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Recognition and Binding of the Human Selenocysteine Insertion Sequence by Nucleolin

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Abstract Prokaryotic and eukaryotic cells cotranslationally incorporate the unusual amino acid selenocysteine at a UGA codon, which conventionally serves as a termination signal. Translation of selenoprotein gene transcripts in eukaryotes depends upon a "selenocysteine insertion sequence" in the 3′-untranslated region. We have previously shown that DNA-binding protein B specifically binds this sequence element. We now report the identification of nucleolin as a partner in the selenoprotein translation complex. In RNA electromobility shift assays, nucleolin binds the selenocysteine insertion sequence from the human cellular glutathione peroxidase gene, competes with binding activity from COS cells, and shows diminished affinity for probes with mutations in functionally important, conserved sequence elements. Antibody to nucleolin interferes with the gel shift activity of COS cell extract. Antibody to DNA-binding protein B co-extracts nucleolin from HeLa cell cytosol, and the two proteins co-sediment in glycerol gradient fractions of ribosomal high salt extracts. Thus, nucleolin appears to join DNA-binding protein B and possibly other partners to form a large complex that links the selenocysteine insertion sequence in the 3′-untranslated region to other elements in the coding region and ribosome to translate the UGA "stop" codon as selenocysteine. J. Cell. Biochem. 77:507–516, 2000.

Key words: DNA-binding protein B; glutathione peroxidase; RNA-binding protein; selenium; selenoprotein; translation

Selenoproteins constitute a unique group of eukaryotic and prokaryotic polypeptides, all incorporating selenium as selenocysteine [Stadtman, 1996; Burk and Hill, 1993; Sunde, 1990]. Selenium closely resembles sulfur, a congener located immediately above it in the periodic table, so selenocysteine functions identically to cysteine except that it is more reactive towards nucleophilic substrates [Hu and Tappel, 1987]. Prokaryotic selenoproteins include formate dehydrogenases from obligate and facultative anaerobes; selenophosphate synthetases from *Escherichia coli* and *Haemophilus influenzae*; a hydrogenase from *Methanococcus vannielii*; and clostridial glycine reductase, nicotinic acid hydroxylase, and xanthine dehydrogenase [Stadtman, 1990; Wilting et al., 1997; Wilting et al., 1998]. Mammalian selenoproteins include the glutathione peroxidase family [Chambers et al., 1986; Schuckelt et al., 1991; Esworthy et al., 1991; Chu et al. 1993], the iodothyronine 5′-deiodinase family [Berry et al., 1991b; Larsen and Berry, 1995], selenoprotein P [Hill and Burk, 1997], selenoprotein W [Vendeland et al., 1995], and thioredoxin reductase [Gladyshev et al., 1996].

All selenoproteins incorporate selenocysteine cotranslationally at a UGA codon [Böck et al., 1991; Sunde, 1990], formerly known only as a termination codon, and utilize a unique selenocysteine-charged tRNA, termed $tRNA^{[Ser]SeC}$, containing the appropriate UCA anticodon [Lee et al., 1989; Diamond et al., 1993]. A critical question in the understanding of this "recoding" process is how the ribosomal translation assembly can make the discrimination between a UGA codon in a selenoprotein

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gene transcript versus the same codon serving as a termination signal in another mRNA. This mechanism directs translational read-through by $tRNA^{[Ser]SeC}$ only in the former and permits releasing factors to terminate translation in all other transcripts.

In prokaryotic mRNAs encoding selenoproteins, translation of the UGA codon as selenocysteine depends upon an immediately adjacent 40-base stem-loop structure, the bacterial selenocysteine insertion sequence (SECIS), immediately downstream within the open reading frame [Zinoni et al., 1990; Böck et al., 1991]. A specialized translation elongation factor, SelB (M_r 68.8 kDa), which is required for selenocysteine incorporation in bacteria [Forchhammer et al., 1989], specifically recognizes the loop portion of the bacterial SECIS secondary structure [Baron et al., 1993]. The C-terminal 17 kDa of SelB contains the mRNA-binding domain [Kromayer et al., 1996]; the remaining SelB amino acid sequence exhibits extensive homology with the sequence of elongation factor (EF)-Tu [Forchhammer et al., 1989; Hilgenfeld et al., 1996].

