Evidence for a novel cytoplasmic tRNA–protein complex containing the KH-multidomain protein vigilin

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Vigilin, a protein found predominantly in cells and tissues with a high biosynthetic capacity, was isolated in its native form from human HEp-2 cells (A.T.C.C. CCL23) by immunoaffinity chromatography. Vigilin forms part of a novel ribonucleoprotein complex that also contains additional, as yet uncharacterized, proteins. Experimental evidence suggests that the nucleic acids entrapped in this complex are protected from RNase and belong to the tRNA family. Using either a pool of total human RNA or radioactively labelled tRNA (tRNA^{Asp*}) in rebinding experiments, we could show that tRNA is selectively recaptured by the RNA-depleted vigilin-containing complex.

INTRODUCTION

Vigilin is a multidomain protein that has been reported to be present in chicken and human, and is homologous to the HX protein from *Saccharomyces cerevisiae* [1–3]. In the core of the 155 kDa protein from either chicken or human, the so-called KH- or vigilin domain occurs 14 times in series, with each one tandemly arranged with an adjacent stretch of 21–33 amino acids of presumptive α-helical structure. Comprehensive sequence analysis has shown that a large variety of proteins apparently contain the characteristic vigilin or KH-domain in monomeric or multimeric form [4–6]. There is circumstantial evidence that these proteins interact with and bind to RNA, presumably through the KH-domain [4,6,7].

Using immunocytochemistry and immunohistochemistry with an antiserum directed against a vigilin–β-galactosidase fusion protein, vigilin was localized within the cytoplasm and its expression pattern was analysed in a variety of organisms and tissues [8,9]. Furthermore, in accordance with the molecular mass derived from the complete cDNA, a protein band of approx. 155 kDa was found by immunoblotting of tissue extracts [8]. These studies also showed that human vigilin was constitutively expressed and synthesized in moderate amounts by the established cell line HEp-2 (A.T.C.C. CCL23). In the present study we describe the isolation of native human vigilin and provide the first evidence that vigilin forms a composite structure in cells with other proteins and tRNA.

EXPERIMENTAL

Preparation of tissue extracts

Tissue samples were homogenized using a glass homogenizer on ice and centrifuged at 15000 ***g*** for 15 min at 4 °C. Each of the supernatants was electrophoresed, blotted and stained for vigilin with anti-vigilin antibodies.

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 in Dulbecco's modified Eagle's minimum essential medium (Biochrom, Berlin, Germany) supplemented with 10 % (v/v) fetal calf serum (Biochrom), 100 units/ml penicillin, 100 μg/ml streptomycin, 4.5 mg/ml D-glucose and 2 mM glutamine. For SDS/PAGE analysis, 10^6 cells were lysed for at least 1 h in 50 μl of a lysis buffer consisting of 5.63 mM Na_2HPO_4, 100 μM PMSF and 0.5 % Nonidet P-40. The cell extract was centrifuged (15000 ***g***, 10 min) and the supernatant was immediately used or stored at −70 °C [8].

Electrophoresis and immunoblotting

The supernatant from lysed and centrifuged cells (1×10^6) was treated for 10 min at 95 °C in Laemmli sample buffer in the presence of 0.1 mM β-mercaptoethanol and used as a control for PAGE and immunoblotting of vigilin. Individual fractions were analysed on SDS/7 %-PAGE [10] and stained with Coomassie Blue G250 or analysed by immunoblotting [8]. Immunoblots from agarose gels were prepared essentially by the same techniques as used for polyacrylamide gels.

Affinity chromatography

Affinity-purified anti-vigilin antibodies were coupled to an affinity membrane chromatography cartridge (Millipore, Eschborn, Germany). The ConSep LC 100 elution and monitoring system (Millipore) was used for affinity chromatography. The cartridge was operated with a pressure of $(4.83–5.52) \times 10^5$ Pa.

The cartridge was loaded with the supernatant from lysed HEp-2 cells (1×10^7) and washed with water until the absorbance (280 nm) reached baseline values. The cartridge was subsequently developed with gradients as follows: (1) with 0–1 M glycine (pH 1.5) to elute a complex containing vigilin, additional proteins and nucleic acids (pool 1); (2) alternatively, with a NaCl gradient from 0 to 0.8 M to wash off weakly bound proteins from the cartridge, so that the vigilin core complex (VCC; containing vigilin and core proteins) only was left on the cartridge; (3) with a 0.8–1 M NaCl gradient to elute nucleic acids bound specifically to the VCC.

