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Research Article

The multi-KH protein vigilin associates with free and membrane-bound ribosomes

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Abstract. The-multi-KH domain protein vigilin has been identified by ex vivo experiments as both a tRNA- and/or mRNA-binding protein. We show here that in vitro under conditions previously shown to allow tRNA binding, recombinant vigilin also binds to selected mRNA species and ribosomal RNA. An in vivo link of vigilin to mRNA and rRNA was elucidated by several approaches. (i) Coexpression/costimulation of vigilin was found with many other proteins independently of whether their mRNA was translated on free or membrane-bound ribosomes. (ii) A close codistribution of vigilin with free ribosomes was seen in the cytoplasm while nucleoli were a major organelle of vigilin accumulation in the nucleus. (iii) Furthermore, free and membrane-bound ribosomes can be enriched for vigilin which suggests that this binding does not depend on the class of mRNA translated. Therefore, we suggest that vigilin does not distinguish between free or membrane-bound ribosomes but is generally necessary for the localization of mRNAs to actively translating ribosomes.

Key words. RNA export; KH protein; KH domain; RNA transport; polyribosomes; microsome.

KH proteins are known as RNA-binding proteins apparently with diverse cellular functions [1–4]. The functional importance of the KH domain in one of the better-known RNA-binding proteins, FMR1 (fragile X mental retardation gene product), is underscored by the observation that a single point mutation of a conserved residue within the KH domain leads to a severe form of the fragile X syndrome, the most frequent type of heritable mental retardation [5]. Vigilin, with a string of 14/15 KH domains [2, 6], is of particular interest with respect to its molecular function(s) and its interacting partners. Being ubiquitously distributed assigning any distinct function is difficult, although recent studies demonstrated that in vitro, vigilin binds tRNA [7, 8] as well as vitellogenin mRNA [9, 10]. Further evidence for vigilin tRNA binding was derived from ex vivo studies which argued for a multiprotein complex with vigilin and tRNA in specific contact [8], and from experiments demonstrating stimulation of the export of tRNAs from the nucleus to the cytoplasm [8]. Based on these observations, we hypothesize that tRNA packaged with vigilin provides a bridging vehicle between the nucleocytoplasmic export of tRNAs [8] and the channeled tRNA cycle on ribosomes [11–13]. Here we show to the best of our knowledge for the first time that a KH protein binds in vitro different native RNAs and that in vivo the colocalization of vigilin and ribosomes does not depend on the binding of ribosomes to membranes of the rough endoplasmic reticulum. This again implies that vigilin associates with RNA without distinguishing between mRNAs coding either for secretory or cytoplasmic proteins.

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Materials and methods

Recombinant expression and purification of vigilin

Vigilin cDNA was amplified by polymerase chain reaction (PCR) using the sense primer 5′-CATGCA TGGGGAGTTCCGTTGCAGTTTTGACC and the antisense primer 5′-GAAGATCTTCGTTTGGGGCCCAA GGGAG, introducing *Nco*I and *Bgl*II restriction sites (underlined) used for insertion of the PCR product into the expression vector pQE 60 (Qiagen, Hilden, Germany). The identity of the cloned gene was verified by DNA sequencing. Expression of recombinant vigilin, carrying a C-terminal His-tag, in *Escherichia coli* C 600$^-$ [14] was induced at 30 °C for 3 h by incubation in LB broth containing 0.5 mM IPTG. Cells were collected by centrifugation, sonicated in 50 mM NaH_2PO_4 pH 6.0, 300 mM NaCl, and the lysate was loaded onto an NiNTA superflow column (Qiagen). After washing with 30 column volumes of wash buffer (50 mM NaH_2PO_4 pH 8.0, 1 M NaCl) and 10 column volumes of wash buffer containing 40 mM imidazole, the protein was eluted in the wash buffer containing 80 mM imidazole. Western blot analysis of the purified recombinant protein using the vigilin-specific antiserum FP-III (see below) revealed a protein with a molecular weight indistinguishable from native vigilin from HEp-2 cells.