In contrast, translation of eukaryotic selenoprotein mRNA depends upon an 80–90 nucleotide SECIS element in its 3′-untranslated region [Berry et al., 1993; Berry et al., 1991a; Shen et al., 1993], which directs translation of selenocysteine from a position ranging from a few hundred nucleotides to more than 4 kb downstream from the UGA codon [Martin et al., 1996; Buettner et al., 1998]. The eukaryotic SECIS forms a well-conserved secondary structure that is predicted by RNA folding programs to form a stem-loop featuring a long stem incorporating non-Watson-Crick base-pairing, several bulges, and an apical loop [Berry et al., 1991a; Grundner-Culemann, et al. 1999; Walczak et al., 1996]. The primary nucleotide sequence is highly variable except for three very short (2–4 bases), highly conserved sequences [Berry et al., 1991a; Berry et al., 1993]. Function of the SECIS depends upon both these short conserved sequences and, to an even greater degree, the secondary structure of the entire element [Shen et al., 1995a].

Previous studies have shown that the SECIS element is necessary for translation of mRNA encoding selenoproteins [Berry et al., 1991a; Berry et al., 1993; Shen et al., 1993; Bermano et al., 1996] as well as sufficient to direct the translation of an *opal* (UGA) mutation as selenocysteine in a protein not normally containing that amino acid [Shen et al., 1993; Leonard et al., 1996; Lesoon et al., 1997]. Attention has now turned to what molecules—the eukaryotic equivalents of SelB—recognize the SECIS element to permit selenocysteine insertion at the UGA codon.

We have previously identified DNA-binding protein B (dbpB) as a SECIS-binding protein [Shen et al., 1998]. This protein was originally isolated and named in accordance with its DNA-binding properties [Sakura et al., 1988; Didier et al., 1988; Horwitz et al., 1994]. However, it also contains four arginine-rich motifs characteristic of a group of RNA-binding proteins [Burd and Dreyfuss, 1994] and specifically binds to the SECIS element of human cellular glutathione peroxidase mRNA. However, this protein lacks properties, such as $tRNA^{[Ser]SeC}$ binding and GTPase activity, associated with the prokaryotic translation factor SelB [Hüttenhofer and Böck, 1998; Tormay et al., 1996]; so eukaryotic selenoprotein translation is likely to require additional proteins to form a selenocysteine-specific elongation complex. Other SECIS and $tRNA^{[Ser]SeC}$ binding activities have been observed [Fujiwara et al., 1999; Gelpi et al., 1992; Lesoon et al., 1997; Hubert et al., 1996; Yamada et al., 1994], but specific candidate proteins are not yet identified.

We now report the identification of nucleolin as a partner in the selenoprotein translation complex. Like dbpB, this protein is best known for other functions; but the current studies show that it both specifically binds the SECIS and also pairs with dbpB in cellular and ribosome-associated protein extracts.

MATERIALS AND METHODS

Expression Library Screening

λgt11 cDNA expression libraries from human K562 and HeLa cell lines were plated on *E. coli* cultures and expression induced by overlaying the plaques with nitrocellulose membranes presoaked in 10 mM IPTG and then incubating at 37°C for 6 h. The membranes were removed from the culture plates, blocked in 3% bovine serum albumin (5× Denhardt's reagent) at room temperature for 1 h, and air-dried at room temperature for 15 min. All subsequent procedures were carried out at 4°C. The membranes were submerged twice for

10 min in 200 ml of HEPES binding buffer (25 mM HEPES, pH 7.9, 25 mM NaCl, 5 mM $MgCl_2$, 0.5 mM DTT, 6 M guanidine HCl) then submerged twice for 5 min in the same buffer with five serial two-fold dilutions of guanidine HCl, then HEPES binding buffer without guanidine HCl. After 30 min incubation with BLOTTO, the filters were soaked for 5 min in "buffer K" (100 mM potassium phosphate, pH 7.0, 10 mM $MgCl_2$, 50 mM KCl, 2mM DTT, 0.5 mM PMSF, 0.5 mM ATP and GTP) and hybridized for 2 h with 10^6 cpm/ml of ^{32}P-labeled *GPX1* SECIS RNA probe, supplemented with 0.5× Denhardt's reagent, 0.5 mg/ml *E. coli* total tRNA, 1000 unit/ml RNasin (Promega), and 0.25 mg/ml heparin sulfate. The membranes were washed twice for 1 h with buffer K and then subjected to autoradiography. Synthesis of ^{32}P-labeled RNA probes for library screening and electrophoretic mobility shift assays was performed as previously described [Shen et al., 1995b]. Human nucleolin cDNAs in λgt11 plaques positive by two consecutive screenings were excised from λgt11 by *Not*I digestion and ligated into pBluescript KS or the His-tag vector pRSET B (Invitrogen).