For RNA rebinding experiments, a matrix-bound VCC was prepared as follows: (1) native vigilin from a HEp-2 cell extract was loaded on to the cartridge, (2) the VCC was completely depleted of innate RNA with 0–1 M NaCl, and (3) prior to reloading with the pool of total RNA or radioactively labelled

Abbreviations used: VCC, vigilin core complex; GuSCN, guanidinium isothiocyanate; tRNA^{Asp*}, a radioactively labelled tRNA.
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tRNA, the cartridge was washed with water to desalt it, and subsequently equilibrated.

Treatment of chromatographic fractions

Fractions collected from the affinity cartridge were dialysed, lyophilized, dissolved in TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) and divided into aliquots which were processed as follows: without further treatment (control); with 0.5 % SDS; with 0.5 μg/μl DNase-free RNase A (EC 3.1.27.5) and RNase T1 (EC 3.1.27.3) for 30 min at 37 °C plus 0.5 % SDS; with 0.5 μg/μl DNase I (EC 3.1.21.1) for 30 min at 37 °C plus 0.5 % SDS; with RNase A plus 0.5 % SDS; with DNase I plus 0.5 % SDS; with 0.5 μg/μl proteinase K (EC 3.4.21.64) for 30 min at 37 °C plus 0.5 % SDS. The nucleic acids of pool 1 were extracted from the complex by an acid guanidinium isothiocyanate (GuSCN)/phenol/chloroform treatment [11].

Characterization of vigilin-containing fractions

To characterize the fractions collected from the anti-vigilin antibody cartridge, different gel electrophoresis procedures were applied to identify proteins and nucleic acids: (1) TAE (40 mM Tris/acetate, 2 mM EDTA, pH 8.5) agarose gel electrophoresis (0.8 %), which is suitable for the analysis of nucleic acids and protein–nucleic acid complexes in a non-denaturing gel matrix separation system; (2) SDS/PAGE with 7 % polyacrylamide for protein analysis, and (3) PAGE with 12 % polyacrylamide and 8 M urea to identify the bound RNA species.

Detection of the separated components was achieved as follows: (1) ethidium bromide staining of agarose gels to identify nucleic acids, (2) Ponceau S staining of proteins after blotting from agarose gels (a procedure suitable for the detection of proteins in a protein–RNA complex) or from polyacrylamide gels on to nitrocellulose membranes, (3) immunostaining of nitrocellulose membranes with anti-vigilin antibodies, and (4) silver staining of urea/polyacrylamide gels. Staining of the blotting membranes was performed in such a manner as to demonstrate coincidence of protein bands and immunoreactive bands. The amounts of RNA and protein were evaluated densitometrically by whole-band scanning (Optoquant; Computer & Vision, Lübeck, Germany) by using purified tRNA from yeast and BSA as reference standards.

RNA binding studies

Human total RNA was prepared from MG63 cells by GuSCN lysis and CsCl gradient centrifugation [12].

Yeast $tRNA^{Asp*}$ (20 pmol), functionally tested by aminoacylation [13], was dephosphorylated with bacterial alkaline phosphatase and radioactively labelled with 0.37 MBq of [γ-^{32}P]ATP (1.11×10^5 GBq/mmol) by T4 polynucleotide kinase. Radioactively labelled $tRNA^{Asp*}$ was separated from non-incorporated nucleotides by 12 %-PAGE/8 M urea. After autoradiography, the band corresponding to undegraded tRNA was cut out of the gel and eluted from the gel slice with 400 μl of 0.5 M ammonium acetate, 0.1 % SDS and 1 mM EDTA. After ethanol precipitation the $tRNA^{Asp*}$ was dissolved in 100 μl of diethyl pyrocarbonate-treated water to a final concentration of 2×10^6 c.p.m./100 μl.

The VCC bound to a solid matrix (see above) was depleted of innate RNA and used for rebinding studies. Human total RNA (50 μg) containing low amounts of tRNA, or radioactively labelled $tRNA^{Asp*}$ (5×10^5 c.p.m.), was loaded on to the cartridge, which was then developed again with a 0–0.8 M NaCl gradient followed by a glycine gradient (0–1 M). As a carrier RNA for the radioactively labelled $tRNA^{Asp*}$, 10 μg of unlabelled vigilin cRNA (a 0.65 kb sense *in vitro* transcript from a vigilin cDNA clone, Vig-g-8.1 [14]) was used. Proteins and RNA were eluted in a single peak which was collected and analysed by agarose (0.8 %)-gel electrophoresis, followed by blotting and staining with both Ponceau S and anti-vigilin antibodies and visualization of radioactive samples by autoradiography. Prior to loading on to the gel, relevant fractions were dialysed, lyophilized and dissolved in TE buffer.

The entire pool 1, tRNA from yeast and radioactively labelled $tRNA^{Asp*}$ were used as markers for gel electrophoresis.