Preparation of [^{32}P]RNAs

RNAs were prepared in vitro by runoff transcription from plasmids pT7HAV1 linearized with *Nco*I (hepatitis A virus 5′-nontranscribed region Ia RNA = HAV Ia RNA) [15], pGEM3Z5SrRNA linearized with *Hin*dIII giving human 5S rRNA (construction of the plasmid: PCR product with the primers 5′-CGAATTCGTCTACGGCCA ACCACCCT and 5′-CCCCAAGCTTAAAGCTACAGC ACCCGGT on cDNA from human placenta ligated into pGEM3Z via *Eco*RI and *Hin*dIII restriction sites in both the PCR product and the vector) and from the following PCR-generated templates: T7-vitellogenin-3′ NTR: sense primer: 5′-TAATACGACTCACTATAGGG, antisense primer: 5′-TTGAGATCA-GTTTATCACATCAG TGAC TATAGTGAGTCGTATTA, comprising the vigilin core binding site (27 nt) of the 3′-nontranscribed region of *Xenopus* vitellogenin mRNA [16]; T7-polypyrimidine: sense primer: as with T7-vitellogenin-3′ NTR, antisense primer: 5′-GGAAAGGGAAAAGGGAAAGGGAAA GAAAGGGAAAAGGGAAAGGGAAACCTATAG GAGTCGTATTA; T7-Lys3: sense primer 5′-TAATA GACTCACTATAGGCCCGGATAGCTCAGTC, antisense primer 5′-TGGCGCCCGAA-CAGGGAC, using plasmid ptRNALys3 [17] encoding human tRNA^{Lys} (UUU) as a template. Except for HAV Ia RNA (transcribed with SP6 RNA polymerase), transcripts were synthesized with T7 RNA polymerase, and RNAs were internally labeled to equal specific molar activities with α[^{32}P]CTP (NEN, Zarenten, Belgium). Oxytocin 3′ NTR mRNA from rat was a generous gift of E. Mohr (UKE, Hamburg, Germany). After transcription, RNAs were purified by denaturing 8–10% PAGE (8.3 M urea, 1 × TBE), eluted from gel slices by incubation at 4 °C overnight in 200 mM Tris-Cl (pH 6.5) and 0.1 mM EDTA, precipitated with ethanol and redissolved in water.

Gel retardation assays

Samples were incubated in a volume of 10 µl 1 × shift buffer (50 mM glycine pH 7.5, 20 mM NH_4Cl, 10 mM $MgCl_2$, 5 mM DTT and 0.2 mM EDTA) for 7 min at room temperature. After addition of 1/10 volume of sample buffer (50% glycerol, 1 µM EDTA pH 8.0, 0.05% bromophenol blue), samples were electrophoresed on 5% nondenaturing PAA gels in 1 × TBE buffer for 45 min at room temperature. Detection and quantification were performed with a Bio-Imaging Analyzer BAS-1000 (Fujifilm, Fuji Düsseldorf, Germany). The relative shift was determined as the proportion of shifted activity relative to the total amount of activity in the lane, in relation to the shift of tRNA^{Lys} (set to 100%).

Cell culture and preparation of cell extracts

The established cell line HEp-2 (ATCC CCL23, derived from human epithelial larynx carcinoma) and human primary fibroblasts were grown as described previously [18, 19]. Fibroblasts were harvested 2 and 5 days after seeding. For SDS-PAGE, 10^6 cells were lysed for at least 1 h [18]. The cell extract was centrifuged and the supernatant was immediately used or stored at –70 °C.

Analysis of collagen and proteins in fibroblasts

Cells growing on the bottom of 25-cm^2 tissue culture flasks were preincubated for 24 h in DMEM supplemented with 100 U/ml penicillin, 50 µg/ml L-ascorbate, 2 mM L-glutamine, 1% FCS. Incubation with 370 kBq/ml L-[2,3-^{3}H]proline (1.6 GBq/mmol; Amersham, Freiburg, Germany) in incubation medium I (DMEM with 50 µg/ml L-ascorbate, 100 U/ml penicillin, 0.15 mg/ml β-aminoproprionitrile, 1% FCS, 25 mM HEPES, 15 mM MOPS, pH 7.4) was then carried out for 24 h.

For analysis of quantitative collagen synthesis, cell lysates were concentrated and washed with Microsep ultrafiltration units with an exclusion size of 10 kDa (Filtron, Karlsruhe, Germany) to remove unbound radioactivity, resuspended in 6 N HCl, hydrolyzed (110 °C, 24 h) and dried in a desiccator. After resuspension in amino acid sample dilution buffer (Beckmann, Munich, Germany), the amounts of [^{3}H]proline and [^{3}H]hydroxylproline were determined by amino acid analysis using ion exchange chromatography with a solid-phase scintillation detector. Quantitative collagen synthesis was expressed as hydroxyproline counts per cell. The [^{3}H]hydroxyproline counts per cell directly reflected the quantity of

newly synthesized collagen normalized to cell number [20].