Overexpression of His-Tagged Nucleolin Protein

The plasmid pRSET B containing 6-His-tagged truncated nucleolin cDNA (encoding amino acids 274–707) was used to transform *E. coli* strain BL21(DE3) carrying the plasmid pLysE, so that the expression of the fused gene was controlled by bacteriophage T7 RNA polymerase promoter [Studier et al., 1990]. The cells were grown at 37°C to OD_{600} = 0.5–0.8, induced with 0.4 mM IPTG for 3 h, then lysed by sonication. Recombinant His-tagged nucleolin was purified by nickel-nitrilotriacetic acid resin column chromatography, according to the manufacturer's protocol (Qiagen, Inc.) and then further purified by electrophoresis on a 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel.

Production of Nucleolin-Specific Antisera

Antibodies against a nucleolin peptide were raised in rabbits immunized with a synthetic peptide corresponding to the C-terminal 15 amino acids of human nucleolin (GGGDHKPQGKKTKFE), coupled to keyhole limpet hemocyanin at an additional N-terminal tyrosine. Peptide-specific anti-nucleolin antibodies were purified from rabbit serum by affinity chromatography on peptide-Affigel 10 affinity matrices and stored frozen at −70°C until use. Concentrated rabbit polyclonal anti-nucleolin immunoglobulin G (IgG) was prepared by precipitation of rabbit serum with 40% ammonium sulfate, followed by resuspension in buffer K and concentration by Micron (Amicon) ultrafiltration. For affinity purification of anti-nucleolin IgG, an affinity column was prepared by coupling the His-tagged nucleolin protein to cyanogen bromide-activated Sepharose 4B (Sigma) following standard procedures [Harlow and Lane, 1988]; then, immunoaffinity purification of the antibody was accomplished by standard methods [Harlow and Lane, 1988].

Western Blots

Western blots were performed according to the protocol of the ProtoBlot Western Blot AP System (Promega). Briefly, protein samples were fractionated by 10% SDS-PAGE, transferred to nitrocellulose membranes, blocked with 3% bovine serum albumin, and incubated with antibody. Detection of antibody-targeted protein bands was achieved by either alkaline phosphatase-mediated color development (Promega) or by horseradish peroxidase-mediated chemiluminescence (ECL, Amersham) after second antibody incubation with appropriately conjugated goat antibodies against rabbit IgG.

Electrophoretic Mobility Shift Assays

Gel shifts were performed as previously described [Shen et al., 1995b]. For antibody competition assays, 5–10 μl of concentrated, affinity-purified anti-nucleolin antibody was added to the gel shift assay mixture and incubated, with or without excess free C-terminal peptide, for 20 min at 30°C, then 20 min at 4°C, prior to loading on the gel. Probes of *GPX1* wild type and mutant SECIS elements were prepared as previously described [Shen et al., 1995a].

Immuno-Affinity Purification

Concentrated anti-dbpB or pre-immune rabbit IgG was prepared by ammonium sulfate precipitation, as described above, and bound to SHA Sepharose resin using the manufacturer's protocol for "The Linx AP System" (Invitrogen, Inc.). HeLa cell extract (S-100 fraction by differential centrifugation) was incubated with

the column resin overnight at 4°C, washed thoroughly with phosphate-buffered saline, then bound proteins were eluted with 5 M LiCl in 20 mM phosphate buffer (pH 7.2). Eluted proteins were centrifuged in a Centricon or Microcon (Amicon, Inc.) filter apparatus for removal of LiCl and concentration for further analysis.

