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A protein binds the selenocysteine insertion element in the 3′-UTR of mammalian selenoprotein mRNAs

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ABSTRACT

Several gene products are involved in co-translational insertion of selenocysteine by the tRNA^{Sec}. In addition, a stem–loop structure in the mRNAs coding for selenoproteins is essential to mediate the selection of the proper selenocysteine UGA codon. Interestingly, in eukaryotic selenoprotein mRNAs, this stem–loop structure, the selenocysteine insertion sequence (SECIS) element, resides in the 3′-untranslated region, far downstream of the UGA codon. In view of unravelling the underlying complex mechanism, we have attempted to detect RNA-binding proteins with specificity for the SECIS element. Using mobility shift assays, we could show that a protein, present in different types of mammalian cell extracts, possesses the capacity of binding the SECIS element of the selenoprotein glutathione peroxidase (GPx) mRNA. We have termed this protein SBP, for Secis Binding Protein. Competition experiments attested that the binding is highly specific and UV cross-linking indicated that the protein has an apparent molecular weight in the range of 60–65 kDa. Finally, some data suggest that the SECIS elements in the mRNAs of GPx and another selenoprotein, type I iodothyronine 5′ deiodinase, recognize the same SBP protein. This constitutes the first report of the existence of a 3′ UTR binding protein possibly involved in the eukaryotic selenocysteine insertion mechanism.

INTRODUCTION

Incorporation of selenocysteine into selenoprotein is a co-translational event occurring in prokaryotes and eukaryotes. In bacteria, the mechanism is rather complex but has been widely elucidated (reviewed in ref. 1). Four gene products, SELA, SELB, SELC and SELD are required for incorporation of selenocysteine. Selenophosphate synthetase (SELD) synthetizes selenophosphate, the activated selenium moiety (2,3). A specialized tRNA, the selenocysteine tRNA^{Sec} (SELC) is first charged with serine by the conventional seryl-tRNA synthetase (4). Subsequently, conversion of the seryl-tRNA^{Sec} to selenocysteyl-tRNA^{Sec} is catalyzed by selenocysteine synthase (SELA) which uses selenophosphate as the selenium donor (5,6). SELB is the selenocysteine specific translation factor, homologous to EF-Tu, which binds to the selenocysteyl-tRNA^{Sec} (7). Selenocysteine incorporation results from translation of a UGA codon, which is specified for that function by the occurrence of an adjacent RNA stem–loop structure. SELB binds to the loop of this RNA motif, thereby bringing the charged tRNA^{Sec} to its proper codon (8).

Several selenoproteins have been identified in mammals (reviewed in ref. 1), but a function has been assigned to only two of them, the glutathione peroxidases and the iodothyronine 5′ deiodinases. The family of glutathione peroxidases (GPx) is of pivotal role for the protection against oxidative damage by free radicals (9). The type I iodothyronine 5′-deiodinase (5′DI) converts thyroxine to the active hormone (10). Data are, however, comparatively scarce when it comes to the selenocysteine insertion mechanism in eukaryotes. A tRNA^{Sec} has been found which participates in the conversion of the seryl to selenocysteyl-residue and further donates it to selenoproteins (11–13). The eukaryotic tRNA^{Sec} possesses features of secondary and tertiary structures distinct from canonical tRNAs, but also from its bacterial counterpart (14,15). Its uniquely long aminoacyl acceptor stem constitutes one structural determinant crucial for the serine to selenocysteine conversion step (16). The human homologue of SELD has recently been characterized and cloned (17,18) and detection of proteins possibly homologous to SELA and SELB has been described (19–21). Deciphering of the UGA selenocysteine codon appears to utilize a eukaryote-specific mechanism, distinct from that found in bacteria. Indeed, studies have identified regions within the 3′ untranslated regions (3′ UTR) of the 5′DI, GPx and selenoprotein P mRNAs containing short stretches of sequence conservation and required for recognition of the selenocysteine UGA codon (22–24). Based on computer folding, these stretches of RNA sequences were proposed to adopt a stem–loop structure termed selenocysteine insertion sequence (SECIS) or selenium translation element (STE), different in sequence, structure and location from that found in bacteria (23,25).

