**Human Ribosomal Protein L5 Contains Defined Nuclear Localization and Export Signals**

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Ribosomal protein L5 is part of the 60 S ribosomal subunit and localizes in both the cytoplasm and the nucleus of eukaryotic cells, accumulating particularly in the nucleoli. L5 is known to bind specifically to 5 S rRNA and is involved in nucleocytoplasmic transport of this rRNA. Here, we report a detailed analysis of the domain organization of the human ribosomal protein L5. We show that a signal that mediates nuclear import and nucleolar localization maps to amino acids 21–37 within the 297-amino acid L5 protein. Furthermore, carboxy-terminal residues at positions 255–297 serve as an additional nuclear/nucleolar targeting signal. Domains involved in 5 S rRNA binding are located at both the amino terminus and the carboxyl terminus of L5. Microinjection studies in somatic cells demonstrate that a nuclear export signal (NES) that maps to amino acids 101–111 resides in the central region of L5. This NES is characterized by a pronounced clustering of critical leucine residues, which creates a peptide motif not previously observed in other leucine-rich NESs. Finally, we present a refined model of the multidomain structure of human ribosomal protein L5.

The biogenesis of eukaryotic ribosomes occurs at a specific subnuclear compartment, the nucleolus, and requires the coordinated assembly of four different rRNAs and approximately 80 ribosomal proteins (1, 2). The 5.8, 18, and 28 S rRNAs are synthesized by RNA polymerase I in the nucleolus, whereas, in contrast, 5 S rRNA is transcribed by RNA polymerase III in the nucleoplasm. The ribosomal proteins are encoded by mRNAs that are synthesized by RNA polymerase II. After translation, these proteins are imported from the cytoplasm into the nucleolus for assembly of four different rRNAs and approximately 80 ribosomal subunits. These, in turn, are then exported to the cytoplasm. Thus, multiple intracellular transport activities between the nucleus and cytoplasm are required for de novo ribosome synthesis.

In eukaryotic cells, the 5 S rRNA is part of the 60 S ribosomal subunit. In addition, a significant amount of 5 S rRNA is complexed with various proteins to form nonribosome-associated ribonucleoprotein particles. After transcription, 5 S rRNA is able to transiently bind the La antigen, a 50-kDa protein that acts in the termination of polymerase III transcripts, in the nucleus (3, 4). Furthermore, in the nucleus, 5 S rRNA also binds either its own transcription factor IIIA or ribosomal protein L5, forming 7 or 5 S ribonucleoprotein particles, respectively (reviewed in Ref. 5). In particular, it has been suggested that the 5 S rRNA–L5 complex (5 S ribonucleoprotein particle) acts as a precursor to ribosome assembly by delivering 5 S rRNA from the nucleoplasm to the nucleolar assembly site of 60 S ribosomal subunits (6). Studies in *Xenopus* oocytes have shown that 5 S rRNA can be exported from the nucleus to the cytoplasm for subsequent accumulation at distinct cytoplasmic storage sites by either transcription factor IIIA or L5 (7, 8). As a consequence of increased ribosomal subunit synthesis, stored 5 S rRNA must be reimported from the cytoplasm into the oocyte nucleus. In contrast to nuclear export, however, the nuclear import of 5 S rRNA appears to be exclusively mediated by L5 protein (9–11). Although cytoplasmic storage sites for 5 S rRNA have not been observed in mammalian cells, the data so far raised in *Xenopus* oocytes demonstrated that L5 protein is an intracellular 5 S rRNA transport factor.

In addition to its 5 S rRNA transport activity, L5 has also been shown to bind to other cellular proteins that participate in various intracellular transport pathways. For example, L5 interacts with the Mdm2 oncoprotein (12). Mdm2 is an inhibitor of the p53 tumor suppressor gene product that, upon binding to it, promotes the degradation of p53 via the ubiquitin-dependent proteasome pathway (13, 14). Importantly, Mdm2 has been shown to shuttle between the nucleus and cytoplasm of mammalian cells, and Mdm2-mediated nuclear export of p53 has been suggested to lead to p53 degradation (15, 16). Furthermore, L5 has been shown to bind to eukaryotic initiation factor 5A (17), which is a critical cellular cofactor of the Rev trans-activator of human immunodeficiency virus type 1 (HIV-1) (18, 19). Rev is a nucleocytoplasmic shuttle protein that is required for the nuclear export of unspliced and incompletely spliced HIV-1 mRNAs (reviewed in Ref. 20). Interestingly, competition experiments in *Xenopus* oocytes have previously demonstrated that the nuclear export pathways for Rev and 5 S rRNA share common components (21). Taken together, these data indicate that L5 protein may play a role in nucleocytoplasmic trafficking, in addition to its role in 5 S rRNA transport.