RESULTS

Vigilin is present in tissues showing high protein synthesis

We have demonstrated specific staining by anti-vigilin antibodies in all tissues studied, mainly in cells known to produce and secrete at times substantial quantities of proteins, e.g. liver parenchymal cells and pancreatic secretory cells from various species, the placenta and the secretory epithelial layer of the uterus (Figure 1).

The native vigilin complex contains RNA

When denaturing conditions for the isolation of vigilin from HEp-2 cell lysates were applied, it was possible to purify vigilin to homogeneity. Whenever vigilin was isolated under native conditions, by making use of different physicochemical properties of the protein (gel-filtration chromatography, ion-exchange chromatography, native preparative electrophoresis, etc.), a similar set of proteins/nucleic acids was co-purified, indicating that vigilin may maintain specific interactions with other macromolecules as part of a cytoplasmic protein complex.

In an approach to isolate and characterize this putative multiprotein complex, an immunoaffinity cartridge loaded with monospecific anti-vigilin antibodies was used for purification. Since the monospecific polyclonal antibodies attached to the cartridge were shown to bind vigilin alone by immunoblotting of

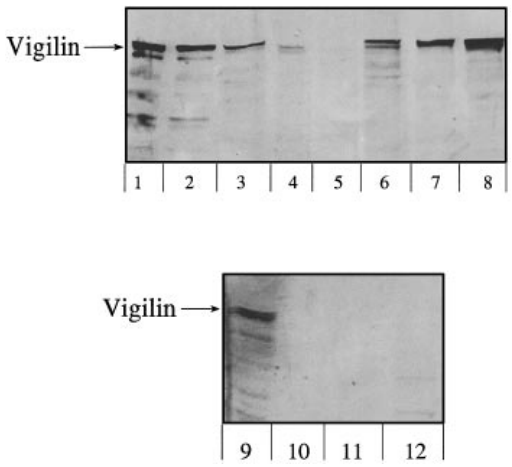

Figure 1 Detection of vigilin in immunoblots of different tissues

Supernatants of a variety of homogenized and centrifuged tissue samples with a high or low level of protein synthesis were electrophoresed and blotted. Blots were stained with anti-vigilin antibodies. The following tissues were used: lane 1, pancreas of rainbow trout; lane 2, liver of rainbow trout; lane 3, human placenta; lane 4, secretory epithelia of rat uterus; lane 5, human muscle; lanes 6 and 9, HEp-2 cells; lane 7, rat liver; lane 8, canine pancreas; lane 10, human skin; lane 11, uterus from ovariectomized rat; lane 12, pancreas from starved rat