The fact that the SECIS element can be situated >1 kb downstream of the selenocysteine codon (22) raises several questions regarding the mechanistic role of this element. Does it exert its function through long range RNA–RNA interactions or upon binding protein factor(s) or both? In an attempt toward resolving the issue, we sought protein(s) that bear SECIS RNA-binding capacity. In the work presented here, we describe detection of one such protein in mammalian cell extracts.

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MATERIALS AND METHODS

DNA constructs

The SECIS element from the rat type I iodothyronine 5′deiodinase (5′DI) cloned downstream of a T7 promoter was obtained by PCR of a rat liver cDNA library in λgt10 (a gift of Dr S. Cereghini, Institut Pasteur-Paris). Primer 1, 5′-CGCGGATCCTAATACGACTCACTATAGGGTTCATCTGTCATGTC-3′ is complementary to the lower strand from positions 1497 to 1514; primer 2, 5′-CCGGAATTCTAGCCTGACGGATTTTAATCG-3′ is complementary to the top strand from positions 1610 to 1630 (10). A construct carrying the rat glutathione peroxidase (GPx) SECIS element behind a T7 promoter was obtained by PCR of the parental construct pKs-cGP-1 (a gift of Dr Ye-Shih Ho) containing the rat GPx cDNA (26). Primer 3, 5′-CGCGGATTCTAATACGACTCACTATAGGTGATGGCTGGCTGGCTGCCCTC-3′ and primer 4, 5′-CCGGAATTCTTTAAATGGACGAGACCAGCGCCCATC-3′ were complementary to the lower strand from positions 980 to 999 and to the top strand from positions 1078 to 1100, respectively. Primers 1 and 3 incorporated a *Bam*HI cloning site and the T7 RNA polymerase promoter. Primers 2 and 4 contained an *Eco*RI cloning site, primer 4 containing in addition a *Dra*I linearization site. PCR reactions were performed for 30 cycles, 2 min at 94°C, 2 min at 50°C and 2 min at 72°C. The amplified fragments were ligated to *Bam*HI/*Eco*RI cleaved pUC119 vector to yield pT7RDIS and pT7RGPxS for the rat 5′DI and GPx SECIS elements, respectively. After linearization with *Dra*I, T7 transcription of pT7RGPxS generates a 121 nucleotide long RNA fragment starting at position 980, ending at position 1100 which contains the SECIS motif (26). Transcription of pT7RDIS yields a 139 nucleotide long RNA starting at position 1497 and ending at position 1630 of the gene (10), containing the SECIS motif.

In vitro transcription with T7 RNA polymerase

Uniformly ^{32}P-labeled SECIS RNAs were transcribed in a 50 µl medium containing 2 µg of linearized DNA template (*Eco*RI for pT7RDIS and *Dra*I for pT7RGPxS), 40 mM Tris–HCl pH 8, 6 mM $MgCl_2$, 10 mM NaCl, 2 mM spermidine, 10 mM DTT, 250 µM of GTP, CTP and UTP, 30 µM ATP, 125 µCi [α-^{32}P]ATP (3000 Ci/mmol), 20 U RNasin and 3 µl T7 RNA polymerase (prepared from an overproducing strain). The mixture was incubated for 3 h at 37°C, the RNA phenol extracted and gel purified. Large scale production of T7 SECIS RNA transcripts was performed as follows. A 250 µl volume contained 25 µg of linearized DNA, 40 mM Tris–HCl pH 8, 20 mM $MgCl_2$, 1 mM spermidine, 0.1% Triton X-100, 5 mM DTE, 4 mM each NTP, 60 U RNasin and 15 µl T7 RNA polymerase. The mixture was incubated for 3 h at 37°C and phenol extracted. The RNAs were purified on 10% preparative polyacrylamide gels and electroeluted.

