**tRNA is entrapped in similar, but distinct, nuclear and cytoplasmic ribonucleoprotein complexes, both of which contain vigilin and elongation factor 1α**

Charli KRUSE*, Arnold GRÜNWELLER*, Dagmar K. WILLKOMM*, Thomas PFEIFFER†, Roland K. HARTMANN† and Peter K. MÜLLER*

*Department of Medical Molecular Biology, Medical University of Lübeck, Ratzeburger Allee 160, D-23538 Lübeck, Federal Republic of Germany, and †Department of Biochemistry, Medical University of Lübeck, Ratzeburger Allee 160, D-23538 Lübeck, Federal Republic of Germany

Vigilin, which is found predominantly in cells and tissues with high levels of protein biosynthesis, was isolated in its native form from human HEp-2 cells (A.T.C.C. CCL23) by immunoadfinity chromatography. Here we demonstrate that vigilin is part of a novel large tRNA-binding ribonucleoprotein complex (tRNP), found not only in the cytoplasm, but also in the nuclei of human cells. Compositional differences in the protein pattern were detected between the nuclear and cytoplasmic tRNPs, although some properties of the purified nuclear tRNP, such as tRNA protection against nuclease attack, were identical with those of the cytoplasmic tRNP. By using either a pool of total human nuclear RNA or radioactively labelled yeast tRNA in vitro in rebinding experiments, we could show that tRNA is specifically recaptured by the RNA-depleted, vigilin-containing nuclear complex. We could also show that vigilin is capable of binding tRNA in vitro. Another tRNA-binding protein is elongation factor 1α, which appears to be enriched in the cytoplasmic and nuclear tRNP complexes. This suggests that the cytoplasmic tRNP may be involved in the channelled tRNA cycle in the cytoplasm of eukaryotic cells. Our results also suggest that the nuclear vigilin-containing tRNP may be related to the nuclear export of tRNA.

**INTRODUCTION**

Ribonucleoprotein complexes (RNPs) are substrates for RNA export from the nucleus to the cytoplasm [1,2] through nuclear-pore complexes, an energy-dependent process which is mediated by specific saturable factors [3–8]. These findings have stimulated efforts to identify RNA-binding proteins involved in RNA export. A number of RNA-binding motifs has been described in recent years, such as the RNP motif, the arginine-rich motif, the RGG box and the heterogenous nuclear ribonucleoprotein K homologous (KH) domain [9]. The multi-KH-protein vigilin has a nuclear localization sequence, and has been localized in the nucleus and cytoplasm of a wide variety of cells [10,11]. NMR analysis of the vigilin KH-domain structure has suggested that the protein consists of 15 KH domains [12], rather than the 14 domains described previously [13]. The occurrence of an additional fifteenth N-terminal KH domain is supported by the observation that the exon/intron boundaries also define the structure of this domain, as observed for the remaining 14 domains [13]. Since the KH-domain sequence motif is highly degenerate, and to avoid confusion with the numbering of the 14 KH domains of vigilin described previously, we propose to denote this newly identified domain with the affix ‘0’. So far, the vigilin tRNA complex has only been characterized from a cytosolic extract [14].

Although vigilin is a ubiquitous protein, it should be emphasized that highest expression is observed in those cells which are known to produce high quantities of protein, such as liver parenchymal cells or pancreatic secretory cells [14,15].

From the increase of vigilin production in cells and tissues with stimulated translational activity, and owing to the fact that anti-vigilin antibodies may inhibit the synthesis of proteins during translation in vitro [14,16], it is reasonable to assume that vigilin may have an, as yet unknown, function in protein translation. We have previously shown that vigilin is part of a large tRNA–protein complex in the cytoplasm of mammalian cells [14]. By binding studies in vitro, we provide evidence that vigilin is able to bind tRNA.

Since vigilin had also been shown to be present in the nucleus, we analysed a native nuclear extract for the presence of a similar complex containing vigilin and tRNA. Because such a complex may participate in the mechanism of tRNA nuclear export, which is, at present, completely elusive and apparently different from export mechanisms described for other RNA species [4,17,18], the identification of nuclear tRNA-binding proteins is of paramount importance. In the present study, we have identified a vigilin-containing RNP in the nucleus. Moreover, we could demonstrate that the elongation factor 1α (EF-1α) is enriched in the purified nuclear, as well as cytoplasmic, vigilin-containing tRNPs.