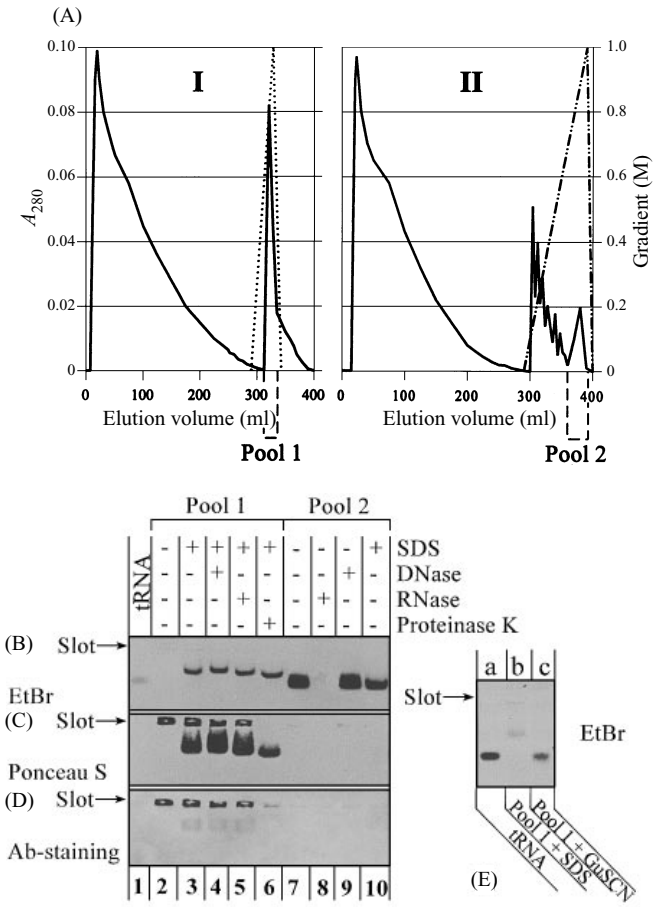

Figure 2 The native vigilin complex contains RNA

Elution profile of HEp-2 lysates eluted from an anti-vigilin antibody affinity cartridge by a glycine gradient (**A**, panel I). The vigilin-containing fraction (pool 1) was analysed for the presence of nucleic acids on an ethidium bromide-stained agarose gel (**B**). In a second experiment, nucleic acids were separated from proteins bound to the affinity cartridge using a NaCl gradient (0–1 M, pool 2). Vigilin is present in the fraction eluting between 0.8 and 1 M NaCl. Aliquots of the pooled fractions (pools 1 and 2) were analysed by agarose-gel electrophoresis and subsequent ethidium bromide staining (EtBr, **B**). The same gel was stained with Ponceau S (**C**) and immunostained (Ab-staining, **D**). Staining of bands coincided for all staining procedures used. Prior to electrophoresis the fractions were treated with various enzymes and reagents, and the amounts of RNA and protein were estimated densitometrically. Lane 1, tRNA from yeast (marker; 0.2 μg of RNA); lane 2, untreated fraction (not measurable); lane 3, with 0.5% SDS (0.71 μg of RNA/2.29 μg of protein); lane 4, with DNase and 0.5% SDS (0.58 μg of RNA/2.57 μg of protein); lane 5, with RNase and 0.5% SDS (0.66 μg of RNA/2.39 μg of protein); lane 6, with proteinase K and 0.5% SDS (0.65 μg of RNA/1.43 μg of protein); lane 7, untreated fraction (1.3 μg of RNA); lane 8, with RNase (no RNA); lane 9, with DNase (1.46 μg of RNA); lane 10, with 0.5% SDS (1.1 μg of RNA). (**E**) Deproteination of RNA by treatment with GuSCN. Lane a, tRNA from yeast (marker; 0.4 μg of RNA); lane b, vigilin-containing glycine eluent treated with 0.5% SDS, as for sample shown in lane 3 of (**B**) (0.26 μg of RNA); lane c, RNA shown in lane b following extraction with GuSCN (0.41 of μg RNA). Solid line, absorbance at 280 nm; dotted line, glycine gradient; dotted/dashed line, NaCl gradient.

cell lysates (Figure 1), retention of any macromolecules other than vigilin by the cartridge must be due to their direct or indirect interaction with vigilin, thus identifying these molecules as constituents of a vigilin-containing complex. Figure 2(A) shows a characteristic elution profile where vigilin immunoreactive bands, along with a pool (pool 1) of other proteins (results not shown), were eluted by a glycine gradient (Figure 2A, left panel). In order to analyse the fractions eluted with glycine for the presence of nucleic acids, we treated aliquots of the eluate with nucleic acid-degrading enzymes and subsequently performed an electrophoretic separation of the samples by agarose-gel electrophoresis and staining with ethidium bromide (Figure 2B). In addition, the same agarose gel was blotted on to a nitrocellulose membrane and stained with Ponceau S (Figure 2C) and with anti-vigilin antibodies (Figure 2D). Without SDS, the eluted material remained in the sample slot and was not stained with ethidium bromide (Figure 2B, lane 2). However, the eluted material was stained after blotting with both Ponceau S (Figure 2C, lane 2) and with antibodies against vigilin (Figure 2D, lane 2). Following incubation with SDS (0.5%), a single ethidium bromide-stainable band migrated into the agarose gel (Figure 2B, lanes 3–5), indicating that the isolated vigilin complex contains a nucleic acid. The nucleic acid is well protected against nuclease treatment when bound to the complex, since it was not susceptible to either RNase (Figure 2B, lane 4) or DNase (Figure 2B, lane 5). The proteins of the complex, when mobilized with SDS, migrated to the same point in this gel system as the nucleic acid (Figure 2C, lanes 3–6), as demonstrated by Ponceau S staining after blotting. Antibody staining revealed that vigilin was also present in this nucleoprotein complex (Figure 2D, lanes 3–5). The complex is partially resistant to proteinase K (Figure 2C, lane 6). Vigilin, however, is apparently one of the proteinase-sensitive proteins of the complex, since it was no longer detected after proteinase K treatment (Figure 2D, lane 6), although it is open to debate whether vigilin was completely digested or only its immunoreactive epitopes. Furthermore, the migration of the nucleic acid was slightly affected by proteinase K treatment, as shown by a faster-migrating and denser band (Figure 2B, lane 6).