Preparation of cell extracts

S100 HeLa cell extracts (a post-ribosomal supernatant prepared at 150 mM KCl) were prepared from HeLa cells (produced by the cell culture group at the IGBMC Illkirch, France) as described in ref. 27. COS-7 and Faza extracts were prepared by the freeze–thaw method. Faza (H4-II-E-C3) is a 8-azaguanine resistant cell line derived from the Reuber H-35 rat hepatoma (28). Protein concentration was determined by the Bradford assay.

RNA binding and gel retardation assays

The RNA–protein reactions were conducted on ice. *In vitro* transcribed ^{32}P-labeled RNA (100 000 c.p.m.; ~1 pmol) was incubated in 20 µl containing 50 mM Tris–HCl pH 7.5, 5 mM $MgCl_2$, 600 mM KCl, 10 µg total *E.coli* tRNA, and variable amounts of protein extracts for 15 min. Heparin (50 µg) was then added to prevent unspecific RNA–protein interactions and incubation was continued for 5 min. For competition assays, the appropriate amount of unlabeled RNAs was added prior or after the protein extracts. Samples were electrophoresed through 4% non-denaturing polyacrylamide gels (acrylamide/bisacrylamide ratio, 60:1) in 0.5× Tris-borate buffer containing 5% glycerol. Band intensities were quantified with a Fuji BioImage Analyzer BAS 2000 and the values normalized.

UV cross-linking assays

Incubation of ^{32}P-labeled transcripts (500 000 c.p.m.) with HeLa S100 extracts was as described for gel retardation assays. The samples were exposed to UV light at 254 nm (5 cm away from a 100 W UV lamp) for 10 min on ice, followed by treatment with RNase T1 (final concentration 1 U/µl) for 2.5 h at 37°C. ^{32}P-labeled proteins were analyzed by 12% SDS–PAGE. In some cases, the protein extract was digested by proteinase K for 1 h at 37°C prior to addition of the RNA probe.

RESULTS

Complex formation with the glutathione peroxidase and type I iodothyronine 5′ deiodinase selenocysteine insertion motifs in HeLa extracts

Based on computer folding, the SECIS element was defined by others as a sequence portion capable of forming a stem–loop structure, essential for selenocysteine incorporation (22–25). However, neither the minimum length required for function nor experimental determination of its structure are known. For this reason, we used for this study slightly longer RNA transcripts in which the proposed folded structure is embedded.

The ability of a HeLa S100 extract (a post-ribosomal supernatant prepared at 150 mM KCl) to bind the SECIS RNA elements contained in the 3′ UTR of the glutathione peroxidase (GPx) and type I iodothyronine 5′-deiodinase (5′DI) mRNAs was examined by using a mobility shift assay. The ^{32}P-labeled 121 nucleotide long GPx RNA probe produced by T7 transcription of pT7RGPxS contains the SECIS motif. It was assayed with increasing amounts of protein. Figure 1A shows that addition of 16 µg of extract provokes the appearance of a retarded band with a weak signal (lane 3). The signal intensity increases when increasing amounts of protein extracts are added (lanes 4–10). We next asked whether the 139 nucleotide long RNA fragment arising from pT7RDIS, containing the 5′DI SECIS element, can also bind a trans-acting factor. To answer the question, the ^{32}P-labeled 5′DI probe was incubated with increasing amounts of the HeLa S100 extract. Figure 1B indicates that the 5′DI SECIS element leads to complex formation as well, but the situation differs from that observed with the GPx SECIS element. With 32 µg (lane 3) of HeLa S100 extract a diffuse, but well detectable, low mobility complex C2 forms, the intensity of which increases with increasing amounts of protein (lanes 3–6). However, a second, higher mobility complex C1 appears when higher amounts of extracts are added (130 µg). C1 migrates slightly