Glycerol Gradient Centrifugation

A ribosomal fraction of HeLa cells was prepared by differential centrifugation (60,000 rpm pellet of the 10,000 g supernatant) and the "translation initiation fraction" removed from resuspended ribosomes by high salt wash (0.5 M KCl) followed by dialysis [Barton et al., 1996]. Glycerol gradients were prepared by successive layering of 0.5 ml of solutions of glycerol (30%, 25%, 20%, and 15% in 20 mM HEPES pH 8.0, 100 mM KCl, 100 μM EDTA) and held overnight at 4°C. The ribosomal fraction was loaded as 100 μg protein in 5% glycerol and centrifuged into the gradient at 55,000 rpm in a Beckman TLS rotor for 16 h. The gradient was then analyzed by western blotting aliquots of successive 50 μl fractions.

RESULTS

For direct cloning of genes encoding proteins that bind the SECIS element, we screened two λgt11 cDNA expression libraries from human K562 and HeLa cell lines with a ^{32}P-labeled synthetic RNA transcript of the SECIS element from the human cellular glutathione peroxidase gene, *GPX1*. From approximately 10^7 phage plaques, 60 positive plaques were detected by binding of the radiolabeled probe. For secondary screening, two additional radiolabeled RNA probes were used as negative controls: a 6-nt basal stem deletion mutant *GPX1* SECIS and the HIV TAR element. Based on previous functional and gel shift analyses [Shen et al., 1995a; Shen et al., 1995b], candidate plaques were selected for strong signal with the wild type *GPX1* SECIS probe, weak or absent signal with a 6-nt basal stem deletion mutant probe (which showed no gel shift or functional activity), and none with the HIV TAR probe (a control for nonspecific binding to a different stem-loop structure). After secondary screening, sequencing of phage clones from the remaining positive plaques revealed, in addition to sequences encoding dbpB [Shen et al., 1998], two plaques (one from each library) encoding overlapping segments of human nucleolin. To test nucleolin for SECIS-binding activity, we prepared recombinant 6-His-tagged polypeptide incorporating amino acids 274–707 and including the RNA-binding domains. Gel-shift experiments showed that this polypeptide binds the *GPX1* SECIS (Fig. 1, far right lane) and competes with the native binding activity in the S100 fraction of COS1 cytoplasm (Fig.1, middle lanes).

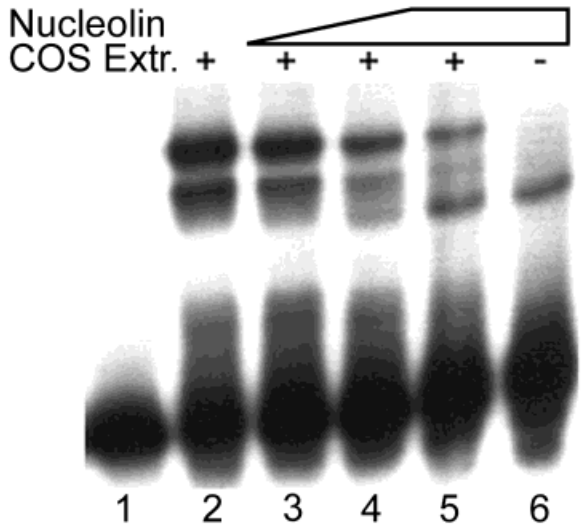

Fig. 1. RNA gel shift assay of SECIS probe with nucleolin with COS cell extract. *GPX1* SECIS probe was incubated with or without COS cell extract (indicated by COS + or —) and increasing amounts of purified recombinant His-tagged nucleolin. Electromobility shift assays were performed as described in Materials and Methods.

To test the role of nucleolin in the SECIS gel shift complex observed with cytosolic extract, the S100 fraction from COS1 cells was incubated with *GPX1* SECIS with or without polyclonal antibody directed against the C-terminal 15 amino acids of nucleolin. The purified anti-nucleolin IgG dramatically reduced the gel shift band intensity (Fig. 2, lane 3), an effect largely reversed by coincubation with free C-terminal peptide. Anti-nucleolin antibody may inhibit RNA binding by steric hindrance and thus remove the probe rather than causing a "supershift" of its electrophoretic mobility.