**EXPERIMENTAL**

**Cell culture and preparation of cell extracts**

The established cell line (HEp-2, A.T.C.C. CCL23) derived from human epithelial larynx carcinoma was grown as described previously [14]. For SDS/PAGE, 10⁶ cells were lysed for at least...
1 h [14]. The cell extract was centrifuged, and the supernatant was used immediately or stored at −70 °C. For preparation of native nuclear and cytosolic fractions, we used a modification [10] of the method originally described by Dignam et al. [19].

**Gel-shift experiments and UV-crosslinking**

For gel-shift experiments, 10 000 to 30 000 Cerenkov c.p.m. of 32P-labelled tRNA\(_{\text{Vig}}\) (tRNA\(_{\text{Vig}}\)) transcribed in vitro in the presence of [\(\alpha\)-32P]CTP and 5-bromo-UTP were incubated with 10 \(\mu\)l of vigilin core complex (VCC) isolated from 300 \(\mu\)g of cytoplasmic protein in a total vol. of 25 \(\mu\)l of 1 × shift buffer [50 mM glycine (pH 7.5)/20 mM NH\(_4\)Cl/10 mM MgCl\(_2\)/5 mM dithioerythritol/0.2 mM EDTA] for 5 min at room temperature (RT). UV-crosslinking was performed at 302 nm for 10 min at RT using a Stratalinker (Stratagene, Heidelberg, Federal Republic of Germany). Samples were then loaded on to a native PAGE with a shift buffer with 0.5 \(\%\) polyacrylamide gel in 1 × sample buffer [5 \(\%\) (v/v) glycerol/0.1\(\%\) EDTA/0.005 \(\%\) Bromophenol Blue], supplemented with 0.5 \(\%\), SDS if indicated, and run at 20 mA. Exposure times of X-ray films (Fuji, Tokyo, Japan) were 12–74 h at −80 °C using an intensifying screen.

**Construction of the vigilin expression plasmid**

The vigilin expression plasmid pTM1Vig was constructed using a PCR product derived from human cDNA. For this purpose, total RNA was isolated from MG63 cells (A.T.C.C. CRL-1427) by guanidine thiocyanate (GuSCN) lysis and CsCl gradient centrifugation [20], and reverse transcription was performed with superscript reverse transcriptase according to the manufacturer’s protocol (Gibco-BRL, Eggenstein, Federal Republic of Germany). The primers used for reverse transcription and PCR were 5′-AGATCAACCATGGGTTCCGTTGCAG (sense) and 5′-CAGCACGGCTCGAGAGGGTTCTGTTC (antisense), corresponding to positions 146–170 and 3972–3996 of the human cDNA sequence [21]. Due to single-point mutations at positions 151 and 3987, the primers introduced NcoI and XhoI restriction sites, that were utilized to clone the PCR product into the vector pTM1 [22] digested with the same enzymes. The identity of the insert was confirmed by DNA sequencing.

**Capturing assays**

Binding assays of labelled vigilin to immobilized tRNA were performed using a modified protocol, on the basis of procedures described by Boelens et al. [23] and Siomi et al. [24]. Streptavidin magnetic particles (Boehringer-Mannheim, Federal Republic of Germany) were coated with total tRNA from yeast (Boehringer-Mannheim, Federal Republic of Germany), and 3′-biotinylated according to von Alsen and Noller [25] in a total vol. of 30 \(\mu\)l of buffer [10 mM Tris/HCl (pH 7.4)/2.5 mM MgCl\(_2\)/1 M NaCl/0.5 \(\%\) (v/v) Triton X-100] per 1.6 mg of beads and 18 \(\mu\)g of tRNA on a rotating wheel for 30 min at RT.