Cotransfection of vigilin and β-galactosidase into COS7 cells

Vectors used for transfection experiments were pADCMV, pADCMVβgal (D. Gründler-Thompson, Bender & Co, Vienna, Austria) and pADCMVVig constructed by ligation of an oligonucleotide linker (sense: 5′-AGCTTGC GCCAGC, antisense 5′-CATGGCTGGC GCA) and the *Nco*I/*Xho*I Vigilin insert of pTM1Vig [18] into *Hin*dIII-cut pADCMV1.

Briefly, 1 µg of each vector was transfected into cells from the established cell line COS7 (ATCC CRL 1651, 3×10^5 cells per transfection) using a commercially available LipofectAmine kit (Life Technologies, Eggenstein, Germany) according to the manufacturer's instructions. Cells were harvested 48 h after transfection, lysed in 250 µl 25 mM Tris-phosphate pH 7.8, 1 mM DTT, 1 mM EDTA, 15% glycerol, 10% Triton X-100 per sample and centrifuged for 30 s at 13,000 g. To determine β-galactosidase activity, 40 µl of lysate supernatant were added to 120 µl 33 mM Na_2HPO_4 pH 7.3, 1.3 mM $MgCl_2$, 133 mM β-mercaptoethanol and 40 µl 6 mg/ml PNPG in 0.1 M Na_2HPO_4 pH 7.3. Relative β-galactosidase activities were calculated from the rates of substrate turnover measured as change in absorbtion at 405 nm. For vigilin detection, lysates were electrophoresed on SDS-PAGE and immunoblotted as described below.

Heat shock experiments with HEp-2 cells

HEp-2 cells were grown as described above in 25-cm^2 cell culture flasks. One day after seeding, cells were exposed to a temperature of 42 °C for 1 h while control cells stayed at 37 °C. Cells were then allowed to grow for another 6 h at 37 °C. After this time, control as well as treated cells were harvested as described above. The relative amount of vigilin or hsp 90 was determined densitometrically by whole band scanning. All experiments were carried out at least three times in parallel.

Preparation of ribosomes from man, rat and chicken

Samples of rat or chicken liver were homogenized and fractionated as described by Adelman et al. [21] to isolate free polysomes and polysomes bound on membranes of the rough endoplasmic reticulum (rough microsomes).

Ribosomes from human placenta were prepared as described by Matasova et al. [22] and quantified by photometrical determination in A_{260} units/ml (sample purity was estimated by the ratio of A_{260} to A_{280}). For calculation of the ribosomal subunits, one A_{260} unit was assumed to correspond to 50 pmol of 40S or to 25 pmol of the 60S subunit.

Electrophoresis and immunoblotting

Supernatants from lysates of fibroblasts, COS7 cells or Hep-2 cells (derived from 1×10^6 cells) [23], as well as fractions of free or membrane bound ribosomes from rat and chicken tissues were analyzed on SDS-PAGE using 7% polyacrylamide gels. Gels were blotted on nitrocellulose membranes and vigilin was visualized by immunodetection with the vigilin-specific antiserum FP3 [23]. In heat shock experiments, hsp 90 was stained with a specific monoclonal antibody (StressGene, Victoria, Canada).

Immunostaining of HEp-2 cells

After growing on cover slips, cells were fixed and permeabilized (see above) and immunostained with different antibodies. As primary antibody, an anti-vigilin antiserum (FP-III, dilution 1:500 in PBS containing 0.1% bovine serum albumin), antibodies against the human ribosomal protein P (Euroimmun, Lübeck, Germany; 1:100 in PBS containing 0.1% bovine serum albumin) or anti-nucleoli antibodies (Leinco Technologies, St. Louis, Mo.; 1:200 in PBS containing 0.1% bovine serum albumin) from rabbits were applied for 1 h at 37 °C. Incubation with FITC-labeled anti-rabbit IgG or Cy3-labeled anti-rabbit IgG, further washes, embedding and fluorescence microscopy were then performed as above.

Gel retardation assay of proteins

Approximately 2–3 µg of 80S ribosomes was incubated with an equal amount of recombinant vigilin in 1 × shift-buffer (50 mM glycine pH 7.5, 25 mM NH_4Cl, 12.5 mM $MgCl_2$, 5 mM DTE, 0.25 mM EDTA) in a total volume of 20 µl for 15 min at 37 °C. As a control, ribosomes were incubated with bovine serum albumin and recombinant importin β, which was recombinantly expressed from the plasmid pQE60 [24] in the bacterial strain TG1 and purified as already described for vigilin.