To evaluate the specificity of nucleolin binding to the SECIS, we performed electromobility shift assays with purified recombinant His-tagged nucleolin and *GPX1* SECIS constructs with deletions of the three short conserved sequences common to all mammalian SECIS el-

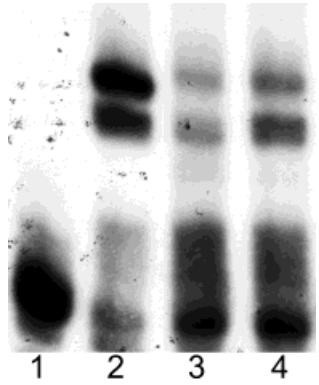

Fig. 2. Effect of anti-nucleolin antibody on the SECIS-binding activity of COS cell extract. Electromobility shift assays were performed with the *GPX1* SECIS probe. Lane 1: without COS cell extract; lane 2: with COS extract; lane 3: with COS extract and IgG raised against the C-terminal 15 amino acids of nucleolin; and lane 4: with COS extract, anti-nucleolin IgG, and free C-terminal peptide.

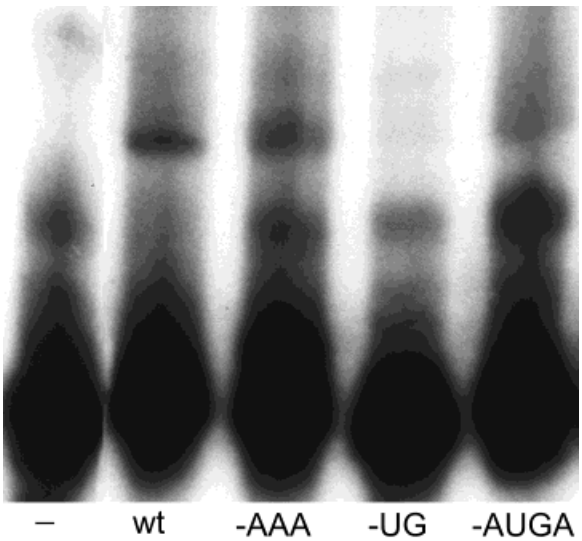

Fig. 3. RNA gel shift assay of nucleolin with short deletion SECIS probes. Electromobility shift assays were performed with or without (— lane) purified recombinant His-tagged nucleolin and the indicated *GPX1* SECIS RNA probes: wild type probe (wt) or deletions of short conserved sequences AAA, UG, and AUGA, as indicated.

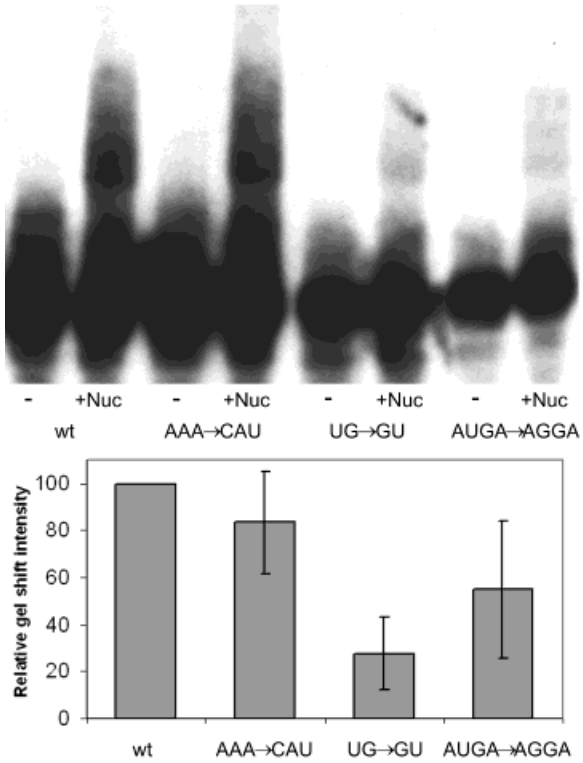

Fig. 4. RNA gel shift assay of nucleolin with SECIS probes mutated in the conserved sequences. Electromobility shift assays were performed without (— lanes) or with (+Nuc lanes) purified recombinant His-tagged nucleolin and the indicated *GPX1* SECIS RNA probes: wild type probe (wt) or mutations of short conserved sequences AAA, UG, and AUGA, as indicated. **Upper panel:** autoradiograph of a representative experiment. **Lower panel:** graph representing compiled data from four experiments; columns indicate means and error bars show standard deviations. Gel shift band intensity was quantitated by phosphorimager and normalized to the signal from the wild type band within each experiment.