Vigilin was transcribed and translated from pTM1Vig in a cell-free reticulocyte lysate system (TNT, Promega, Madison, U.S.A.) according to the manufacturer’s instructions in the presence of [\(\alpha\)-32P]methionine (Amersham, Braunschweig, Federal Republic of Germany). Labelled vigilin aliquots (9 \(\mu\)l) were added to 6 \(\mu\)l of binding buffer [10 mM Tris/HCl (pH 7.4)/2.5 mM MgCl\(_2\)/100 mM NaCl, supplemented with 0.1 \(\%\) (v/v) Triton X-100]. Samples were then preincubated for 20 min at RT in the absence or presence of 3.75 \(\mu\)g (an approximately 5-fold excess over the amount of tRNA bound to the beads) of indicated competitor nucleic acids, followed by addition of 400 \(\mu\)g of coated beads (3 ng of tRNA/\(\mu\)g), and further incubation for 40 min at RT. Beads were washed 4 times with 1 ml TMN buffer containing 0.5 \(\%\) (v/v) Triton X-100. Bound protein was eluted by boiling the beads in SDS sample buffer, and analysed by discontinuous SDS/PAGE (8 \(\%\) gels). Bands were detected by fluorography for 54 h. Non-specific vigilin binding to the beads could be excluded by incubating vigilin with uncoated beads.

**Electrophoresis and electroblotting**

Individual fractions of nuclear and cytoplasmic proteins [10] were analysed by SDS/PAGE on 5–15 \(\%\) gradient gels, and stained with a silver-staining kit (Bio-Rad) following the manufacturer’s protocol, or analysed by immunodetection. The supernatants from centrifuged cell lysates (derived from 1 × 10\(^6\) cells) were boiled in Laemml sample buffer in the presence of 0.1 mM 2-mercaptoethanol and used as controls for SDS/PAGE and immunoblotting of vigilin. Immunoblots of agarose gels were carried out essentially with the same techniques as used for PAGE.

**Affinity chromatography**

Affinity-purified anti-vigilin antibodies were coupled to an affinity membrane chromatography cartridge (Millipore, Eschborn, Federal Republic of Germany). The cartridge was operated with the ConSep LC 100 elution and monitoring system (Millipore, Eschborn, Federal Republic of Germany) with a pressure of 48.3–55.2 kPa. Non-specific adsorption could be excluded as described previously [14].

The cartridge was loaded with either the cytoplasmic or the nuclear fraction of HEp-2 cell extracts, and washed with water until the \(A_{\text{280}}\) reached baseline values. The cartridge was subsequently developed with gradients, as follows: (1) with 0–1 M glycine (pH 1.5) to elute complexes containing vigilin, additional proteins and nucleic acids; (2) alternatively, with a NaCl gradient from 0–0.8 M NaCl to wash off weakly bound proteins, leaving only the VCC, containing vigilin and core proteins, on the cartridge; or (3) with a 0.8–1 M NaCl gradient to elute nucleic acids tightly bound to the VCC.

For RNA-rebinding experiments, a matrix-bound VCC was prepared by a three-step process: (1) native HEp-2 nuclear extract containing vigilin was loaded on to the cartridge; (2) VCC was subsequently completely depleted of innate RNA with 0–1 M NaCl; (3) before reloading with the pool of total nuclear RNA, the cartridge was desalted with water, and subsequently equilibrated with PBS buffer.

**Treatment of chromatographic fractions**

Fractions collected from the affinity cartridge were dialysed, freeze-dried, dissolved in TE buffer [10 mM Tris/1 mM EDTA (pH 8.0)] and divided into aliquots, which were processed sequentially with (1) 0.5 \(\%\) (w/v) SDS, (2) a mixture of 0.5 \(\mu\)g/\(\mu\)l DNase-free RNase A (EC 3.1.27.5) and RNase T1 (EC 3.1.27.3) for 30 min at 37 °C in the presence of 0.5 \(\%\) (w/v) SDS, and (3) 0.5 \(\mu\)g/\(\mu\)l DNase I (EC 3.1.21.1) for 30 min at 37 °C in the presence of 0.5 \(\%\) (w/v) SDS. Nucleic acids were extracted from the complex by acid-GuSCN/phenol/chloroform treatment [26].

**Characterization of vigilin-containing fractions**

To characterize the fractions collected from the anti-vigilin antibody cartridge, different gel electrophoresis protocols were
followed to identify proteins and nucleic acids: (1) Tris/acetate/EDTA (TAE) [40 mM Tris/acetate (pH 8.5)/2 mM EDTA] agarose electrophoresis (0.8°) to analyze nucleic acids and protein–nucleic acid complexes in a non-denaturing gel matrix; (2) gradient SDS/PAGE (5–15% gels) for protein analysis, and (3) PAGE (12% gels) with 8 M urea to identify the bound RNA species.