In another set of experiments, a HEp-2 lysate was affinity purified on the anti-vigilin antibody cartridge and elution was achieved by a 0–1 M NaCl gradient in an attempt to dissociate the protein–nucleic acid interaction (Figure 2A, right panel). Nucleic acid-containing material was found only in the molar range of 0.8–1 M NaCl (pool 2), and the recovered material migrated as a single nucleic acid band both without or with SDS treatment (Figure 2B, lane 7 and 10). This band, now unprotected by complexing proteins, was susceptible to RNase (Figure 2B, lane 8) but not to DNase (Figure 2B, lane 9) treatment. Thus the nucleic acid of the complex was shown to be RNA and not DNA. Neither protein staining nor immunostaining with anti-vigilin antibodies was seen (Figures 2C and 2D, lanes 7–10). The migration distance of this RNA was similar to that of yeast tRNA (Figure 2B, lane 1).

Liberation of RNA from the vigilin complex

When the vigilin complex bound to the affinity cartridge was first developed with a 0–1 M NaCl gradient (Figures 3A and 3B, lanes 1–5), unprotected and digestable RNA was released, while no vigilin-specific band could be recognized by immunoblotting. This demonstrates the firm attachment of the protein to the antibody cartridge up to 1 M NaCl. The band at 60 kDa is the result of cross-reactivity of the unpurified anti-vigilin antiserum with the heat-shock protein HSP60 [8]. Intact vigilin was found only in fractions obtained with the subsequent 0–1 M glycine gradient (Figure 3B, lane 6). Nucleic acids, however, were identified in fractions between 0.8 and 1 M NaCl (Figure 3C, lane 5) and could be completely digested by RNase treatment (Figure 3C, lane 5 for fraction 5). No additional quantities of nucleic acids were found in the glycine eluate (Figure 3C, lane 6). Neither vigilin nor RNA was eluted in the washing fractions before the NaCl gradient was started (Figures 3B and 3C, lanes 1). The vigilin composite, eluted from the affinity cartridge by glycine (Figure 2A, left panel, and Figure 2E), was treated with

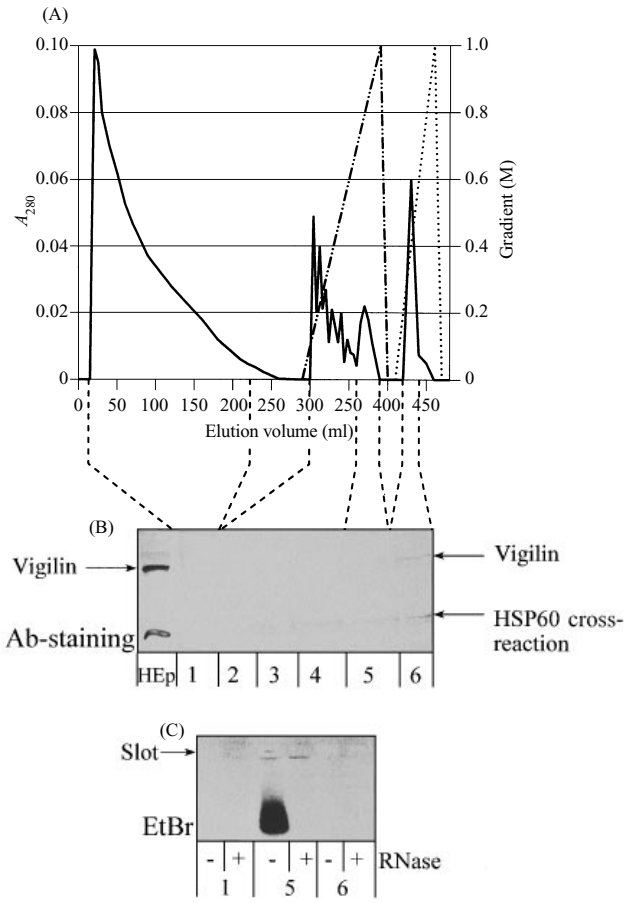

Figure 3 Liberation of vigilin-bound RNA

(**A**) Affinity chromatography of HEp-2-lysates on a cartridge with purified anti-vigilin antibodies. After binding of the vigilin complex to the cartridge-bound antibodies, the cartridge was developed first with a NaCl gradient (0–1 M) and then with a glycine gradient (0–1 M). Solid line, absorbance at 280 nm; dotted line, glycine gradient; dotted/dashed line, NaCl gradient. (**B**) Fractions 1–6 were electrophoretically separated by SDS/PAGE (7% polyacrylamide), blotted and immunostained (Ab-staining). Vigilin was found only within the glycine gradient (1 M glycine, pH 1.5, 0–100%). HEp, HEp-2 lysate. (**C**) Agarose-gel electrophoresis (1% agarose) of nucleic acids in fractions stained with ethidium bromide: —, untreated fractions; +, after RNase treatment.