ements. As shown in Fig. 3, deletion of the conserved sequence UG dramatically decreased nucleolin binding; deletion of AUGA had a lesser effect, and deletion of AAA did not diminish nucleolin binding. Further gel shift experiments utilized less disruptive nucleotide exchange mutations rather than simple deletion of the conserved sequences. As shown in Fig. 4, inversion of the UG sequence or a single substitution in the AUGA sequence produced statistically significant loss of SECIS binding by nucleolin ($P < 0.05$, n = 4, paired t test). Previous studies [Shen et al., 1995a] have shown complete loss of selenoprotein translational function by these mutations. As expected from the deletion experiment, the gel-shift signal did not change significantly with the AAA sequence mutation. Electromobility shift experiments with both dbpB and nucleolin tested their capacity for cooperative binding of the *GPX1* SECIS. Figure 5 demonstrates that incubation of the SECIS with both recombinant proteins eliminates the bands produced by either protein alone and generates a new, distinct, and slower mobility band, indicating that both proteins can attach to the same SECIS molecule and do not compete with each other for binding. They may either recognize differ-

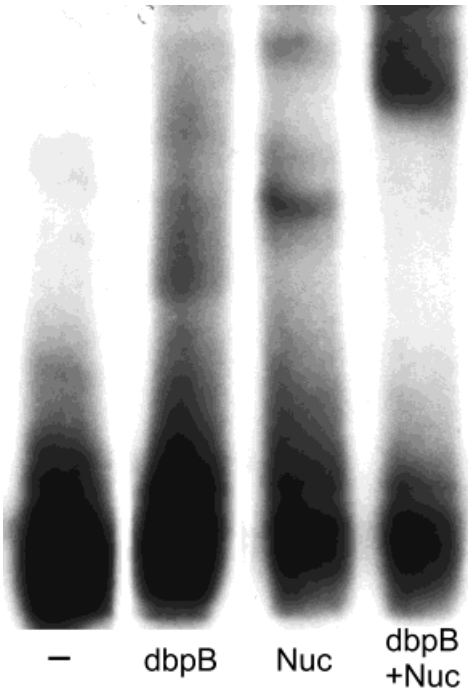

Fig. 5. SECIS-binding activity of dbpB and nucleolin in combination. Electromobility shift assays were performed with *GPX1* SECIS RNA probe incubated without (— lane) protein addition or with (as indicated below each lane) purified recombinant His-tagged dbpB, purified recombinant His-tagged nucleolin, or both.

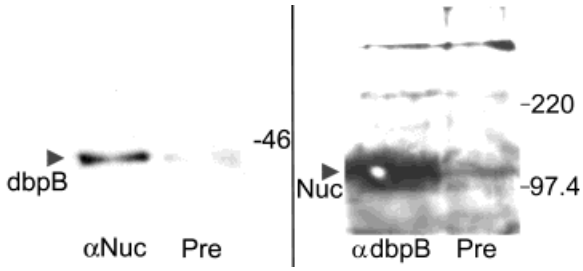

Fig. 6. Association of nucleolin with dbpB. HeLa cell cytosol was extracted by immunoaffinity chromatography using anti-nucleolin IgG (α-Nuc), anti-dbpB IgG (α-dbpB), or their respective pre-immune IgGs (Pre) and then analyzed by SDS-PAGE and western blotting with anti-dbpB antibody (**left panel**) or anti-nucleolin antibody (**right panel**). The coextracted proteins (left panel: dbpB; right panel: nucleolin) are indicated by the arrowheads and labels in the left margins; numbers in the right margins represent protein size markers.