Detection of the separated components was essentially as described [14], using ethidium bromide staining, Ponceau S staining, immunostaining of nitrocellulose membranes with either the anti-vigilin antibody or the anti-(EF-1α) antibody, and by silver staining. The amounts of RNA and protein were evaluated densitometrically by whole-band scanning (Optoquant, Computer and Vision, Lübeck, Federal Republic of Germany) by using purified tRNA from yeast and BSA as reference standards.

**RNA-rebinding studies**

RNA extractions were performed at 60 °C with 1 vol. of phenol [saturated with 10 mM Tris/HCl (pH 4.3)/2 mM EDTA], and were repeated until the interphase remained clear. Aqueous phases were finally extracted twice with equal vols. of chloroform. RNA was precipitated with ethanol and dissolved in water. The quality of the preparation was tested on a 12% polyacrylamide/urea gel, and stained with silver according to the manufacturer’s protocol (Bio-Rad, München, Germany). For rebinding studies, the antibody-associated VCC bound to a solid matrix was depleted of innate RNA (see the Affinity chromatography subsection). Nuclear RNA from HeLa cells (40 μg per injection) was then loaded on to the cartridge, which was developed again with a 0–1 M NaCl gradient. RNA fractions were collected, precipitated with 1 vol. of propan-2-ol and resuspended in TE buffer. RNAs were separated on a 12% polyacrylamide/urea gel and revealed by silver staining.

**RESULTS**

**Gel shifts and UV-crosslinking of tRNAAsp* to the VCC**

Previous tRNA-rebinding studies with the cytoplasmic VCC, immobilized on to an affinity cartridge, demonstrated binding of yeast tRNAAsp* to the VCC after depletion of innate tRNA with 1 M NaCl [14]. As a continuation of our previous study, we analysed rebinding of tRNA to the affinity-purified and tRNA-depleted cytoplasmic VCC by gel-shift and UV-crosslinking experiments. Figure 1 shows that the tRNAAsp* probe (carrying 3-brominylated modifications at all U residues) becomes incorporated into a high-molecular-mass complex, even in the absence of UV-crosslinking (lanes 3 and 4). Migration of this vigilin-containing tRNP (ribonucleoprotein complex) into the gel could only be observed when 0.5% (w/v) SDS was added after UV irradiation (lane 5). The smear in lane 5 suggests that the tRNP complex was partly, but non-uniformly, decomposed by the SDS treatment. These results, combined with the requirement of 1 M NaCl to release endogenous tRNA from immunopurified vigilin complexes [14], support the notion that tRNA is tightly bound to a high-molecular-mass cytoplasmic complex which contains vigilin.

**Binding of vigilin to biotinylated tRNA**

To investigate the role of vigilin in tRNA binding, we used a capturing assay with biotinylated tRNAs coupled to magnetic beads. A eukaryotic full-length vigilin expression vector (pTM1Vig) was transcribed in vitro, and vigilin was labelled during translation with [35S]methionine in a rabbit reticulocyte lysate. Identity of the translated protein with native vigilin of cells and tissues was ascertained by detection with the vigilin-specific antibody FPIII (results not shown). The shorter polypeptide obtained by in vitro translation (Figure 2) is attributable to translational initiation at an internal AUG codon. The results of the binding reaction are shown in Figure 2; vigilin bound to the biotinylated tRNA (tRNAAsp*; lane 3), and competition of binding was readily observed in the presence of excess untagged tRNA (lane 4), but not with excess fish sperm DNA (lane 5) or poly(A) RNA (lane 6), which is consistent with specific binding of vigilin to tRNA.