The purpose of this study was to characterize in detail the regions of ribosomal protein L5 that are required for intracel-

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1 The abbreviations used are: HIV-1, human immunodeficiency virus type 1; GFP, green fluorescent protein; GST, glutathione S-transferase; βGal, β-galactosidase; NLS, nuclear localization signal; NES, nuclear export signal; aa, amino acid(s).
lular trafficking, subcellular localization, and 5 S rRNA interaction.

MATERIALS AND METHODS

Molecular Clones—The plasmid p3L5, expressing human L5 protein, and the plasmid pBrevM10LI-BFP have been described in detail previously (17, 22). Wild-type and mutant L5 genes were cloned into various expression vectors by standard methods using synthetic double-strand oligonucleotides or polymerase chain reaction technology. Plasmids expressing L5–GFP fusion proteins were generated by cloning the respective L5 coding regions in the unique Nhel site of the vector pCF25 (23). Accordingly, various L5 coding regions were subcloned between the XbaI and XhoI site of the vector pBIC2/CMV/βGal (18), resulting in plasmids expressing βGal-L5 fusion proteins. The plasmids pGEX-L5 and pGEX-Rev are bacterial vectors that express the human L5 and HIV-1 rev genes, respectively, fused to the carboxyl terminus of glutathione S-transferase (GST) (17, 19). Variants of pGEX-L5 possessing mutated L5 genes were generated by exchange of the wild-type gene for the respective mutated gene. The vector pGEX-L5-NE8 expresses a short peptide motif (L5 amino acid position 101–111) fused to GST. pGEX-L5-31NES and pGEX-L5-Δ2NES express mutated versions of this fusion protein in which the leucine residues corresponding to L5 amino acid position 103–105 or 103–105, 109, and 110 were mutated into alanines. Bacterial vectors expressing GST-L5–GFP fusion proteins were generated by insertion of double-strand synthetic oligonucleotides or polymerase chain reaction-generated L5-derived DNA fragments between the BamHI and Nhel site of pGEX-GFP (24). The gene encoding human 5 S rRNA was isolated from a human cDNA by polymerase chain reaction, introducing terminal HindIII and BamHI sites. The product was digested with the respective enzymes and inserted in the vector pcDNA3. The isolated cDNA encodes a copy of the previously published 5 S rRNA sequence (25).

Cell Cultures and Transfections—HeLa cells were maintained and transfected with calcium phosphate as described previously (23). Localization studies in living cells were performed by transient transfection of 2.0 × 10^6 HeLa cells, grown in 50-mm glass-bottomed dishes (MatTek Co.) using phenol red-free Dulbecco’s modified Eagle’s medium, with 2 μg of the various L5–GFP expression plasmids. GFP expression in the transfected cell cultures was analyzed at 16 h posttransfection. To determine the subcellular localization of the βGal-L5 fusion proteins, 1.5 × 10^6 HeLa cells were transfected with 5 μg of the respective expression vectors. At 40 h posttransfection, cells were fixed and subjected to βGal-specific indirect immunofluorescence analysis.

Purification of Recombinant Fusion Proteins and RNA Binding Assay—GST fusion proteins were expressed in E. coli BL21 and purified from crude lysates by affinity chromatography on glutathione-Sepharose 4B (Amersham Pharmacia Biotech) as described previously (17, 24, 26). RNA gel retardation assays were performed using an in vitro-transcribed 32P-labeled 5 S rRNA probe, MS2 competitor RNA, and GST fusion protein as described previously (27).

