A Nuclear Transport Signal in Mammalian Target of Rapamycin Is Critical for Its Cytoplasmic Signaling to S6 Kinase 1

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The mammalian target of rapamycin (mTOR) regulates nutrient-dependent cell growth and proliferation through cytoplasmic targets, such as S6 kinase 1 (S6K1). Consistent with its main function in the cytoplasm, mTOR is predominantly cytoplasmic. However, previously we have found that mTOR shuttles between the nucleus and cytoplasm, and we have proposed that the nucleocytoplasmic shuttling of mTOR is required for the maximal activation of S6K1. The intrinsic signals directing mTOR nuclear transport and the underlying mechanisms are unknown. In this study we initially set out to identify nuclear export signals in mTOR. A systematic scan of the mTOR sequence revealed 16 peptides conforming to the canonical leucine-rich nuclear export signal; of which 3 were found by reporter assays to contain leptomycin B-sensitive and leucine-dependent nuclear export activity. Unexpectedly, mTOR proteins with those conserved leucines mutated to alanines were unable to enter the nucleus. Further investigation revealed that the L982A/L1287A mutations likely induced a global impairment of nuclear import activity. Consistent with its role in the cytoplasm and its activation of Akt, mTOR shuttles between the nucleus and the cytoplasm, as evidenced by nuclear accumulation in cells treated with leptomycin B (LMB), a specific inhibitor of the export receptor Crm1 (21). Most importantly, nuclear import was accompanied by the significantly reduced ability of the L545A/L547A mutant to activate S6K1 in cells. When nuclear import was restored in the L545A/L547A mutant by the addition of an exogenous nuclear import signal, signaling to S6K1 was rescued. Taken together, our observations suggest the existence of a nuclear shuttling signal in mTOR and provide definitive evidence for the requirement of mTOR nuclear import in its cytoplasmic signaling to S6K1.

Mammalian target of rapamycin (mTOR) is a Ser/Thr protein kinase that mediates nutrient-dependent intracellular signaling to ultimately govern a wide range of cellular processes, including cell growth, proliferation, and various types of differentiation (1, 2). The most well-characterized function of mTOR is the regulation of protein synthesis (3), primarily mediated by the ribosomal subunit S6 kinase 1 (S6K1) and the eukaryotic initiation factor 4E-binding protein 1 (4E-BP1), both regulators of mitogen-stimulated translation initiation (4, 5). In conjunction with other signaling pathways, notably the phosphatidylinositol 3-kinase pathway, mTOR regulates S6K1 and 4E-BP1 by directly phosphorylating both proteins (6–8). Recent findings have revealed two mTOR-associated protein complexes (9–11). Whereas raptor in the rapamycin-sensitive complex appears to serve a scaffold role to bridge mTOR and its substrates S6K1/4E-BP1 (12–14), the rictor-containing mTOR complex regulates the actin cytoskeleton in a rapamycin-insensitive manner (11). Furthermore, the rictor-mTOR complex has turned out to be the long-sought kinase for Ser-473 of Akt (15), critical for the activation of Akt.

Consistent with its primary target being the translational machinery, mTOR is predominantly localized in the cytoplasm, associated with a variety of intracellular membrane structures (16–20). However, a small fraction of mTOR is found in the nucleus at steady state (21, 22), and mTOR shuttles between the nucleus and the cytoplasm, as evidenced by nuclear accumulation in cells treated with leptomycin B (LMB), a specific inhibitor of the export receptor Crm1 (21). Surprisingly, we have found that the nuclear shuttling of mTOR is critical for the signaling to its cytoplasmic targets S6K1 and 4E-BP1. This conclusion is based on the following observations (21, 23): (a) LMB treatment inhibited S6K activation and 4E-BP1 phosphorylation; (b) mTOR with increased nuclear export activity (via tagging with an exogenous nuclear export sequence (NES)) had diminished capacity to activate S6K/4E-BP1; (c) mTOR with increased nuclear import activity (via tagging with an exogenous nuclear import signal (NLS)) had enhanced signaling capacity toward S6K/4E-BP1; and (d) when mTOR was supplemented by an increasing number of NLSs (one, two, and four copies), its ability to signal to S6K in the cell first increased and then decreased, suggesting that an optimal rate of nucleocytoplasmic shuttling, but not nuclear import or export per se, leads to maximal signaling. This has led us to postulate that mTOR may have distinct functions in the nucleus and that it undergoes shuttling to coordinate its roles in the cytoplasm and nucleus. Such counterintuitive behavior of a cytoplasmic signaling protein has also been observed in Ste5, a scaffold protein in the yeast mating pathway (24). Although the collective evidence for the role of mTOR nuclear shuttling is compelling, the studies so far have largely relied on engineered mTOR proteins with exogenous NLS and NES. Identification of the intrinsic nuclear transport signals and characterization of their role in mTOR function will be pivotal for the validation of the functional significance of mTOR nuclear shuttling and the dissection of the transport mechanism.