The probes were then embedded into a native 0.8% low – melting – point agarose gel and separated in Tris-buffered saline (pH 8.4, 89 mM Tris, 10 mM H_3BO_3, 2 mM EDTA) overnight at 15 V. Subsequently, the gel was stained with Coomassie R 250 (0.1%, 10% acidic acid, 30% methanol) and destained with 10% acidic acid, 15% methanol.

Results

Vigilin binds to small de-novo-transcribed RNAs

The aim of this study was to demonstrate the association of vigilin with various RNA species. Initially, a representative set of small RNA molecules was transcribed in vitro, thereby radioactively labeled, and subsequently assayed for binding to recombinantly produced vigilin (fig. 1 A, B). As shown by the gel shift assay, all RNA species except for HAV Ia were retarded, though to varying extents (fig. 1 B). Apparently, RNA molecules with presum-

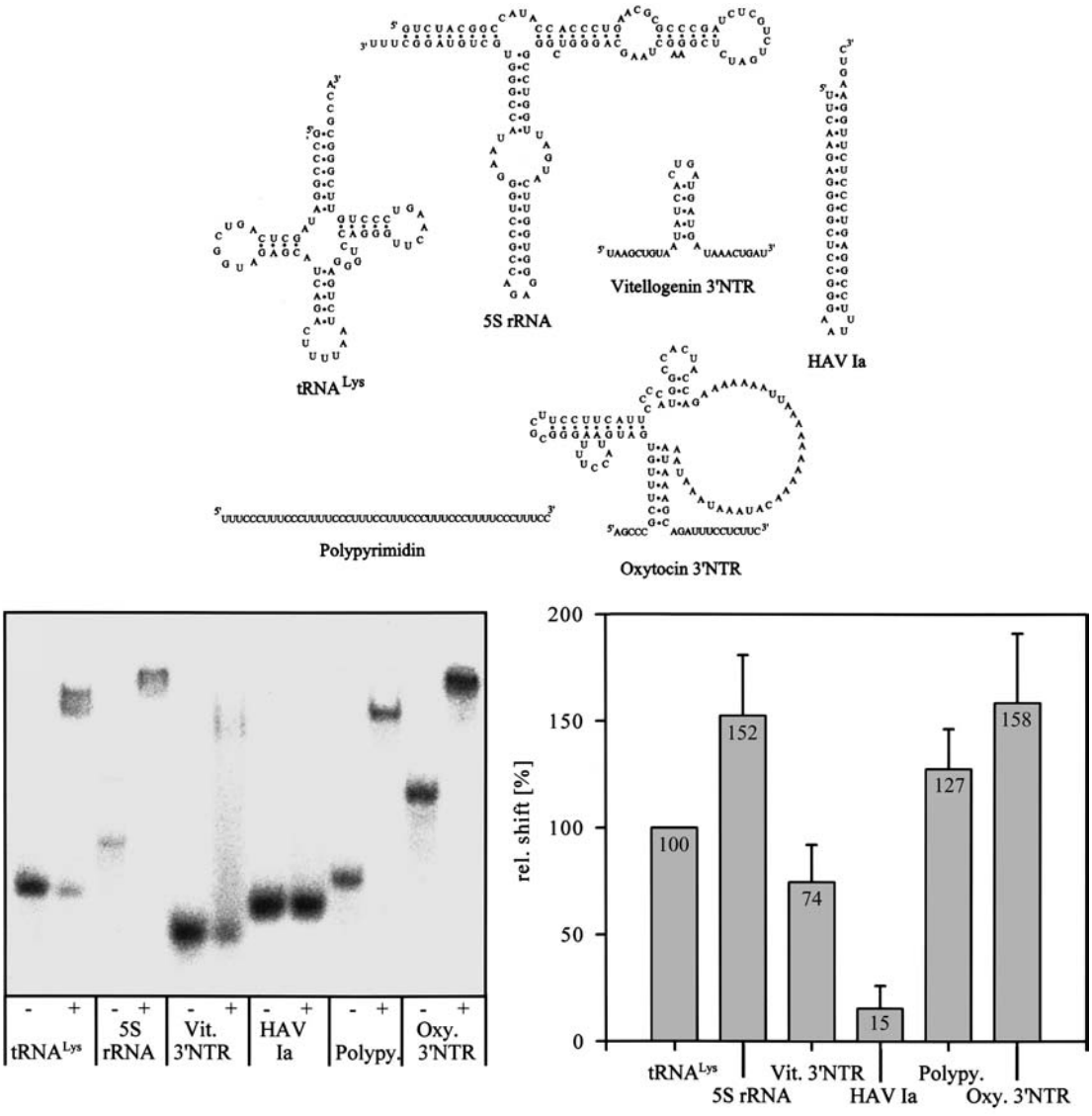

Figure 1. Gel retardation assay of different RNA species and recombinant vigilin. The published (5S rRNA: Sarge and Maxwell [46], HAV Ia: (Brown et al. [47]) or predicted secondary structure of tested RNAs according to Zuker et al. [48] and Mathews et al. [49] is shown (*A*). Comparative gel retardation experiments documenting the binding of different RNA species to recombinant vigilin (*B*). Equal amounts of ^{32}P-labeled RNAs were incubated with (+) or without (–) vigilin and the proportion of shifted activity was quantified relative to the shift of tRNA^{Lys} (set to 100%) (*C*). RNAs used were: tRNA^{Lys}, 5S rRNA, vitellogenin 3′NTR (Vit. 3′NTR), HAV Ia, polypyrimidine (Polypy.) and Oxytocin 3′NTR (Oxy. 3′NTR).

ably only small single-stranded nucleotide stretches showed poor binding to vigilin (HAV Ia), while complete lack of double strands did not preclude binding (polypyrimidine track). Whereas the functional association between vigilin and tRNA had already been shown previously [8, 24], this finding prompted us to investigate a possible functional link between vigilin and other eucaryotic RNA species (mRNA, rRNA) in the following sets of experiments.

Vigilin binds both to ribosomal RNA and ribosomes and colocalizes with ribosomes in the cytoplasm and nuclei