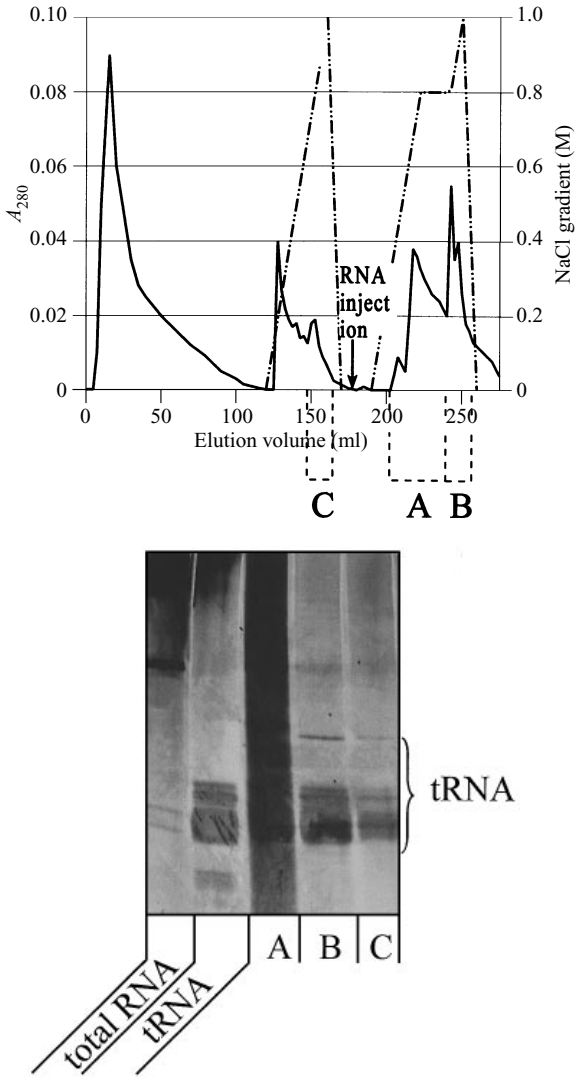

Figure 4 Characterization of RNA rebound to vigilin

Elution profile of HEp-2 lysates upon affinity chromatography. After the affinity cartridge (loaded with the native vigilin–RNA complex) had been developed with a NaCl gradient to liberate RNA (fraction C), re-equilibration to 0 M NaCl was achieved with a water wash. Subsequently an RNA mixture (human total RNA) was loaded on to the cartridge, which was then developed further with a linear NaCl gradient, up to a plateau level of 0.8 M NaCl (this is the reloaded column referred to in the legend to Figure 5). The 0–0.8 M (fraction A) and the 0.8–1.0 M (fraction B) NaCl fractions of the gradients were collected, treated with phenol and analysed by PAGE (12% polyacrylamide, 8 M urea) and silver staining. The lane labelled 'total RNA' contains human total RNA, of which tRNA constitutes a small part; the lane labelled 'tRNA' contains yeast tRNA used as a molecular mass marker. Lane A, phenol-treated NaCl wash obtained from the reloaded cartridge following a low-concentration NaCl gradient (0–0.8 M); lane B, phenol-treated NaCl fraction obtained from the reloaded cartridge by a high-salt gradient (0.8–1 M NaCl); lane C, phenol-treated fraction C (a fraction equivalent to that shown in Figure 2, lane 3). Solid line, absorbance at 280 nm; dotted/dashed line, NaCl gradient.

the strong chaotropic reagent GuSCN and by subsequent extraction with acidic phenol, and was shown to migrate to the same position in the gel as naked tRNA (Figure 2E, lane c). These observations are in agreement with the notion that the bound nucleic acid may represent tRNA, which is shifted in the gel to a higher apparent molecular mass as long as it is part of the native complex. The shift shown for the nucleic acid of pool 1 in the presence of SDS (Figure 2B, lanes 3–10) is similar to that obtained following treatment of pool 1 with GuSCN (Figure 2E). In summary, these experiments show that it is possible to liberate RNA from a vigilin-containing complex by using high ionic strength (1 M NaCl) or with a strong denaturing reagent (GuSCN).

Binding of RNA to the vigilin complex

To investigate whether or not the RNA-depleted yet antibody-bound VCC is able to reassociate with RNA after re-equilibration of the vigilin cartridge, human total RNA was loaded on to the affinity cartridge, rinsed with the loading buffer and finally developed again with a NaCl gradient. Both the originally bound (Figure 4, lane C) and the rebound (Figure 4, lane B) material showed a similar banding pattern as tRNA from yeast (Figure 4, lane tRNA). All of the non-specifically bound RNA from total RNA was eluted as a smear from the cartridge with 0–0.8 M NaCl (Figure 4, lane A). To rule out non-specific background binding to the affinity matrix, human total RNA was applied to the cartridge containing the anti-vigilin antibody only. In this