**A VCC localized in the nucleus is associated with RNA**

Vigilin contains a functional nuclear localization sequence, and is localized in both the nucleus and the cytoplasm [10]. Thus we examined whether a VCC could be isolated from a nuclear protein fraction prepared from HEp-2 cell lysates. The nuclear fraction used as the starting material has recently been documented to be free of endoplasmic membranes [10], thus this includes only soluble nuclear proteins. The nuclear extract was loaded on to an affinity cartridge coupled with affinity-purified anti-vigilin anti-
Figure 2  Specific binding of vigilin to biotinylated tRNA

Binding of $^{35}$S-labelled vigilin, obtained by in vitro transcription/translation, to biotinylated tRNA coupled to magnetic beads was analysed by SDS/PAGE and autoradiography (for details, see the Experimental section). Lane 1, the radiolabelled vigilin protein; lane 2, incubation of vigilin with magnetic beads in the absence of biotinylated tRNA. Lane 3, vigilin bound to immobilized biotinylated tRNA; lanes 4–6, as lane 3, but in the presence of competing nucleic acids: lane 4, unmodified tRNA; lane 5, fish sperm DNA; lane 6, poly(A) RNA.

Figure 3  Comparison of cytoplasmic (VCC-cytopl.) and nuclear (VCC-nucleus) VCCs

Complexes were analysed for protein composition by SDS/PAGE and silver staining (A), as well as immunoblotting with the anti-vigilin antibody FP III (B) and an antibody raised against human EF-1$\alpha$ (C). A HEp-2 total cell lysate was used as the control; note that the total amount of protein in the total cell lysate largely exceeded that in the two VCC complexes (A). Molecular masses (Mw) are indicated on the left.

bodies, and the cartridge was developed with a NaCl gradient from 0–0.8 M NaCl, such that only the VCC remained bound. The antibody-bound VCC was eluted from the cartridge with a 0–1 M glycine gradient (pH 1.5). One part of the eluted protein fraction was separated by SDS/PAGE and silver-stained (Figure 3A). The other part was transferred on to a nitrocellulose membrane after SDS/PAGE, and was immunostained with the vigilin-specific antibody FP III [10] and an (EF-1$\alpha$)-specific anti-

Figure 4  A VCC affinity-purified from the nuclear soluble protein fraction is associated with RNA

The nuclear VLC (VLC$_n$), eluted from the anti-vigilin antibody affinity cartridge by a glycine gradient (0–1 M), was analysed for the presence of nucleic acids by agarose gel electrophoresis and ethidium bromide staining (upper panel). The same gel was then blotted and stained with Ponceau S to detect proteins associated with nucleic acids (lower panel), and the amounts of RNA and protein were estimated densitometrically. Lane 1, 0.4 µg bulk tRNA from yeast used as marker; lanes 2–4, VLC$_n$, either untreated (lane 2; 0.3 µg of RNA/1.5 µg of protein) or treated with RNase (lane 3; 0.27 µg of RNA/1.11 µg of protein) or DNase (lane 4; 0.34 µg of RNA/1.33 µg of protein); lanes 5–7, as lanes 2–4, but after pretreatment with GuSCN to remove proteins; lane 8, untreated cytoplasmic VLC (0.51 µg of RNA/1.52 µg of protein). Samples in lanes 2–8 were adjusted to 0.5% (w/v) SDS before gel loading to allow migration of ribonucleoprotein complexes into the gel matrix.

body [27] (Figures 3B and 3C). Cytoplasmic VCC and HEp-2 total cell lysates were used as controls. The VCC isolated from nuclear extracts shows distinct differences in protein composition compared with the cytoplasmic VCC, although several prominent bands appeared to be present in both complexes (Figure 3A). Note that the amount of protein in the loaded HEp-2 cell-lysate fraction largely exceeded that of the two VCC complexes. Vigilin appears to be enriched in both complexes, as inferred from immunostaining intensities in comparison with the HEp-2 lysate fraction (Figure 3B). EF-1$\alpha$-immunostaining intensities (Figure 3C) indicate that this elongation factor also accumulates in both the nuclear and cytoplasmic vigilin-containing complexes. EF-1$\alpha$ may be identical to the 50 kDa polypeptide which could be shown in the silver staining of VCCs (Figure 3A).