In the next set of experiments, free and membrane-bound ribosomes were prepared from rat and chicken tissues and shown by immunoblotting to contain vigilin in both preparations in about equal amounts (rat) or somewhat enriched in free ribosomes (chicken) (fig. 2A). Accordingly, 80S ribosomes (free ribosomes) incubated with vigilin clearly showed binding to vigilin in a gel retardation assay (fig. 2B). Ribosomal association and codistribution of vigilin with ribosomes was further corroborated by morphological studies. Specifically, vigilin was found

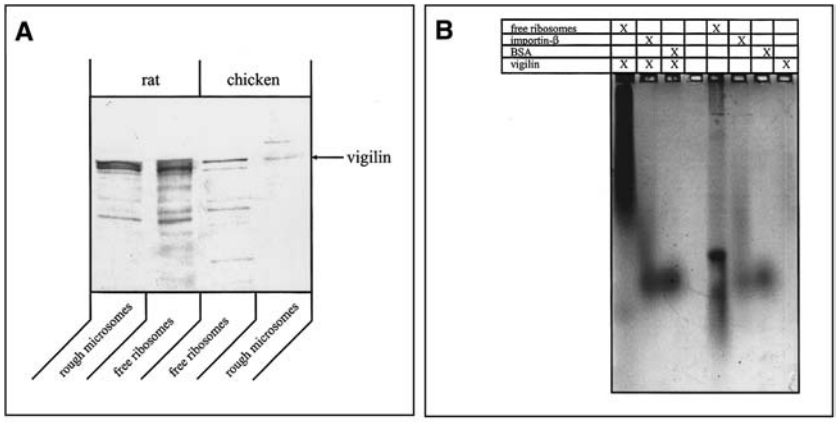

Figure 2. Binding of vigilin to completely assembled ribosomes. (*A*)Immunoblot of vigilin which shows copurification of vigilin with isolated free and membrane-bound ribosomes (rough microsomes) from rat in nearly equal amounts and chicken ribosomal fractions with higher concentrations of vigilin in fractions with free ribosomes. (*B*) Gel retardation assay in a nondenaturating gel system of 80S human ribosomes (free ribosomes) with vigilin. Importin-β and bovine serum albumine (BSA) function as negative controls. Incubation with vigilin resulted in a dramatic shift of ribosomes.

colocalized with the ribosomal protein P seen by coimmunostaining in HEp-2 carcinoma cells (fig. 3, A–C). Similar immunostaining techniques indicated that vigilin concentrates in nucleoli in addition to its occurrence in the cytoplasm (fig. 3, D–F).

Synthesis of vigilin is paralleled by ongoing translational activity of a large range of proteins, independently of whether their corresponding mRNAs are translated on free or membrane-bound ribosomes

Whereas the in vitro binding capacity of vigilin to RNA proved rather unspecific, its subcellular localization seemed to provide a first hint toward a possible vigilin function in vivo. Specifically, based on the localization of vigilin at free and membrane-bound ribosomes, one might expect that, in general, vigilin is involved in both types of translation process. Accordingly, one would assume that changes in vigilin expression also do not discriminate between translation of mRNAs coding for either cytoplasmic or secreted proteins. Vigilin expression would therefore correlate with changing levels of both types of protein.