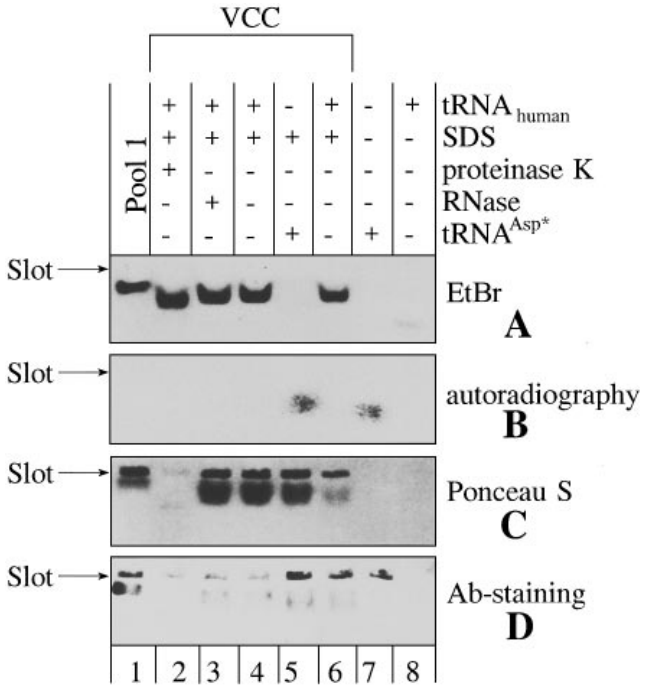

Figure 5 RNA rebinding studies

Reloading of the RNA-depleted VCC with total human RNA was performed as described in the legend to Figure 4. The recharged VCC with the enclosed tRNA, however, was then developed with a glycine gradient (0–1 M; **A–C**, lanes 2–4). In a further experiment, radioactively labelled and functionally tested yeast $tRNA^{Asp*}$ was mixed with vigilin sense cRNA and reloaded on to the RNA-depleted VCC. In these instances vigilin–tRNA complexes were eluted from the affinity cartridge as: (1) a VCC with human tRNA (VCC + $tRNA_{human}$; lanes 2–4) and (2) a VCC with $tRNA^{Asp*}$ (VCC + $tRNA^{Asp*}$; lane 5). Aliquots of the pooled fractions were analysed by agarose-gel electrophoresis and subsequent ethidium bromide staining (EtBr; **A**). The blot of this agarose gel was visualized by autoradigraphy (**B**), Ponceau S staining (**C**) and immunoblotting (Ab-staining; **D**), and the amounts of RNA and protein were estimated densitometrically. Staining of bands coincided for all staining procedures used. The fractions were pretreated with the following enzymes and reagents: lane 1, pool 1 (see Figure 2; 0.55 μg of RNA/1.308 μg of protein); lane 2, VCC + $tRNA_{human}$ with proteinase K and 0.5% SDS (0.8 μg of RNA/0.2 μg of protein); lane 3, VCC + $tRNA_{human}$ with RNase and 0.5% SDS (0.73 μg of RNA/2.61 μg of protein); lane 4, VCC + $tRNA_{human}$ with 0.5% SDS (0.66 μg of RNA/2.27 μg of protein); lane 5, VCC + $tRNA^{Asp*}$ with 0.5% SDS (2.14 μg of protein); lane 6, VCC + $tRNA_{human}$ with 0.5% SDS (0.64 μg of RNA/0.947 μg of protein); lane 7, $tRNA^{Asp*}$ (marker); lane 8, yeast tRNA (marker; 0.15 μg of RNA).

instance, no affinity binding could be observed at high salt concentration (0.8 M NaCl). The applied nucleic acids were completely released from the cartridge–antibody backbone at lower NaCl concentrations (results not shown).

tRNA rebinding studies

Non-specifically bound RNA was washed off the cartridge with a NaCl gradient (0–0.8 M). When, subsequently, the VCC was eluted (1 M glycine) along with rebound RNA, the digestion/protection profile (resistance to RNase and proteinase K; Figures 5A, 5C and 5D, lanes 2–4) was similar to that observed for the native vigilin- and cognate-RNA-containing fraction (Figure 2). After treatment with proteinase K there was a shift in migration, although the protein co-migrating with the ethidium bromide-stained band was hardly visible (Figures 5A, 5C and 5D, lane 2). The reconstituted RNA–protein composite (Figure 5, VCC + rebound RNA, lanes 2–4) migrated in an agarose gel between the tRNA contained in the native complex (pool 1; Figure 2 and Figure 5, lane 1) and naked tRNA (Figure 5, lanes 7 and 8). The ratio of RNA-to-protein mass in the native pool 1 was close to 1:3. The proportion of RNA increased following treatment with proteinase K and decreased after digestion with RNase (Figure 2). Accordingly in the VCC containing rebound tRNA the ratio of RNA-to-protein mass was approx. 1:2.5, and it changed in the same direction as did the pool 1 material after treatment with either proteinase K or RNase (Figure 5).