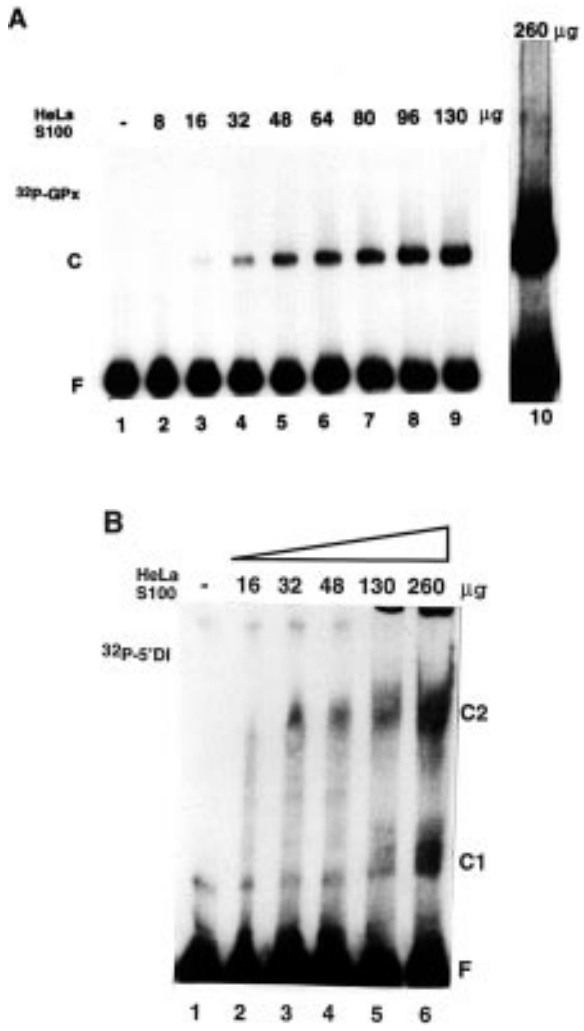

Figure 1. Complex formation between glutathione peroxidase (GPx) and type I iodothyronine 5′ deiodinase (5′DI) SECIS elements and a component contained in HeLa extracts. (**A**) The ^{32}P-labeled RNA probe containing the GPx SECIS element was incubated in the absence (lane 1) or in the presence of increasing amounts of HeLa S100 extracts. Amounts are indicated above lanes 2–10. Lane 10 originates from a separate experiment and was overexposed. (**B**) The ^{32}P-labeled RNA probe containing the 5′DI SECIS element was incubated in the absence of extract (lane 1) or in the presence of increasing amounts (indicated above the lanes) of HeLa extracts (lanes 2–6). F, free RNA probe; C, complex; C1 and C2 represent the two complexes obtained with the 5′DI probe (see text).

above the band present in the no-extract-added lane (lane 1) and possesses an electrophoretic mobility comparable to that of complex C obtained with the GPx SECIS element. The intensity is still low with 130 µg of extract, but becomes higher with 260 µg (lane 6). It is worth noting that no stable low mobility complex equivalent to C2 can form with the GPx SECIS element under identical conditions (Fig. 1A, see the overexposed lane 10). Thus, these results indicate that one complex can form with RNA sections containing the GPx SECIS element, while two complexes appear with the 5′DI SECIS element.