Because of its unique posttranslational hydroxylation of prolyl residues, the production of collagen, a secreted protein of the extracellular matrix, can be easily monitored by metabolic labeling of primary human fibroblasts. De novo collagen synthesis in semiconfluent primary fibroblasts (day 2) is generally found at about 10% of total protein and decreases to trace amounts when fibroblasts are maintained for a prolonged time (day 5) (fig. 4). Concomitantly, vigilin expression was completely lost during the same time span. Similarly, a decrease of total protein synthesis was seen. Chosing a suitable cellular set up, heat shock experiments can be used to induce specific protein synthesis. Apparently, vigilin expression is responsive to an elevated culture temperature and follows a similar stimulatory path as hsp 90. Again, these experiments show that both a secreted protein (collagen) and a cytoplasmic protein (hsp 90) follow a correlative production pattern where stimulated protein synthesis is accompanied by elevated levels of vigilin concentration (fig. 5A). This correlation is more directly

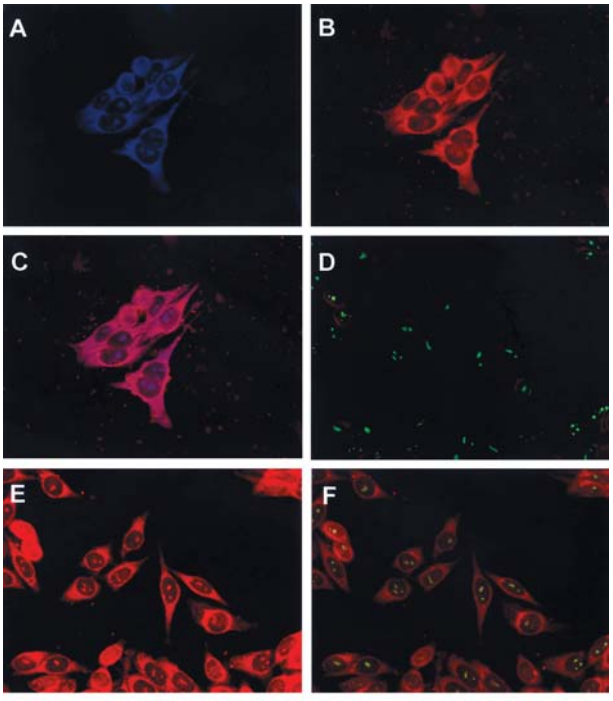

Figure 3. Immunostaining of HEp-2 cells. The ribosomal protein P (*A*), the nucleoli (*D*) and vigilin (*B, E*) were visualized by fluorescence labeled antibodies. Colocalization is shown in the merged pictures (*C, F*).

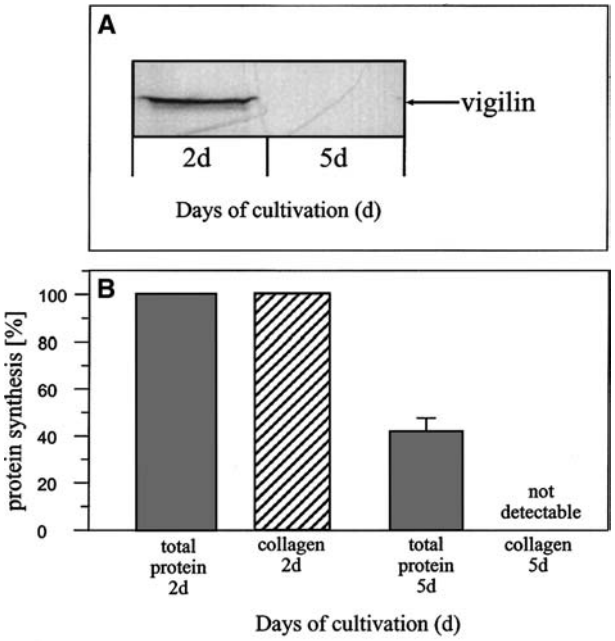

Figure 4. Coregulation of vigilin and the secretory protein collagen during cultivation. Primary human fibroblast were cultivated over a period of 5 days after which the vigilin synthesis ceased completely as shown by immunoblotting (*A*). Total protein synthesis was downregulated approximately 50%, whereas collagen was synthesis abolished (*B*).