Microscopy, Immunofluorescence Studies, and Microinjection—Transfected cells expressing GFP fusion proteins were analyzed either by a Zeiss LSM 410 Micro System in the confocal mode or using a Zeiss Transfected cells expressing GFP fusion proteins were analyzed either by comicroinjection of Vero cells into the nucleus with GST-L5–GFP reporter. Expression of the respective fusion constructs in HeLa cells or microinjection of the GST-L5–GFP fusion proteins into Vero cells allowed the mapping of regions in L5 that mediate nuclear import and nucleolar localization. Of note, GFP alone localizes in the cytoplasm and nucleus, but not in the nucleoli of expressing cells (28). In contrast, the βGal reporter allows monitoring of nuclear import but not nucleolar localization (29). NA, not applicable.

RESULTS

Nuclear Import and Nucleolar Localization of Ribosomal Protein L5—It has previously been observed that the L5 protein accumulates primarily in the nucleus and particularly in the nucleoli of mammalian cells (6). In order to define the sequences in L5 that mediate its nuclear import and possibly nucleolar localization in more detail, we first performed subcellular localization studies on wild-type and mutant L5 proteins in living cells. For this, we constructed a series of expression plasmids that contain various L5 sequences fused in frame to a gene encoding an enhanced version of the green fluorescent protein (GFP) of Aequorea victoria (Table I). These constructs were transfected transiently into HeLa cells and monitored for GFP expression 16 h posttransfection. As published previously (28), expression of GFP alone resulted in cytoplasmic and nucleoplasmic signals, clearly sparing the nucleoli (not shown). In contrast, expression of the 297-amino acid (aa) L5 wild-type–GFP protein resulted in nuclear accumulation of the fusion protein with significant concentration in the nucleoli (Fig. 1A). Inspection of L5–GFP variants revealed that regions in both the L5 amino terminus (aa 1–92) and carboxyl terminus (aa 235–297) mediate strong nucleolar localization (Fig. 1, A and C). As GFP alone is equally distributed between the cytoplasmic and nuclear compartment (28), we also tested the L5 proteins for their ability to target an exclusively cytoplasmic protein to the cell nucleus. A series of vectors expressing βGal-L5 fusion proteins were therefore generated and subcellular localization of the respective gene products visualized 40 h posttransfection, using indirect immunofluorescence microscopy on fixed HeLa cells (summarized in Table I). As expected, βGal localized in the cytoplasm (Fig. 2A), but was directed into the nucleus when it was expressed as a fusion to L5 wild-type protein (Fig. 2B). Nucleolar accumulation was not observed when βGal served as a reporter for L5 subcellular localization. Although

### Table I

| Nuclear import | Nuclear localization |
|----------------|----------------------|
| GFP            | NA                   |
| L5–LGF-GFP     | NA                   |
| L5–1–92–GFP    | NA                   |
| L5–23/37–GFP   | NA                   |
| L5–36/254–GFP  | NA                   |
| L5–235/297–GFP | +                    |
| L5–235/265–GFP | +                    |
| βGal           | –                    |
| βGal-L5        | +                    |
| βGal-L5–1/139  | +                    |
| βGal-L5–1/80   | +                    |
| βGal-L5–1/149  | +                    |
| βGal-L5–1/251  | +                    |
| βGal-L5–21/35  | +                    |
| βGal-L5–30/251 | –                    |
| βGal-L5–36/297 | –                    |
| βGal-L5–150/297| +                    |
| βGal-L5–255/265| –                    |
| βGal-L5–255/284| +                    |
| GST–L5–255/297–GFP| +           |
| GST–L5–255/265–GFP| –           |
| GST–L5–255/265–GFP| –/+            |
the reason for this is still unknown, the observation that βGal cannot be used as a reporter to investigate nuclear localization was also made in a previous study (29). Thus, only sequences mediating nuclear import, but not sequences required for nuclear localization, can be identified by this experimental approach. Inspection of the data obtained revealed that a strong nuclear import signal resides in the amino-terminal half of L5 (Fig. 2, C–F), apparently mapping to residues located at amino acid position 21–35 (Fig. 2H). In addition, fusion proteins containing the carboxyl-terminal part of L5 (aa 150–297) also accumulated in the nucleus (Fig. 2, L and M), indicating the presence of an additional nuclear targeting signal. However, this nuclear accumulation was not observed when L5 variants containing aa residues 36–297 or 255–265 were expressed (Fig. 2, I and K).

