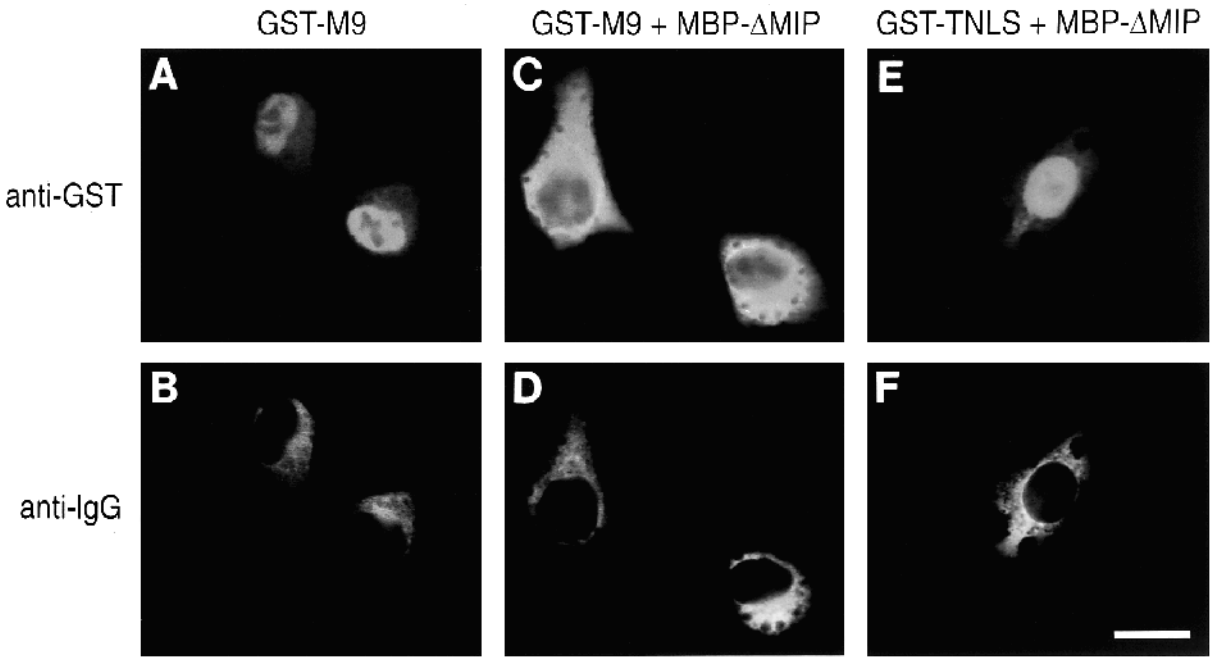

Fig. 5. Microinjection assay for NLS function. Purified recombinant proteins consisting of GST linked to the M9 NLS (GST-M9) or the SV40 'T' antigen NLS (GST-TNLS) were microinjected into the cytoplasm of HeLa cells along with rabbit IgG. In C through F, the cells were also microinjected with a purified fusion protein consisting of MBP linked to residues 541 to 890 of MIP (MBP-ΔMIP). After 30 minutes of incubation at 37°C, the cells were fixed and subjected to double label immunofluorescence to visualize the intracellular localization of GST fusion proteins (A,C,E) and of the rabbit IgG internal control (B,D,F). Although A and B were derived from cells injected with GST-M9 and IgG only, coinjection of purified wild-type MBP did not affect the nuclear translocation of the GST-M9 protein in other experiments. Bar, 20 μm.

protein with the greatest homology to the MIP protein reported here. These data (Aitchison et al., 1996) therefore suggest that there are likely to be several nuclear import pathways in eukaryotic cells, mediated by several distinct karyopherin-β homologs, and further suggest that a nuclear import pathway focused on hnRNP-like proteins has been conserved between yeast and human.

Also recently, Pollard et al. (1996) reported the identification of a human cofactor for hnRNP A1 nuclear import that is identical to the MIP protein reported here. This earlier paper also demonstrated that this cofactor, which they termed transportin, directly interacted with the M9 NLS sequence and that transportin/MIP could mediate the nuclear uptake of M9-containing substrates in vitro. This present study fully confirms these earlier data and extends them by: (1) confirming that transportin/MIP is expressed as a ~101 kDa protein in vivo (Fig. 2); (2) demonstrating that transportin/MIP is localized to the cell nucleus (Fig. 3); and (3) identifying a dominant negative form of transportin/MIP (Fig. 5).

In terms of nomenclature, we believe that this novel karyopherin-β homolog could certainly be named transportin, as suggested earlier by Pollard et al. (1996). However, as it now appears (Aitchison et al., 1996) that there are likely to be at least four, and possibly more, functional homologs of karyopherin-β in eukaryotic cells, we would suggest that it would be preferable for the original karyopherin-β to be renamed karyopherin-β1 while the novel karyopherin-β homolog here termed MIP would be most appropriately named karyopherin-β2. These names would more clearly convey the similar biological roles played by these two related proteins and would also represent a logical extension of the proposed names for different, related forms of the karyopherin-α subunit (α1, α2 etc.) (Moroianu et al., 1995a; Wang et al., 1997).

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