At 289 kDa, mTOR must depend on active import and export mechanisms to traverse the nuclear pore. However, no classic NLS defined by clusters of basic residues is found in the primary sequence of mTOR. It is possible that mTOR may piggyback on another protein that undergoes standard importin-dependent nuclear import, or alternatively, mTOR may employ an unconventional mechanism to enter the...
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nucleus. A growing number of proteins, predominantly signal transducers, have been found to travel through the nuclear pore by directly interacting with the nuclear pore complex (25). Nuclear export of mTOR, on the other hand, appears to be more conventional; inhibition by LMB suggests a Crm1-dependent export mechanism. In this study, we set out to characterize NES-like sequences in mTOR. Our investigation led to the identification of a site required for the nuclear import of mTOR and provided definitive evidence for the functional link between mTOR nuclear shuttling and its signaling to cytoplasmic targets.

EXPERIMENTAL PROCEDURES

Cell Culture—Both monkey kidney epithelial CV-1 cells and human embryonic kidney (HEK) 293 cells were maintained in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum at 37 °C with 5% CO2. Transient transfection was performed at ~60% cell density using PolyFect (Qiagen) according to the manufacturer’s recommendations. The detailed transfection conditions are described below.

Antibodies and Other Reagents—Anti-FLAG monoclonal antibody and anti-HA polyclonal antibody were obtained from Sigma and Clontech, respectively. Anti-Myc 9E10.2 ascites were generated by Covance. All secondary antibodies were from Jackson ImmunoResearch. Rapamycin and LMB were purchased from Calbiochem and Biomol, respectively.

Reporter Assays for Nuclear Export Activity—pBl5-FRB-HA and pBl5-Gal4BD-3xFKBP12-FLAG (26) were generous gifts from Prof. Gerald Crabtree (Stanford University School of Medicine). pBl5-FRB-HA was modified by the insertion of an oligonucleotide linker into the SalI site to introduce a KpnI site between FRB and HA. Double-stranded oligonucleotides encoding putative NES sequences with KpnI and SalI overhangs were ligated into the modified pBl5-FRB-HA at KpnI and SalI sites. CV-1 cells cultured on glass coverslips in 12-well plates were co-transfected with pBl5-FRB-NES-HA and pBl5-Gal4BD-3xFKBP12-FLAG at 1 μg of each DNA/well using PolyFect (Qiagen). 24 h after transfection the cells were replenished with fresh medium and treated by 100 nM rapamycin with or without 20 ng/ml of LMB for 5 h. The cells were then fixed in 3.7% formaldehyde, permeabilized in 0.1% Triton X-100, and incubated with anti-FLAG and anti-HA antibodies followed by fluorescein isothiocyanate-anti-mouse IgG and Texas Red-anti-rabbit IgG antibodies, respectively. The coverslips were mounted on glass slides with FluoroSave (Calbiochem) and examined with a Leica inverted fluorescence microscope. Fluorescent images were mounted with a CCD camera using the SPOT software (Diagnostic Instruments, Inc.).

Construction of Full-length mTOR Mutants—Site-directed mutagenesis was performed with the QuickChange kit (Stratagene). To construct the full-length mTOR L545A/L547A, L982A/L984A, and L1287A/L1289A mutants, the EcoRV-Smal, Smal-EcoRI, and EcoRI-EcoRI fragments of mTOR cDNA were cloned into pGEX-2T, pUC19, and pCR2.1-T-TOPO, respectively, where mutagenesis was carried out. The entire fragments subjected to mutagenesis were sequenced to confirm the presence of the desired mutations and the absence of random mutations. The EcoRI-EcoRI fragment carrying the L1287A/L1289A mutations then replaced the wild-type fragment in pcDNA3-FLAG-mTOR/S2035T (27). For the L545A/L547A and L982A/L984A mutants, the EcoRV-EcoRI fragment was then reconstituted in the mutagenesis plasmids by inserting Smal-EcoRI and EcoRV-Smal fragments, respectively. The mutated EcoRV-EcoRI fragments then replaced the wild-type fragment in pcDNA3-FLAG-mTOR/S2035T without the EcoRI-EcoRI fragment. Finally, the EcoRI-EcoRI fragment of mTOR was inserted to yield full-length cDNA.