Furthermore, radioactively labelled yeast $tRNA^{Asp*}$ was also recaptured by the VCC, in full agreement with the rebinding experiments using human total RNA. Approx. 70% of the total radioactivity loaded was recovered as part of the VCC (Figure 5B, lane 5). The protein–$tRNA^{Asp*}$ composite (VCC + RNA, Figure 5A, lane 5) migrated to a spot similar in position to that shown in Figure 5A, lanes 2–4.

DISCUSSION

Although vigilin is a ubiquitous protein, it should be emphasized that highest expression is observed in those cells that are known to produce at times large quantities of protein [15,16], such as liver parenchymal cells and pancreatic secretory cells (Figure 1). Because of the increase in vigilin production in cells and tissues with stimulated translational activity, and the fact that anti-vigilin antibodies can inhibit the synthesis of proteins during *in vitro* translation [16], it can be assumed that vigilin may have an as yet unknown function in protein translation.

In addition to cells and tissues with a high translational activity, vigilin is constitutively synthesized in transformed cell lines [8] such as HEp-2 cells (A.T.C.C. CCL23), which are thus suitable for the experimental isolation of vigilin in its native state from cell lysates. Using an anti-vigilin antibody cartridge, we succeeded in isolating a vigilin-containing composite that consist, besides vigilin, of a distinct set of additional proteins (results not shown) and RNA (pool 1, Figure 2). When the vigilin composite was desorbed from the column by a glycine gradient, the cognate nucleic acid was susceptible to neither DNase nor RNase. In contrast, when the anti-vigilin affinity cartridge was developed by a 0.8–1 M NaCl gradient, a nucleic acid-containing fraction was obtained which was completely digestable by RNase but not by DNase (Figure 2B, lanes 8 and 9), apparently because the protective protein cover had remained on the cartridge. As a consequence, the gel shift seen for the intact composite after elution with 1 M glycine (Figure 2B, lanes 3–10) did not occur, and the nucleic acids devoid of proteins migrated further into the gel. The same nucleic acid migration pattern was observed when the intact protein–nucleic acid complex was dissociated with GuSCN (Figure 2E, lanes b and c). It is also worth mentioning that treatment of the vigilin–nucleic acid complex with proteinase K destroyed the immunoreactivity with anti-vigilin antibodies, although protein staining was still visible with Ponceau S (Figure 2C, lane 6). A similar pattern of resistance against proteinase K has been observed in experiments designed to liberate peptidyl transferase from a protein–nucleic acid complex [17].

Based on the observation that the affinity cartridge can be depleted of any cognate nucleic acid by a 0–1 M NaCl gradient, we set up experiments to study the rebinding of nucleic acid on to the cartridge-bound vigilin composite. Following re-equilibration, the vigilin composite was charged with a preparation of human total RNA and purged with a 0–0.8 M NaCl gradient to remove non-specifically bound RNA species. Subsequently, the vigilin composite desorbed by 1 M glycine showed all the properties of the native intact vigilin–RNA complex; i.e. (1) the complex was resistant to RNase treatment and (2) the gel shift of the entrapped RNA was similar to that of the native complex.

The resistance to RNase treatment is explained by protection of the rebound RNA by the VCC. One can hypothesize that the physiological function of the complex may be to confer protection against cellular RNase activity to the bound tRNA. These observations imply that the VCC exhibits all the properties required for tRNA binding. In line with the above experiment, and as further proof for tRNA binding, we succeeded in demonstrating rebinding of functionally tested $tRNA^{Asp*}$ to the VCC. At present, however, it is not clear if the structure and/or the composition of the vigilin complex differ depending on the tRNA bound.

In summary, we provide experimental evidence that vigilin, in association with other proteins, binds to and protects tRNA. This is supported by the fact that a depleted vigilin complex can regain RNA from human total RNA and by the specific binding of yeast $tRNA^{Asp*}$ to this complex. Therefore we assume that vigilin is part of a tRNA-containing cytoplasmic ribonucleoprotein complex, as other KH-domain proteins are known to form part of a variety of ribonucleoprotein complexes [18,19]. Future experiments will address whether vigilin alone or, more likely, in concert with other proteins can bind and protect tRNA, and which structural features (if any) of the vigilin domain(s) are involved in tRNA binding. Furthermore, studies on the potential role of vigilin in presenting tRNAs to the translation machinery are vital.

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