The data presented so far measured the steady-state accumulation of transiently expressed L5 proteins. In order to gain insight into the kinetic of the potential nuclear/nucleolar targeting capacity of the L5 carboxyl terminus, we next employed a recently established microinjection-based assay system (24). For this, we generated recombinant transport substrates in which L5-derived sequences were fused to a chimeric GST-GFP tag (Table I). These fusion proteins were microinjected directly into the cytoplasm or nucleoplasm of Vero cells. After 45 min of incubation at 37 °C, cells were fixed with paraformaldehyde, and the injected proteins were visualized by GFP- and IgG-specific fluorescence. As shown in Fig. 3, the L5 carboxyl terminus (aa 255–297) directed the respective GST-GFP hybrid protein into the nucleus (Fig. 3, A and B). In contrast, GST-L5–265/297-GFP and GST-L5–255/265-GFP chimeras were import-deficient and remained in the cytoplasm (Fig. 3, C–F). When the same transport substrates were microinjected directly into the nucleoplasm of Vero cells, strong nuclear accumulation was observed in case of the GST-L5–255/297-GFP protein (Fig. 4, A–C). This accumulation was not detected when L5 aa 255–264 was deleted in GST-L5–265/297-GFP (Fig. 4, D–F). A weak nucleolar accumulation was observed by microinjection of the GST-L5–255/265-GFP protein (Fig. 4, G–I).

Summarizing the data obtained in living and fixed cells, strong nuclear import of L5 is mediated by a region located in the amino terminus of the protein (aa 21–37). This sequence also contributes to nucleolar localization of L5. In comparison, the L5 carboxyl terminus (aa 255–297) contains a rather weak nuclear localization signal and sequences at aa position 255–265 contribute to nucleolar localization. The notion that a weak NLS is located in the L5 carboxyl terminus is also in agreement with the finding that this signal was unable to mediate nuclear steady-state accumulation of βGal-L5 fusion proteins that also contained the L5 NES (aa 101–111, see below; Fig. 2I). In contrast, βGal-L5 fusion proteins that carry the strong amino-terminal NLS were easily detectable in the nucleus, even when the NES was present (Fig. 2, C and D).

5 S rRNA Binding Characteristics of Wild-type and Mutant L5 Proteins—The binding of rat L5 protein to human 5 S rRNA has been investigated previously (29). In this work, biotinylated 5 S rRNA was incubated with radioactive-labeled L5 protein generated by in vitro translation in reticulocyte extracts. Protein–RNA complexes were then precipitated using streptavidin beads and bound proteins were analyzed by protein gel electrophoresis. When we initially used the same experimental system to study 5 S rRNA-L5 interaction, we realized that in vitro translated L5 proteins form homodimers with the wild-type L5 that is present in abundance in the reticulocyte lysates. In fact, subsequent gel-filtration analysis of recombinant wild-type L5 protein also provided independent evidence that L5 has the capacity to form protein homodimers.2 The biological significance of this protein–protein interaction is currently unknown. In order to avoid the effects of indirect binding events we therefore decided to employ RNA gel retardation analysis to assess the direct interaction of L5 proteins to 5 S rRNA in a defined system. For this, wild-type and selected mutant L5 proteins were expressed and purified in the context of fusions to GST and then analyzed in combination with an in vitro transcribed human 5 S rRNA probe (Fig. 5). The addition of increasing amounts of GST-L5 wild-type protein to the binding reaction mixture resulted in the appearance of a 5 S RNA-protein complex with slower mobility in nondenaturing gel electrophoresis (Fig. 5A). Control experiments demonstrated that GST alone does not bind to 5 S rRNA (Fig. 5B). Furthermore, the addition of anti-L5 antibody (17) to a preformed RNA–protein complex resulted in the detection of a supershift-signal (Fig. 5C, lane 2 versus lane 3), confirming that the retarded complex contains L5 protein. As expected, addition of an unrelated antibody (α-βGal) did not affect the 5 S RNA–L5 complex (Fig. 5C, lane 4). In order to validate the 5 S RNA-based gel retardation assay system further, we next investigated the binding characteristics of the HIV-1 Rev trans-activator protein. As shown in a previous study by time-re-
solved fluorescence spectroscopy, Rev appears to interact with 5 S rRNA in a similar manner to its interaction with its homologous viral Rev response element RNA target (30). In close agreement with these data, GST-Rev protein clearly bound 5 S rRNA in our gel retardation assay system (Fig. 5D) and, moreover, preformed complexes were supershifted using a Rev-specific antibody (31) (Fig. 5D, lane 4). Addition of increasing amounts of Rev to the binding reaction mixture resulted in the successive appearance of 5 S rRNA–protein complexes with slower mobilities (Fig. 5E, lanes 3–6). The clear resolution of the retarded 5 S rRNA probe in multiple distinct bands has been shown to be due to the cooperative binding of multiple Rev molecules to a single RNA target (26, 32). Interestingly, L5 wild-type protein competed efficiently for 5 S rRNA binding when the protein was added to preestablished 5 S rRNA-Rev complexes (Fig. 5E, lane 7), suggesting that L5 binds with higher affinity and both proteins target the same binding site on 5 S rRNA. Whether or not this is indeed the case will be the subject of future studies in which the affinities of both proteins for 5 S rRNA will be determined in detail. The affinity of HIV-1 Rev for 5 S rRNA, however, may be the reason why this viral regulatory protein displays a pronounced nucleolar steady-state accumulation in expressing cells (33, 34).