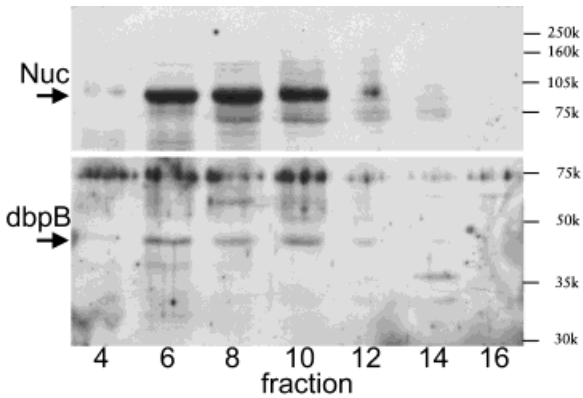

Fig. 7. dbpB and nucleolin in fractions from HeLa ribosomal salt extract. High salt washes of ribosomal cell fractions were centrifuged through a glycerol gradient and then analyzed by western blot for nucleolin (**upper panel**) and for dbpB (**lower panel**). The nucleolin (Nuc) and dbpB bands are indicated by the arrows in the left margin; size markers, by numbers in the right margin. Numbers in the lower margin refer to fractions collected from the glycerol gradient. Neither protein was detectable in higher or lower fractions.

ent regions of the SECIS RNA or form an intermolecular complex that then interacts with the SECIS.

Further experiments examined whether the two proteins also associate with each other in vivo. In copurification assays, proteins were extracted from HeLa cytosol by column-immobilized polyclonal antiserum against dbpB or nucleolin. The extracted proteins were then tested for the presence of the other protein by western blot. As shown in the left panel of Fig. 6, the anti-nucleolin Sepharose column (left lane) coextracted far more dbpB than the control pre-immune IgG column (right lane). Inversely, as shown in the right panel of Fig. 6, the anti-dbpB Sepharose column (left lane) co-extracted more nucleolin than control pre-immune IgG column (right lane). Western blots against whole cell extracts and purified recombinant proteins (not shown) showed that each antiserum was specific for its target antigen and did not cross-react with the other protein.

Finally, we examined a ribosome-derived subcellular fraction for both dbpB and nucleolin to determine whether they associate with each other and with the translational apparatus in the cytoplasm. HeLa cell lysates were subjected to differential centrifugation and high salt wash [Barton et al., 1996] to generate a ribosomal "translation initiation" fraction, which contains both initiation factors and other ribosome-associated translational mediators [Lee et al., 1998; Shibutani et al., 1996]. This material was then fractionated on a glycerol gradient for nondenaturing separation of protein complexes, and fractions analyzed by western blot, as shown in Fig. 7. Both dbpB and nucleolin were detected at high levels in the same fractions of the ribosomal high salt

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EF-1β: 83  DVEDTTGSGATDSKDDDDIDLFGSDDEEESEE---------AKRLRE--ERLAQYESKKAK 132
           D ED       D +DDDD D    D+EEE EE          KR +E  ++ A  E+KK K
Nuc  : 241 DDEDEDDDDDEDDEDDDDED----DEEEEEEEEEEPVKEAPGKRKKEMAKQKAAPEAKKQK 297

EF-1β: 94  DSKDDDDIDLFGSDDEEESEEAKRLREERLAQYESKKAKKPA 135
           D  DDDD D    DDE++ EE +   EE + +   K+ K+ A
Nuc  : 244 DEDDDDDEDDEDDDDEDDEEEEEEEEEEPVKEAPGKRKKEMA 285
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Fig. 8. Putative elongation factor domain in nucleolin. Regions of homology are indicated between the amino acid sequences of nucleolin (Nuc) and human elongation factor 1β (EF-1β), aligned by the BLASTP 2.0.9 program at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST/). + indicates a conservative difference.

wash. Together with the coimmunoextraction data, these findings provide evidence for an intermolecular interaction that would be necessary for the two SECIS-binding components to participate in the larger selenoprotein translation complex.

DISCUSSION

The current data indicate that nucleolin serves as another component of the selenocysteine translation complex. It binds to the *GPX1* SECIS element with specificity for functionally significant sequence elements and physically associates with dbpB, the other known and identified SECIS-binding protein [Shen et al., 1998]. Although the sequence specificity of nucleolin binding does not include all functionally significant regions, we hypothesize that the selenocysteine translation complex includes multiple elements, none of which alone would provide complete, specific recognition of the SECIS element.