We further investigated the properties of the nuclear VCC following the experimental protocol used for its cytoplasmic counterpart [14]. A nuclear extract was loaded on to the affinity cartridge, and the bound material was eluted with a 0–1 M glycine gradient (pH 1.5). The eluted fraction was dialysed, freeze-dried, resolved in TE buffer and divided into two subfractions. One subfraction was analysed on a 0.8% TAE buffer–agarose gel stained with ethidium bromide, as shown in Figure 4 (upper panel), using tRNA from yeast as a marker (lane
Figure 5  Rebinding of tRNA to the nuclear VCC

(A) Elution profiles after loading of a HEp-2 cell nuclear extract on to an anti-vigilin antibody affinity cartridge. After binding of the native nuclear vigilin–RNA complex, the affinity cartridge was first developed with a NaCl gradient to liberate bound RNAs (fraction 1), followed by re-equilibration to 0 M NaCl by washing with water. Subsequently, an RNA mixture (40 µg of total nuclear RNA from HeLa cells, 8 µg in fraction n in (B)) was loaded on to the cartridge; unbound flow-through material eluted in fraction 2. The cartridge was again developed with a linear NaCl gradient (0–1 M) to remove weakly, as well as tightly, bound RNAs (fractions 3–5). The RNA-depleted vigilin complex was then released from the cartridge by applying a glycine gradient (0–1 M). (B) Fractions 3 (0–0.6 M NaCl), 4 (0.6–0.8 M NaCl) and 5 (0.8–1 M NaCl), derived from the second NaCl gradient after rebinding of RNA, as well as the vigilin complex released in the final step [fraction 6 in (A)] were extracted with phenol and analysed by 12% PAGE in the presence of 8 M urea, followed by silver staining. Lane n, 8 µg of human nuclear RNA; individual RNA species, as well as the bulk tRNA fraction, were assigned tentatively according to known sizes; fractions in (B) correspond to those in (A). Key to traces: (— — —) NaCl gradient; (........) glycine gradient.

1). In lanes 2–4, an ethidium-bromide-stainable band, resistant to degradation by DNase I (lane 4) or RNase (lane 3), migrated into the gel. After extraction with GuSCN, the material migrated to the same position as tRNA in the absence of nucleases (lane 5), and could be digested completely with RNase (lane 6). Blotting of the nuclear vigilin large complex (VLC, lanes 2–4), followed by Ponceau S staining (lower panel), revealed the co-migration of proteins with RNA, which explains the retardation of innate RNA in ethidium-bromide-stained gels (upper panel). The ratio of RNA-to-protein mass in the untreated nuclear VLC was close to 1:5. Accordingly, in the cytoplasmic VLC, the ratio of RNA-to-protein mass was approximately 1:3 [14].

Rebinding of RNA to the nuclear vigilin complex
To investigate whether the antibody-bound nuclear VCC, like its cytoplasmic counterpart, specifically reassociates with tRNA after RNA depletion (Figure 5A), total nuclear RNA was loaded on to the re-equilibrated cartridge until RNA was detected in the flow-through, indicating saturation of binding (Figure 5B, lane 2). The cartridge was then rinsed with the loading buffer, and finally developed again with a NaCl gradient. Both the innate RNA (lane 1) and the rebound RNA (lane 5) eluted with 0.8–1 M NaCl, and contained almost exclusively tRNA, whereas other subfractions of nuclear RNA, partly degraded, were eluted at 0–0.8 M NaCl (lanes 3 and 4). To rule out non-specific background binding to the affinity matrix, nuclear RNAs were applied to the cartridge containing the anti-vigilin antibody alone. In this instance, all RNA material was completely released from the cartridge–antibody backbone at NaCl concentrations lower than 0.8 M NaCl (results not shown).

DISCUSSION

Previous investigations have shown that oestrogen-stimulated expression of vigilin in the uterus of rats correlates with morphological features, suggesting increased protein synthesis [15]. An immunocytochemical study revealed that vigilin co-localizes with the cytoplasmic side of the rough endoplasmic reticulum (ER) and rough microsome membranes in rat exocrine pancreatic cells, suggesting its association with ribosomes [11]. Moreover, vigilin was found to co-purify with the rough ER and ribosomes during cell fractionation [16]. A close coincidence of vigilin synthesis and periods of substantial protein synthesis has also been observed in developing chicken cartilage and bone [28]. Thus a body of experimental evidence has pointed towards a role of vigilin in protein synthesis of vertebrates.