We next examined the 5 S rRNA binding-characteristics of GST-L5 mutant proteins using this RNA gel retardation assay system. Again, titration of GST-L5 wild-type protein to 5 S rRNA resulted in the appearance of a retarded protein–nucleic acid complex, whereas, in contrast, no 5 S rRNA binding was observed when a GST fusion protein containing the internal region of L5 protein (aa 39–251) was used (Fig. 5F). The subsequent addition of the amino-terminal or carboxyl-terminal L5 regions that contain sequences involved in nuclear import and nucleolar localization of L5, reestablished 5 S rRNA binding (Fig. 5G). In both cases, addition of increasing amounts of GST-L5 mutant protein caused the subsequent appearance of two distinct signals, which may reflect dimer-formation by L5.

Taking the binding data together, our studies suggest that two distinct regions mediate 5 S rRNA binding (Fig. 5G). In both cases, addition of increasing amounts of GST-L5 mutant protein caused the subsequent appearance of two distinct signals, which may reflect dimer-formation by L5.
wild-type L5 protein was low and varied between different batches of recombinant protein preparations (not shown). However, when we microinjected a mutant fusion protein, which contained the central region of L5 (aa 39–251) but lacked the RNA binding regions of L5, efficient nuclear export was always observed. As shown in Fig. 6, a significant amount of the nuclear L5–39/251 protein was transported to the cytoplasm, whereas co-microinjected rabbit IgG control protein remained in the nucleus (Fig. 6, A and B). The nuclear export of proteins is believed to be mediated by NESs, of which the most common type is characterized by a typical pattern of evenly spaced leucine residues (see below). However, this type of sequence motif is not present within the L5 protein (17, 35). In fact, only a single leucine-rich region exists in L5; this region is characterized by a cluster of five leucine residues that map to amino acid positions 103, 104, 105, 109, and 110 (depicted in Fig. 6A). We therefore microinjected GST fusion proteins, containing this L5-derived leucine-rich cluster into the cell nucleus. As shown in Fig. 6, a short L5 region of 11 amino acids (aa 101–111) directed a significant amount of the heterologous GST protein from the nucleus into the cytoplasm of Vero cells (L5-NES; Fig. 6, C and D). Residual export activity only was observed upon injection of the L5-D1NES fusion protein, in which the leucine residues at L5 amino acid position 103–105 were mutated into alanines (Fig. 6, E and F). Clearly, nuclear export was completely abrogated when all leucine residues present in this L5-derived region were simultaneously substituted by alanines (L5-D2NES; Fig. 6, G and H). Moreover, when the cell cultures were supplemented 2 h prior to microinjection with leptomycin B, which has been previously shown to be an inhibitor of the general export receptor CRM1 (36–39), the injected wild-type fusion protein (L5-NES) remained in the nucleus (Fig. 6, I and K). In order to analyze the activity of this leucine-rich sequence in the context of full-length L5 we also introduced the respective leucine to alanine mutations at aa positions 103–105, 109, and 110 in our L5-GFP expression construct. As shown before (Fig. 1A), transient transfection of HeLa cells with the L5-GFP vector resulted in cytoplasmic and nuclear localization of the expressed fusion protein (Fig. 7A). Coexpression of the RevM10-BFP hybrid protein, which has been previously reported to be an exclusively nucleolar protein (22), also demonstrated in this experiment that the L5-GFP wild-type protein indeed accumulates at the nucleoli (Fig. 7B).
In contrast, however, mutation of the potential L5 NES sequence in the L5-Δ2NES-GFP hybrid protein resulted in an exclusively nuclear/nucleolar fusion protein (Fig. 7C). The complete absence of any cytoplasmic GFP-signal indicated a nuclear export defect in the respective protein due to the mutational inactivation of an essential NES sequence.