Subcellular Localization of mTOR—Full-length mTOR constructs containing various mutations were transfected into CV-1 cells plated on glass coverslips in 12-well plates at 1 μg/well using PolyFect. 24 h after transfection, some of the cells were treated with 20 ng/ml of LMB for 8 h. The cells were immunostained with the anti-FLAG antibody and analyzed by microscopy as described earlier.

mTOR Kinase Assay—Various mTOR constructs were transfected into HEK293 cells cultured in 60-mm plates at 5 μg/plate. After 24 h, the cells were lysed and anti-FLAG immunoprecipitation was performed. Autophosphorylation assays were carried out with the immunocomplex as described previously (27).

S6K1 Kinase Assay—Various mTOR constructs and Myc-S6K1 (28) were co-transfected into HEK293 cells cultured in 6-well plates at 2 μg of mTOR and 0.5 μg of S6K1 using PolyFect. 24 h after transfection, the cells were switched to serum-free medium. After serum starvation for 24 h, the cells were treated with 100 nM rapamycin for 30 min, followed by stimulation with 20% fetal bovine serum for 30 min. The cells were then lysed, Myc-S6K1 was immunoprecipitated using the anti-Myc ascites, and the immunocomplex was subjected to in vitro S6 kinase assay as described previously (21).

RESULTS

Identification of Potential Nuclear Export Signals in mTOR—Because the nuclear export of mTOR is inhibited by LMB, the specific inhibitor of Crm1, we reasoned that mTOR might contain one or more leucine-rich NESs. These classic NESs are characterized by several conserved leucines with defined spacing between them, initially identified in human immunodeficiency virus 1 (HIV-1) Rev (29) and protein kinase A inhibitor (30). A survey of NESs identified to date suggested that other hydrophobic residues could substitute for some of the leucines (26, 31–33), allowing us to generate a more encompassing motif (see Fig. 1A). A thorough scan of the mTOR primary sequence using the pattern identification program Patatinpro (34) revealed 16 sequences (9–13 amino acids in length) as potential NESs in mTOR (Fig. 1A).

To test whether any of these NES-like sequences in mTOR could function as a nuclear export signal, we utilized a reporter system developed by Klemm et al. (26), depicted in Fig. 1B. This reporter system is composed of two main elements: the first is a fusion protein containing the putative NES and the FKBP12-rapamycin binding (FRB) domain of mTOR (amino acids 2015–2114) (35), which diffuses freely through the nuclear pore because of its small size (<13 kDa); the second is a fusion protein of GAL4 DNA binding domain (amino acids 1–147) and three tandem copies of FKBP12, which localizes to the nucleus due to the presence of a strong NLS in GAL4. The two fusion proteins are co-expressed in cells, and they form a complex in the presence of rapamycin, the small molecule that simultaneously binds FRB and FKBP12. If the putative NES indeed functions as a nuclear export signal and is strong enough to counter the NLS of the GAL4 fusion, the complex, and thus the GAL4 fusion, will be translocated from the nucleus to the cytoplasm. Thus, the GAL4 fusion protein serves as a reporter for nuclear export activity conferred by the putative NES. The epitope tags HA and FLAG are present on the FRB-NES and the reporter, respectively, to allow immunostaining for visualizing protein localization in the cells. We fused FRB to each of the 16 putative NESs and introduced the fusions into CV-1 cells together with the reporter by transient transfection. Subsequently, the transfected cells were treated with rapamycin for 2–5 h, and the reporter localization was examined. As a positive control, in >90% of the doubly transfected cells Rev-NES induced a significant nucleus-to-cytoplasm translocation of the reporter protein upon the addition of rapamycin (Fig. 2). As shown in Fig. 2, 3 of the 16...
candidate sequences displayed nuclear export activity comparable with that of Rev-NES in the reporter assays: peptides number 5 (amino acids 535–547), 10 (amino acids 975–984), and 12 (amino acids 1281–1289), whereas the others had no or very low export activity; results for peptide 15 (amino acids 2115–2123) are shown as an example. Similar results were obtained in COS-7 cells (data not shown). In conclusion, three peptide sequences from mTOR can function as NESs exogenously.