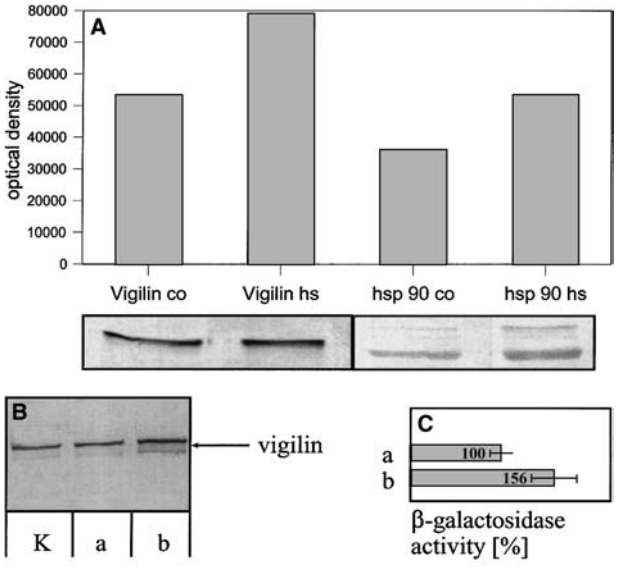

Figure 5. Connection of vigilin synthesis and expression of cytoplasmic proteins. Costimulation of the cytoplasmic protein hsp 90 and vigilin after heat shock treatment (hs) of HEp–2 cells. The detected proteins were quantified densitometrically. (co., untreated control) (*A*). cDNA plasmid cotransfection of vigilin with the cytoplasmic protein β-galactosidase into COS7 cells resulted in an increase in β-galactosidase activity in comparison to $_galactosidase cDNA cotransfected with a noncoding control vector as detected by measurement of β-galactosidase activity (*B*). Vigilin expression was monitored by immunoblotting (*C*). K, COS7 cells without transfection; a, COS7 cells cotransfected with noncoding vector and β-galactosidase encoding vector; b: COS7 cells cotransfected with vigilin-encoding vector and β-galactosidase-encoding vector.

demonstrated by cotransfection of COS7 cells with plasmids encoding vigilin and β-galactosidase: upon cotransfection with the vigilin-encoding plasmid, the activity of β-galactosidase was stimulated approximately 1.5-fold in comparison to a noncoding control vector (fig. 5B). Further parallels between stimulation of vigilin synthesis and stimulated expression of secretory or cytoplasmic proteins are listed in table 1.

Discussion

Ribonucleoprotein complexes (RNPs) are substrates for RNA export from the nucleus to the cytoplasm [25–28] through nuclear pore complexes, an energy-dependent process which is mediated by specific saturable factors [24, 28–32]. These observations have stimulated major efforts to identify and characterize RNA-binding proteins involved in RNA export. A number of RNA-binding motifs have been described in recent years, such as the RNP motif, the arginine-rich motif, the RGG box and the KH domain [1, 4]. There is circumstantial evidence that proteins of the KH domain protein family interact with and bind to RNA presumably through their evolutionarily conserved KH domain [1, 2, 4, 5]. Vigilin is an outstanding member of this family because of its high number (14/15) of consecutive KH domains and its increased expression in cells with ongoing translational activity [7, 9, 18, 33–35]. In recent studies, vigilin was described as being localized at ribosomes [36–38] and to bind both tRNA [8, 33] as well as mRNA [10]. Furthermore, an RNA-stabilizing effect was described [9, 18] and vigilin seems to be involved in RNA export [8, 25]. Whereas the role of vigilin in tRNA me-

Table 1. Experimental evidence for costimulation of vigilin and secretory or cytoplasmic protein.

tissues/ cells analysed	
Secretory proteins	
Primary human fibroblasts	fibronectin, collagen I
Chondrocytes	collagen II
Perspiration gland	glycoproteins
Sclerotic skin	proteins of the extracellular matrix, cytokines
Exocrine pancreas	digestive enzymes
Human plaque cells	apolipoprotein E
Xenopus liver	vitellogenin
Phytohemaglutenin-stimulated lymphocytes	membrane-bound proteins, cytokines
Carcinoma cell lines	membrane-bound proteins
Cytoplasmic proteins	
Human hair follicle	cytokeratins
Keratinocytes	cytokeratins
Transfected COS7 cells	β-galactosidase
HEp-2 cells after heat shock treatment	hsp 70, hsp 90

tabolism is sufficiently understood, its influence on mRNA metabolism has so far been described for vitellogenin mRNA only. Here we investigated, to our knowledge for the first time, if vigilin plays a role in mRNA function/metabolism and if it shows any distinctive binding to ribosomes.