Specificity of the complexes formed with the GPx and 5′DI SECIS elements

To examine the specificity of binding to the GPx and 5′DI SECIS motifs, we carried out competition gel mobility shift assays. Competition assays in the case of GPx used an unlabeled fragment arising from pT7RGPxS, identical to that used as the labeled probe, or *E.coli* 5S ribosomal RNA. The former acted as the specific competitor, the latter as an irrelevant RNA (unspecific competitor). *E.coli* 5S RNA is 120 nucleotide long, therefore identical in size to the GPx SECIS or very close to the length of the 5′DI SECIS element. The competition abilities of the specific and unspecific competitors were assessed by quantitation and normalization of band intensities. In Figure 2A, comparison of lane 3 with lane 2 shows that adding a 10-fold molar excess of unlabeled GPx specific competitor severely inhibits formation of complex C. The calculated values indicate that the intensity of complex C in lane 3 drops to 30% of the value measured in lane 2. Adding more competitor leads to gradual diminution of the intensity of the retarded band: a 50-fold excess leaves 7% of residual activity and a 250-fold excess provokes an almost complete inhibition. Complete abolition was obtained at a 2500-fold molar excess (lane 6). Competitive abilities of the 5S RNA showed a marked contrast. Addition of a 10-fold molar excess (lane 7) produces no effect on the intensity of the retarded band (compare lane 7 with lane 2). A 250-fold molar excess of 5S RNA (lane 9) still leads to 80% of residual complex, while the same molar excess of unlabeled GPx SECIS inhibited almost completely the formation of the complex (compare lane 9 with lane 5). Remarkably, one had to introduce a 2500-fold molar excess of 5S RNA to observe a drop to 30% (normalized value) of residual binding in the retarded band (lane 10). This value was obtained with only a 10-fold molar excess of unlabeled GPx SECIS. Therefore, the concentration of the unspecific 5S RNA competitor required to achieve a 70% inhibition of binding is 250-fold greater than the concentration of the unlabeled GPx SECIS element needed to attain the same rate of inhibition. Another unspecific RNA, the antisense GPx SECIS, was also tested, giving similar results (data not shown). This, in addition to the fact that all the binding reactions were performed in the presence of a 400-fold molar excess of bulk *E.coli* tRNA to prevent unspecific binding (see Materials and Methods), unambiguously demonstrates that the complex C formed with the GPx SECIS element is specific.

Identical experiments were performed to evaluate the specificity of complexes C1 and C2 formed with the 5′DI SECIS element. The unlabeled 5′DI SECIS fragment and the 5S RNA were used as specific and unspecific competitors, respectively. Owing to the smear provoked by the C2 complex, quantitation of the gel bands was difficult and source of inaccuracy in the measurement. Therefore, we relied on visual inspection of the gel for the analysis. Figure 2B, lanes 3 and 4, indicates that the intensity of the band in complex C2 is reduced by the addition of 10- and 50-fold molar excesses of 5′DI SECIS competitor, respectively. A 250-fold excess abolishes almost completely the formation of complex C2 (lane 5). The abilities of the unlabeled 5′DI SECIS to challenge formation of complex C1 are different. The intensity of band C1 is unaffected at a 10-fold molar excess, decreases at a 50-fold molar excess, but a residual level of binding persists at a 250-fold molar excess, whereas C2 disappeared under that same condition. Under competition with the 5S RNA, the intensity of