The activity of a putative NES in mTOR Requires Conserved Leucines and Is Inhibited by LMB—To examine whether the export activity of peptides described above is dependent on the conserved leucines, the last two leucines in the putative NESs were changed to alanines (designated “AA”) and their effect on export activity was investigated. As shown in Fig. 3A, all three putative NESs in mTOR were abolished in all three peptides in >90% of the doubly transfected cells. Hence, these putative NESs have sequence requirements comparable with the canonical NES.

Although we considered the possibility that none of these three sequences served as a strong NES for full-length mTOR, we recognized that such a conclusion could only be drawn if these mutants of mTOR proteins retained the normal nuclear import activity. To address this issue, we treated the cells expressing wild-type or various mutants of mTOR with LMB and examined the localization of the recombinant proteins. The wild-type protein displayed increased nuclear staining upon LMB treatment as previously reported, but this localization shift was completely absent in all of the mutants (Fig. 4A). Therefore, these mutants were not able to enter the nucleus as efficiently as the wild-type, suggesting that the NESs in these mutants were not able to enter the nucleus as efficiently as the wild-type, suggesting that the NESs in these mutants were not able to enter the nucleus as efficiently as the wild-type, suggesting that the NESs in these mutants were not able to enter the nucleus as efficiently as the wild-type, suggesting that the NESs in these mutants were not able to enter the nucleus as efficiently as the wild-type, suggesting that the NESs in these mutants were not able to enter the nucleus as efficiently as the wild-type. A simple explanation for this observation was that the mutations that targeted the putative NESs abolished nuclear localization signals in mTOR as well. The combined presence of NLSs and NESs in the same primary sequences have been previously reported.
labeled as nucleocytoplasmic shuttling signals and are not readily defined by known motifs (36).

Disruption of mTOR Nuclear Import Leads to Impaired Downstream Signaling—To further characterize the full-length mTOR alanine mutants, we assessed their catalytic activity by measuring in vitro auto-phosphorylation. Previously we found that the catalytic activity of mTOR was independent of its nucleocytoplasmic shuttling activity (21). Thus, if the only defects of the alanine mutants were in nuclear transport, one would expect them to display wild-type catalytic activity. However, 10/AA and 12/AA suffered different degrees of reduction in their activity (Fig. 5A). Because both the kinase domain and the major autophosphorylation site (Ser-2481) (37) are located in the C-terminal half of mTOR and the N-terminal half of the protein has been shown to be dispensable for autophosphorylation (27), the 10/AA and 12/AA mutations are not likely to directly affect the kinase activity measured by autophosphorylation. Thus, global protein structural alterations in mTOR-10/AA and mTOR-12/AA cannot be ruled out, even though it seems unlikely that two leucine-to-alanine conversions would elicit such a drastic change. mTOR-5/AA, on the other hand, autophosphorylated as well as the wild-type protein (Fig. 5A), confirming the structural integrity of this mutant.