Taken together, these data demonstrated that the leucine-rich region, which maps to L5 amino acid position 101–111, constitutes a NES that is required for nucleocytoplasmic trafficking of human ribosomal protein L5.

**DISCUSSION**

In the present study, we have investigated the domain organization of ribosomal protein L5. L5 is a nucleocytoplasmic shuttle protein that is involved in the intracellular transport of 5 S rRNA (6, 7, 10). This transport activity requires the interaction of L5 with nuclear import and export factors. However, the regions in L5 that mediate these protein-protein interactions, as well as the region required for 5 S rRNA binding, have to date been poorly defined. In fact, evidence for a distinct domain organization in mammalian L5 originated from a previous study in which the domain structure of the L5 protein from rat was investigated (29). In this work, it was shown that the L5 amino terminus (aa 1–93) contains a 5 S rRNA binding domain and that the carboxyl terminus (aa 151–296) harbors a signal that targets L5 to the nucleus/nucleolus.

By investigating the subcellular localization of L5 fusion proteins in living as well as in fixed cells, we were able to map two independent regions that serve as nuclear/nucleolar targeting signals. The region located in the L5 amino terminus maps to amino acid residues at position 21–37 (depicted in Fig. 8A). Fusion of a corresponding amino acid sequence to otherwise cytoplasmic βGal resulted in nuclear accumulation of the respective fusion protein. Because the βGal reporter protein is not suitable to investigate L5 signals that confer nucleolar localization (29), we also fused this L5 sequence to GFP. This resulted in nucleolar accumulation of the respective L5-GFP fusion protein, whereas, in contrast, no nucleolar localization was detected when wild-type GFP was expressed (28). The combined data indicated that this amino-terminal region in L5 serves as both a strong nuclear localization and nucleolar targeting signal. A second region that is involved in directing L5 to specific subcellular compartments is located in the protein carboxyl terminus and maps to amino acid position 255–265 (Fig. 8A). As with the amino-terminal signal, this region mediates the nucleolar localization of GFP. In contrast, this signal failed to target the ~116-kDa βGal protein to the nucleus. Fusion of the carboxyl-terminal half of L5 (aa 150–297) to βGal, however, resulted in nucleolar steady-state accumulation of this fusion protein. Subsequently, the microinjection of GST-GFP fusion proteins into the cytoplasm or nucleoplasm of Vero cells revealed that the complete L5 carboxyl terminus (aa 255–297) is able to mediate nuclear import as well as nucleolar accumulation. However, the kinetic with which the respective GST-L5-GFP fusion protein translocated from the cytoplasm into the nucleus was slow when compared with the kinetic of the NLS fusion protein, whereas, in contrast, no nucleolar localization resulted in nucleolar accumulation of this fusion protein. Consequently, the microinjection of GST-GFP fusion proteins into the cytoplasm or nucleoplasm of Vero cells revealed that the complete L5 carboxyl terminus (aa 255–297) is able to mediate nuclear import as well as nucleolar accumulation. However, the kinetic with which the respective GST-L5-GFP fusion protein translocated from the cytoplasm into the nucleus was slow when compared with the kinetic of the NLS found within the SV40 large T-antigen (40). In fact, significant nuclear import of cytoplasmic GST-L5-255/297-GFP hybrid protein was observed ~45 min postinjection, whereas, in contrast, the translocation of a similar GST-SV40 NLS-GFP construct was complete after ~10 min. Thus, the L5 carboxyl terminus (aa 255–297) exerts weak nuclear import activity when compared with a classical NLS. Moreover, the sequence located between L5 aa positions 255 and 265 appears to be required, although not sufficient, to direct a heterologous protein into the nucleus. Note that the amino acid composition of this L5 region resembles loosely the NLS found within the SV40 large T-antigen. This prototypic NLS is characterized by a short peptide motif highly enriched with positively charged amino acids (40). In contrast, the NLS located in the L5 amino terminus (aa 21–37) resembles a bipartite NLS of the type originally identified in nucleolusimin. This type of signal is defined by two basic peptide regions that are separated by a spacer region of ~10 amino acid residues (40). With respect to nuclear import it is also important to note that the L5 protein appears to form homodimers. Because the regions responsible for dimerization are not known, we cannot rule out the possibility that nuclear import of some reporter constructs may have occurred due to dimerization of the respective L5 fusion proteins with endogenous wild-type L5 protein.