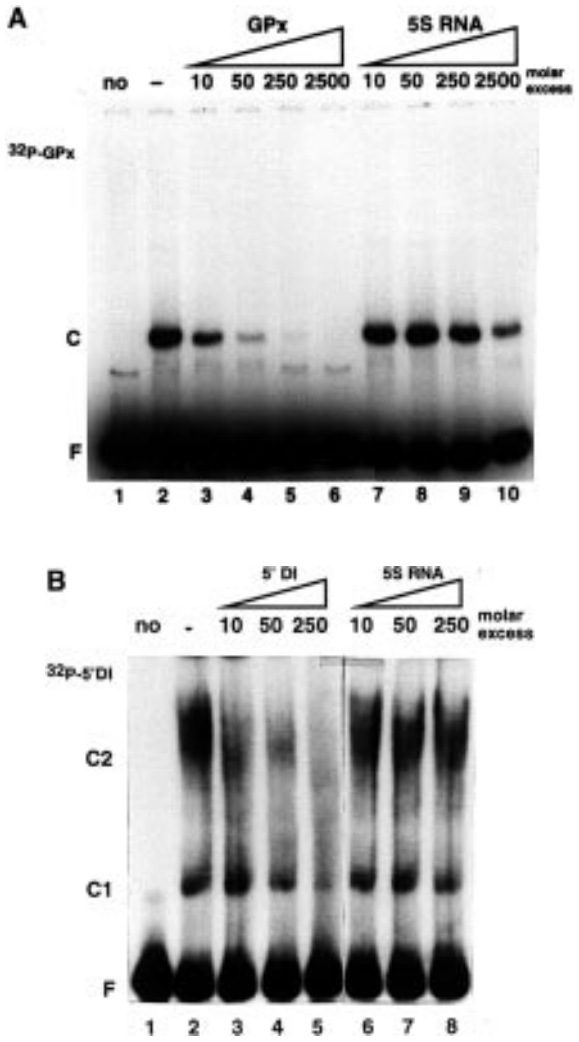

Figure 2. Specificity of the complexes formed with the GPx or 5′DI SECIS elements and a component contained in HeLa extracts. (**A**) The ^{32}P-labeled GPx probe was incubated with increasing molar concentrations of specific (lanes 3–6) or unspecific (lanes 7–10) competitors. Lane 1, incubation of the probe without extract (no). Lane 2, incubation of the probe with extract in the absence of competitor. The specific competitor is the unlabeled GPx SECIS RNA; the unspecific competitor is the *E.coli* 5S ribosomal RNA. The molar excess is indicated above the lanes. (**B**) The ^{32}P-labeled 5′DI probe was incubated without extract in lane 1 (no) or with the extract in the absence of competitor (lane 2) or in the presence of molar excess of specific (lanes 3–5) or unspecific (lanes 6–8) competitors. The molar excess is indicated above the lanes. The specific competitor is the unlabeled 5′DI RNA, the unspecific competitor is the 5S RNA. Symbols are as in Figure 1.

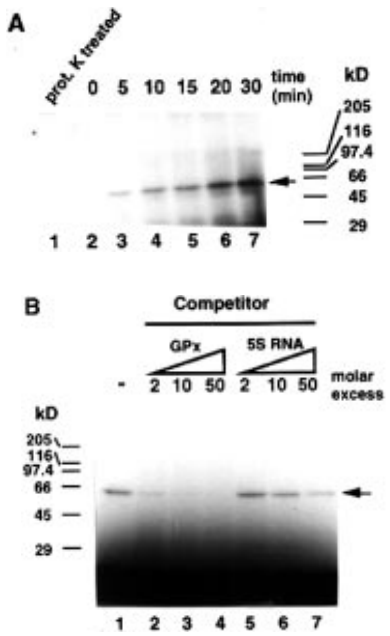

Figure 3. UV cross-linking identifies a protein component binding to the GPx SECIS fragment. (**A**) Lane 1, HeLa S100 extract treated with proteinase K prior to UV light exposure. Lanes 3–7, HeLa S100 extract and ^{32}P-GPx SECIS probe irradiated for 5, 10, 15, 20 and 30 min, respectively, or untreated (lane 2) with UV light. (**B**) HeLa cell extract and ^{32}P-GPx probe treated with UV light for 10 min in either the absence (lane 1) or the presence of specific (GPx) (lanes 2–4) or unspecific (5S RNA) (lanes 5–7) competitors. The cross-linked protein is indicated by an arrow. Protein molecular size markers were run in parallel.

complex C2 is slightly diminished at a 10-fold molar excess (lane 6), but then stays unchanged at 50- and 250-fold molar excesses (lanes 7 and 8, respectively). The intensity of complex C1 is mildly affected at a 250-fold molar excess, only (lane 8). This indicates that both complexes are specific.