We went on to examine the ability of the mTOR mutants to signal to the downstream effector S6K1 in cells. The S2035T mutation that confers rapamycin resistance to mTOR was present in all mTOR variants used here, including the wild-type, which allowed assessment of the recombinant mTOR activity upon “chemical knock-out” of endogenous mTOR function by rapamycin (35, 38). Recombinant mTOR and S6K1 were co-expressed in HEK293 cells treated with rapamycin, and the recombinant S6K1 activity was measured by in vitro kinase assays. As shown in Fig. 5B, the 5/AA and 10/AA mutations in full-length mTOR diminished serum-stimulated S6K1 activity by 75 and 60%, respectively, whereas mTOR-12/AA was completely inactive toward S6K1. The latter observation also confirmed that the rapamycin-insensitive rictor-mTOR activity had no impact on S6K1 activity in this assay. mTOR proteins with various combinations of these mutations displayed signaling activities as would be expected: whenever 12/AA was present the signaling activity of mTOR was abolished; the 5/AA and 10/AA mutations had additive effects on inhibiting S6K1 activation. 4E-BP1 hyperphosphorylation assessed by gel mobility shift was similarly affected by those mutations (data not shown), although a quantitative measurement was not feasible. The partial and complete loss of signaling capacity in 10/AA and 12/AA, respectively, did not correlate with the degree of reduction in their catalytic activity and might be...
An Exogenous NLS Restores Nuclear Import of 5/AA but Not 10/AA or 12/AA—We reasoned that if any of the mutations described above indeed resulted in ablation of both nuclear export and import, the potential loss of nuclear export might be revealed by providing the mutant protein an exogenous NLS. Four copies of NLS from SV-40 large T antigen (39) were engineered to express at the N terminus of the 3AA mutant, designated 4xNLS-3AA. As shown in Fig. 6A, whereas 4xNLS-mTOR displayed enhanced nuclear staining and almost exclusive nuclear staining upon LMB treatment, 4xNLS-3AA stayed in the cytoplasm even in the presence of LMB. Because it is highly unlikely that this mutant gained LMB-insensitive nuclear export activity, the best explanation for its behavior is cytoplasmic retention, due either to the impairment of a normal release mechanism or simply misfolding of the protein. To find out whether mutations of the three putative NES regions contributed equally or differentially to this behavior, we generated NLS-tagged individual mutants, namely 4xNLS-5/AA, 4xNLS-10/AA, and 4xNLS-12/AA. The nuclear distribution of the recombinant proteins in the absence or presence of LMB is summarized in Fig. 6B. 4xNLS-10/AA and 4xNLS-12/AA behaved similarly to 4xNLS-3AA, i.e. they localized predominantly in the cytoplasm, even in the presence of LMB. The strong cytoplasmic retention of 10/AA and 12/AA, together with their reduced catalytic activity, implies global protein structural alterations in these mutants. On the other hand, 4xNLS-5/AA displayed strong nuclear localization just like 4xNLS-wt and accumulated in the nucleus in >90% of the transfected cells. Thus, 5/AA most likely eliminates a major nuclear import signal, and possibly a shuttling signal, in mTOR.

Restoration of mTOR Nuclear Import Leads to Rescue of Downstream Signaling—Although we observed significantly reduced signaling activity in mTOR-5/AA, the mutant that was specifically impaired in nuclear import, it could not be excluded that those double AA mutations affected mTOR signaling by a mechanism unrelated to nuclear import. If the reduced signaling capacity of 5/AA was a direct consequence of impaired nuclear import, one would expect that restoring nuclear
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FIGURE 7. Exogenous NLS restores signaling activity of mTOR-L545A/L547A. Various FLAG-tagged mTOR proteins were co-expressed with Myc-S6K1 in HEK293 cells. The cells were treated as described in the Fig. 5 legend. In vitro S6 kinase assays were performed with immunoprecipitated Myc-S6K1. Relative S6K1 activities were calculated with that of those described above. Strikingly, the tagging of 4xNLS increased the signaling activity of 5/AA from ~25 to ~75% of the wild-type activity (Fig. 7). This close correlation between nuclear import and signaling activity was further validated by the inability of the same NLS tagging to significantly affect the signaling capacity of 10/AA and 12/AA (Fig. 7). Taken together, our observations provide strong support for the idea that nuclear import of mTOR is required for its downstream signaling.

DISCUSSION

Our previous finding that mTOR shuttles between the cytoplasm and nucleus led us to investigate the signals that enable this transport and the functional consequence of disrupting the translocation of mTOR. Previously we proposed that nucleocytoplasmic shuttling is required for mTOR signaling to its cytoplasmic targets. This hypothesis was supported by the sensitivity of mTOR signaling to LMB and the behaviors of mTOR recombinant proteins engineered to contain exogenous NLS or NES (21, 23). Here, in a systematic search for LMB-sensitive NESs, we have identified a site in mTOR critical for nuclear transport. The double point mutation L545A/L547A markedly impaired the nuclear import of mTOR and inhibited mTOR signaling to S6K1. Most importantly, when the import activity of the L545A/L547A mutant was restored by the addition of an exogenous NLS, its signaling capacity was significantly rescued. Although we are not able to directly confirm the nuclear export activity of this mutant currently (our original intention), the striking behavior of the L545A/L547A mutant, in which there is a perfect correlation between nuclear translocation and cytoplasmic signaling capacity, provides definitive proof for the unusual concept that nuclear entry (and exit) of mTOR is required for its cytoplasmic signaling. Future investigation to identify nuclear targets of mTOR and to probe a potential coordination of nuclear and cytoplasmic functions by mTOR shuttling is warranted. It should be pointed out that our conclusions here only apply to the rapamycin-sensitive aspect of mTOR signaling in the cytoplasm, specifically the regulation of S6K1 (and most likely 4E-BP1). It will be interesting to examine the impact of mTOR nucleocytoplasmic shuttling on other cytoplasmic functions of mTOR, such as Akt phosphorylation.