To date, no clear consensus sequence has been reached on what directs nuclear proteins to the nucleolus. Delineation of the sequence requirements for subcellular localization of chicken nucleolin has revealed that the RNA binding motifs in nucleolin also cause its accumulation in the nucleoli (41). These data suggest that nucleolin is not transported actively into the nucleus by way of a classical NLS. However, nucleolin also shows properties that are reminiscent of a classical NLS. This is particularly the case when nucleolin is coexpressed with a NLS-containing reporter protein. In this case, the nuclear accumulation of nucleolin also causes its accumulation in the nucleoli (41). These data suggest that nucleolin is not transported actively into the nucleus by way of a classical NLS. However, nucleolin also shows properties that are reminiscent of a classical NLS. This is particularly the case when nucleolin is coexpressed with a NLS-containing reporter protein. In this case, the nuclear accumulation of nucleolin also causes its accumulation in the nucleoli (41).
The nuclear export of proteins is mediated by NESs, the prototypic signal of which was originally identified in the HIV-1 Rev protein (21, 42). Subsequently, structurally and functionally equivalent export signals have been described in various proteins, including human T-cell leukemia type I Rex (43), cAMP-dependent protein kinase inhibitor (42), mitogen-activated protein kinase kinase (44), and the hdm-2 oncoprotein (15) (depicted in Fig. 8B). Moreover, this type of NES is composed of a short stretch of hydrophobic, mainly leucine residues, which appear to be required for the interaction of the NES with the export receptor CRM1 (36–38, 45). In particular, the spacing of these hydrophobic (leucine) residues is considered to be a hallmark feature for this type of leucine-rich NES. The delineation of the NES of ribosomal protein L5 in this study, however, has revealed a different leucine-rich export signal. Obviously, the L5 NES (aa 101–111) is composed of critical leucines arranged in two separated clusters with no spacing between the individual leucine residues (Fig. 8B). Efficient nuclear export was observed with this signal, particularly when nucleolar retention of L5 was abolished by removing the protein regions that are responsible for 5 S rRNA binding and nucleolar targeting. Moreover, mutation of these leucine residues in the context of full-length L5-GFP demonstrated that this signal indeed affects the intracellular distribution of L5. This is in perfect agreement with the previous finding that the deletion of a short stretch of six amino acid residues in yeast ribosomal protein L1, which is the yeast homologue of L5, caused lethality in vivo (46). Taking our data into consideration, it is now obvious that this deletion (L1 aa 103–108: NH2-LIARRR-COOH) removed the central part of the L1 NES, a domain that is conserved among the eukaryotic 5 S rRNA-binding proteins. Finally, leptomycin B, a drug that has previously been shown to be an inhibitor of CRM1 (36–38) blocked the nuclear export of the L5 NES in our experiments. These data further support the notion that CRM1 is an export receptor for leucine-rich NESs in general. However, it remains to be seen whether additional factors are required for the nucleocytoplasmic translocation of specific RNAs such as 5 S rRNA.

In summary, ribosomal protein L5 is characterized by a multidomain structure. The delineation of the regions that are required for nuclear import, nucleolar localization, and nuclear export provides a tool to search for as yet unidentified L5 interaction partners. Moreover, we expect that sequences similar to the L5 NES will soon be identified in other proteins that traffic between the nucleus and cytoplasm of eukaryotic cells.

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Note Added in Proof—Since submission of this work a similar study on Xenopus and rat L5 protein was published (Claussen, M., Rudt, F., and Pieler, T. (1999) J. Biol. Chem. 274, 33951–33958.

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