Vigilin is a ubiquitous protein consisting of 15 KH domains. The KH motif is present in a family of proteins, of which several have been shown to be capable of RNA binding. Recently, poly(G) binding has been assigned to single vigilin/KH domains [29], and the KH domain of Escherichia coli ribosomal protein S3
crosslinks to ribosomal RNA [30]. Siomi et al. [31] showed that a single point-mutation (Ile\textsuperscript{106} → Asn) in the KH domain of the fragile X mental retardation (FMR1) gene product prevents RNA binding to the protein, and may cause mental retardation.

We previously isolated a vigilin-containing tRNP from the cytoplasm of HEP-2 cells by immunoaffinity chromatography [14]. Innate tRNAs could be completely released from the multiprotein complex by high salt concentrations, and exogenously added tRNA rebound to the complex, restoring its native properties, such as protection of the tRNA against RNase degradation and retardation of tRNA mobility in agarose gels. Here we have further shown by crosslinking and mobility-shift experiments that the tRNA is embedded in a large RNA–protein complex, and only crosslinking followed by SDS treatment allows the tRNA to enter a native 4\% polyacrylamide gel in association with its covalently attached, yet uncharacterized, protein constituent (Figure 1).

The fact that vigilin contains a nuclear localization sequence, and has been found in the cytoplasm and nucleus [10,11], prompted us to look at whether an RNP complex similar to the cytoplasmic complex may occur in mammalian nuclei. As shown in this study, we were able to isolate such a complex from HEP-2 nuclei by immunoaffinity chromatography. Comparison of the cytoplasmic with the nuclear complex revealed distinct differences in their protein composition, although several polypeptides appeared to be present in both complexes (Figure 3A). A number of common features of the nuclear and cytoplasmic complexes are observed. First, as identified with the cytoplasmic VCC [14], its nuclear counterpart is capable of rebinding tRNA (Figure 5B). Secondly, both complexes render bound tRNA resistant to RNase digestion, whereas protection is lost when tRNP complexes in order to understand the cellular role of vigilin.

A central question is concerned with which protein components of the cytoplasmic and nuclear vigilin-containing tRNP complexes physically interact with tRNA. Vigilin itself may, in part, fulfill this role, owing to its tandemly repeated KH domains, which allow the tRNA to enter a native structure of synthetases, trans-translation factors and ribosomes without transient diffusion into the cytoplasm (called the channelled-tRNA cycle) [32,33]. Direct experimental evidence for a close association of EF-1 and aminocyl-tRNA synthetases has been provided in the case of mammalian valyl-tRNA synthetase [34]. Enrichment of EF-1\(\alpha\) (Figure 3), as well as the presence of tightly bound tRNAs in the affinity-purified cytoplasmic VCC [14], suggests that synthetases, in addition to EF-1\(\alpha\) and tRNA, are integral constituents of the cytoplasmic vigilin complex, which appears to form a kind of ‘superstructure’ closely associated with the ribosome [27,35,36]. The apparent differences, as well as similarities, in the protein composition of cytoplasmic and nuclear vigilin complexes (Figure 3), as well as the fact that tRNAs are an integral part of both complexes (Figure 4 and 5 and [14]), support the notion that the nuclear vigilin–tRNP complex may be the precursor of the cytoplasmic complex. Our finding that EF-1\(\alpha\) is also enriched in the nuclear complex is in line with the nuclear localization of EF-1\(\alpha\) in different cell types, as observed in previous studies [32,37]. In conclusion, it is reasonable to assume that our identification of a nuclear vigilin–tRNA-containing complex represents an important step towards the characterization of components involved in nuclear tRNA export.

It is interesting to note that in the yeast system, several enzymes involved in tRNA maturation are localized at the nuclear membrane in association with nuclear pore components and nucleoporins. This suggests that tRNA maturation and export are coupled processes in yeast [38]. Assuming a similar scenario exists in mammalian cells, the mammalian nuclear vigilin–tRNP may include, or may interact with, proteins involved in tRNA maturation and export. We are currently scrutinizing the protein composition of cytoplasmic and nuclear vigilin–tRNP complexes in order to understand the cellular role of vigilin.

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tRNA binding in the nucleus and in the cytoplasm


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