A thorough motif search in mTOR revealed 16 candidate sequences for Crm1-mediated NESs, but only 3 displayed export activity in reporter assays. This is consistent with the general consensus that not all sequences containing the leucine-rich motif have export function (40). All three sequences have the characteristics of classic Crm1-dependent NESs: the strong export activity is abolished by mutating two of the conserved leucines, as well as by LMB treatment. Unexpectedly, mutating those two leucines in each of the three putative NESs led to the abolition of nuclear import of the full-length mTOR, precluding the assessment of their effect on export activity in the full-length protein. L982A/L984A and L1287A/L1289A resulted in an apparent cytoplasmic retention of mTOR, because the addition of a strong NLS, four tandem copies of SV40 NLS, was not sufficient to support nuclear import of the mutant proteins. It is not difficult to envision cytoplasmic retention of mTOR as a part of the regulation of its nuclear transport, because a large fraction of mTOR associates with intracellular membranes (16, 17). L982A/L984A and/or L1287A/L1289A may disrupt the release of mTOR from, or increase its affinity for, the membranous structures. However, while those two mutants had indistinguishable effects on mTOR nuclear import, L1287A/L1289A impaired mTOR signaling more severely than L982A/L984A (Fig. 5A) and the latter had a more negative effect on the catalytic activity of mTOR (Fig. 5B). Thus, neither the reduced catalytic activity nor the loss of nuclear import alone is sufficient to account for the functional impairment. Global protein structural alteration remains a strong possibility in those mutants. The L545A/L547A mutant, on the other hand, clearly had a defect in nuclear import, which was fully restored by the addition of an exogenous NLS. Full catalytic activity of this mutant suggests that a global structural change is unlikely. Although it is possible that a region containing L545/L547 serves as a novel nuclear import signal in mTOR, given the export activity of that peptide in the reporter assays and the sequence adherence to the NES motif, we favor the idea that it may be part of a nucleocytoplasmic shuttling (NS) signal. It should be mentioned that although the short peptide (amino acids 535–547) characterized for export activity is required for nuclear import, it is insufficient to direct nuclear import of an exogenous protein. Thus, the fully functional NS likely spans a larger region or requires additional sequences. The first member and prototype of the expanding family of NS signals was found in the heterogeneous nuclear ribonucleoprotein A1 protein, termed M9, a 38-amino acid sequence devoid of a classic NLS motif, which directs Ran-dependent nuclear import and export (36). The import and export activities in M9 could not be separated despite exhaustive mutagenesis efforts (41); the behavior of the L545A/L547A mutant of mTOR is consistent with that phenomenon. Although it is thought that NS signals lack consensus motifs (36), our results suggest that at least one particular class of NS may share the consensus of an embedded leucine-rich NES, raising the possibility that other NES-containing sequences may function as NSs.

Nuclear import directed by a classic NLS is mediated by importins. Importin-β translocates through the nuclear pore by interacting with the nuclear pore complex, taking along the cargo that is associated with it either directly or through importin-α. Tandem HEAT (a helical motif of 39 amino acids) (42) repeats in importin-β are responsible for interaction with Ran-GTP, importin-α, and nucleoporins. In particular, a specific set of HEAT repeats in importin-β interacts with the FG repeats of nucleoporins (reviewed in Ref. 25). In recent years a growing list of signaling proteins that undergo importin-α and Ran-independent nuclear import has emerged that includes β-catenin, Smad proteins, and extracellular signal-regulated kinase 2 (25). These proteins apparently travel through the nuclear pore by directly interacting with the nuclear pore complex without the help of any importins. The HEAT

4 J. Kim and J. Chen, unpublished observation.
Interestingly, mTOR contains several nucleoporins through the FG repeats, and the binding is responsible for nucleocytoplasmic shuttling of the Smad proteins (45, 46). Interestingly, mTOR contains several nucleoporins through the FG repeats, and the binding is responsible for nucleocytoplasmic shuttling of the Smad proteins (45, 46). Interestingly, mTOR contains several nucleoporins through the FG repeats, and the binding is responsible for nucleocytoplasmic shuttling of the Smad proteins (45, 46). Interestingly, mTOR contains several nucleoporins through the FG repeats, and the binding is responsible for nucleocytoplasmic shuttling of the Smad proteins (45, 46).

Examination of potential interactions between mTOR and various nucleoporins may prove fruitful in future efforts to unravel the mechanisms of mTOR nuclear transport.

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