For testing the in vitro RNA binding properties of recombinant vigilin, gel retardation assays were used. Several RNA species differing in structure and sequence – tRNA, 5S rRNA, mRNAs and a polypyrimidine RNA – were shifted by vigilin in the applied gel system (fig. 1). Only the double-stranded HAV Ia RNA was not bound by vigilin (fig. 1). Therefore, we assume that in vitro, vigilin binds different RNAs in a rather unspecific manner as long as they form complex loop structures or contain single-stranded regions and that in vivo binding specificity might require the presence of other proteins.

Grosshans et al. [39] were able to demonstrate that tRNAs which were not allowed to leave the nucleus enriched within the nucleoli. These data fit with our hypothesis of a connection between tRNA and ribosome export. If this is true, the described tRNP comprising vigilin [7] would have to show binding properties to ribosomes. Accordingly, we found vigilin to be enriched in fractions of both free and membrane-bound ribosomes from all species analyzed (dog, human, rat, chicken, trout) as displayed in figure 2A for rat and chicken. For native rebinding studies, ribosomes were isolated from human placenta and freed from membrane structures and mRNAs. In subsequent gel retardation assays, we were able to demonstrate that vigilin shifted these ribosomes but neither bovine serum albumin nor importin-β (fig. 2B). The ribosomal association of vigilin was supported by immunofluorescence studies of HEp-2 cells with antibodies against vigilin, ribosomal protein P and nucleoli. Here we found that vigilin shows an identical distribution pattern to ribosomes and that vigilin can accumulate within nucleoli (fig. 3).

These results are in line with investigations of Scp160p, the vigilin homologue in yeast, which was also found in association with ribosomes [38]. However, whether vigilin binds to ribosomes by protein-protein or protein-RNA interaction was not part of our investigation. Nevertheless, vigilin evidently binds to ribosomes in an mRNA-independent manner.

As a first approach to obtain data for binding of vigilin to mRNA in vivo, the correlation between vigilin and the two types of mRNA species, translated on free (for cellular proteins) or membrane-bound (for secretory proteins) ribosomes was tested. As a typical example of a secretory protein, we analyzed the extracellular matrix protein collagen. Figure 4 shows that in primary human fibroblasts, collagen synthesis was down-regulated during cultivation paralleled by a decrease in vigilin levels. In addition to collagen, the expression of several other secretory proteins has been shown to follow a similar pattern (table 1) [9, 18, 33, 34, 40–44].

Whereas this demonstrated a correlation between the expression of vigilin and secretory proteins, we then investigated a similar relationship between vigilin and intracellular proteins. Therefore, HEp-2 cells were incubated at 42 °C to induce the cytoplasmic heat shock proteins [45]. The synthesis of hsp 90 was stimulated under these conditions, accompanied by an increase in vigilin expression (fig. 5A). A second approach to test the influence of vigilin on cytoplasmic proteins was cotransfection of vigilin and the nonsecretory protein β-galactosidase into COS7 cells, which demonstrated a vigilin-dependent increase in β-galactosidase activity (fig. 5B).

Thus, by different experimental approaches, vigilin expression levels were found to be coregulated with both secretory and intracellular proteins (table 1), indicating that as one of the putative mRNA-related functions of vigilin, a possible mRNA binding would be independent of the type of mRNA as specified by its translation locality.

From the in vivo data discussed so far, the binding of vigilin to mRNA detected in vitro appears not to show a main physiological function of vigilin in itself. Rather, the mRNA binding is probably to be understood in the context of RNA transport to the ribosomes as part of an ongoing translational activity of cells.

In summary, we can conclude that in vitro, vigilin displays a capacity to bind different species of RNA with little discrimination of structure and sequence. In vivo, although vigilin is not associated with mRNA export from the nucleus, high vigilin levels are found in cells and tissues with increased translational activity of both secretory and cytoplasmic proteins. Additionally, vigilin is cosituated not only with active ribosomes in both modes of translation, transmembrane and cytoplasmic, but also with ribosomal precursors in the nucleoli. The already well-characterized role of vigilin in tRNA export and metabolism taken together with these findings suggest its additional influence on the coordinate export of newly synthesized ribosomes and tRNAs in cells with stimulated translational machinery. The final evidence for these function of vigilin in tuned RNA export and a possible connection to the cytoplasmic mRNA transport has yet to be provided and must be part of further investigations.

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