The differing requirement for SelB, a single specialized elongation factor, in prokaryotes versus a large, multi-component complex in eukaryotes probably derives from the different locations of the respective SECIS elements. The position of the prokaryotic SECIS immediately downstream from the UGA codon assures direct interaction with the translational apparatus. The eukaryotic counterpart, located at a variable distance downstream in the 3′-untranslated region, requires recognition and assembly steps to bring it to the site of peptide synthesis.

Nucleolin protein, as the name implies, comprises an important component of the nucleolus, where it participates in the assembly of the ribosome by binding and processing rRNA and ribosomal proteins [Ginisty et al., 1999; Tuteja and Tuteja, 1998; Bouvet et al., 1998]. Like dbpB, nucleolin also serves multiple additional roles, including function as a component of a B lymphocyte-specific transcription and switch region binding complex [Hanakahi et al., 1997], as a DNA helicase [Tuteja et al., 1995], as a nuclear-cytoplasmic shuttle protein [Borer et al., 1989] that interacts with RNA and DNA viral genomes [Qiu and Brown, 1999; Waggoner and Sarnow, 1998] and even as a cell surface protein [Ginisty et al., 1999] with viral receptor capacity [Nisole et al., 1999].

Thus, for the selenocysteine translation complex, nucleolin probably serves as a link to the ribosome. In addition, it might provide elongation factor function, analogous to that of SelB in prokaryotic selenoprotein synthesis. As shown in Fig. 8, human nucleolin also contains a domain with homology to a sequence from human elongation factor 1β that is highly conserved from humans to brine shrimp [von der Kammer et al., 1991]. Human EF-1β is functionally equivalent to the prokaryotic translation factor EF-Tu, for which SelB substitutes in selenoprotein synthesis [Forchhammer et al., 1989; Hilgenfeld et al., 1996].

Many laboratories have searched for putative eukaryotic SelB equivalents involved in selenocysteine insertion. One candidate $tRNA^{[Ser]SeC}$ binding factor is a 48-kDa polypeptide recognized by autoantibodies present in a subgroup of patients with a severe form of autoimmune chronic active hepatitis [Gelpi et al., 1992]. The antisera coimmunoprecipitate $tRNA^{[Ser]SeC}$ along with the 48-kDa protein, which does not exhibit tRNA synthetase activity. A second candidate is a 50-kDa protein from bovine liver extract that protects $tRNA^{[Ser]SeC}$ against alkaline hydrolysis [Yamada et al., 1994]. However,

neither of these proteins is reported to be able to bind to the SECIS.

Two other groups [Hubert et al., 1996; Lesoon et al., 1997; Copeland and Driscoll, 1999] have identified cytoplasmic binding factors in mammalian cells that specifically recognize the SECIS element. Neither binding factor has been specifically identified by biochemical or molecular methods. A 60–65-kDa protein in human and rat cells binds the SECIS elements from rat glutathione peroxidase and type I iodothyronine 5′-deiodinase transcripts, with somewhat higher affinity for the former [Hubert et al., 1996]. A 120-kDa rat testis cytosol protein, termed SECIS-binding protein 2, specifically recognizes the SECIS of mRNA encoding both the phospholipid hydroperoxide and cellular forms of glutathione peroxidases and requires one of the short conserved sequences [Lesoon et al., 1997; Copeland and Driscoll, 1999]. Gel filtration chromatography demonstrated migration of SECIS-binding protein 2 in a high molecular mass fraction of approximately 500 kDa, perhaps corresponding to the multiprotein complex we observed by glycerol gradient centrifugation. One or more of these additional $tRNA^{[Ser]SeC}$- and SECIS-binding proteins may combine with dbpB and nucleolin to form a large complex that links elements in the coding region, 3′-untranslated region, and ribosome to translate the UGA codon as selenocysteine.

Nucleolin is best known for its interactions with ribosomal RNA and protein. The identification of its properties as a eukaryotic SECIS-binding protein indicates an additional role in the cytoplasm as a member of the complex necessary for the recoding of a UGA “stop” codon as selenocysteine.

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