Detection of a protein binding to the GPx SECIS element

UV cross-linking experiments were performed aiming at identifying the factor bound to the GPx SECIS element. In Figure 3A, HeLa S100 extracts were submitted to UV light for increasing periods of time in the presence of the labeled GPx SECIS RNA fragment. When compared to the control lane 2, not submitted to UV light, lanes 3–7 loaded with UV-treated extracts show the presence of one prominent band ranging between 60–65 kDa. Digestion of the HeLa S100 extract with proteinase K prior to incubation with the probe and UV cross-linking, obliterates the band at 60–65 kDa (lane 1), attesting that the binding factor is a protein. We have not identified the RNase T1 oligonucleotide that was cross-linked to the protein. However, the largest one would not contribute more than 5 kDa of the total estimated molecular weight. Figure 3B shows that the intensity of the signal is strongly affected by the addition of the unlabeled GPx SECIS probe. Addition of a 2-fold molar excess reduces the intensity of the band (lane 2), while a 50-fold excess abrogates it (lane 4). The crosslink is unaffected by the presence of the 5S RNA competitor at a 2-fold molar excess (lane 5) and very moderately at a 10-fold molar excess (lane 6). A slight decrease in the intensity of the band is observed at a 50-fold molar excess (lane 7). Minor bands migrating below 66 kDa appeared in Figure 3A and at 25 kDa in Figure 3B. They very likely arose from incomplete RNase T1 digestion and to differential susceptibility of GPx SECIS and 5S RNAs to RNase T1. These experiments, in conjunction with the competition bandshift assays presented in Figure 2A, provide evidence that a protein, ranging between 60–65 kDa on SDS–PAGE gels, binds specifically to the GPx fragment carrying the SECIS element. We have called this protein SBP, for Secis Binding Protein.

A competition gel shift assay was carried out in an attempt to determine whether the same SBP or a different protein binds to the GPx and 5′DI SECIS elements. The labeled GPx probe was

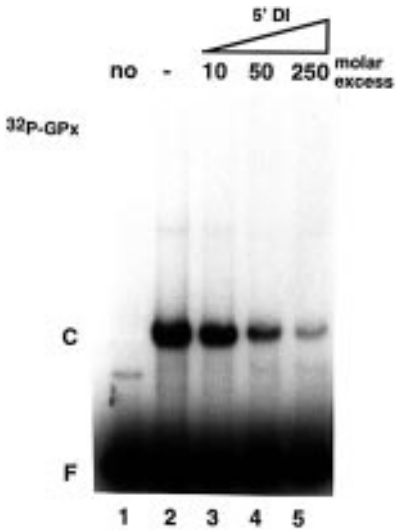

Figure 4. The 5′DI SECIS element can challenge the interaction between SBP and the GPx SECIS element. The labeled GPx probe (lane 1, no extract) was incubated with the extract in the absence (lane 2) or the presence of increasing concentrations of unlabeled 5′DI SECIS element (lanes 3–5). Symbols are as in Figures 1 and 2.

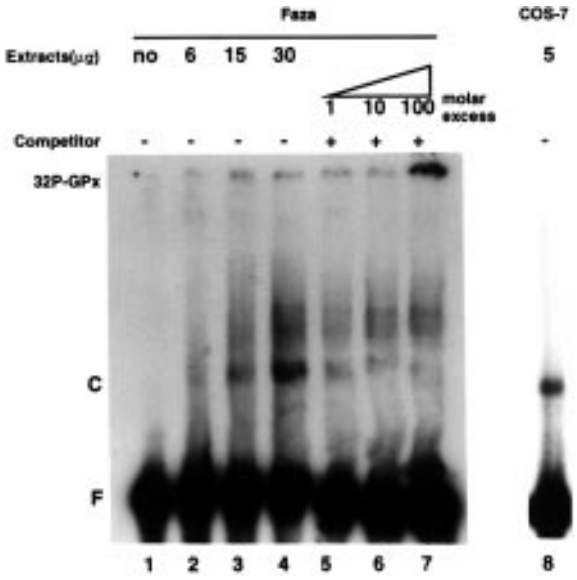

Figure 5. A complex can form between the GPX SECIS element and various cell type extracts. A complex was also observed with Faza (lanes 2–7) and COS-7 (lane 8) extracts. The Faza cell type is described in Materials and Methods. Lane 1, incubation in the absence of extract (no). Lanes 2–4, incubation with increasing amounts (indicated above the lanes) of Faza extracts. Lanes 5–7, competition experiment performed with the unlabeled GPx fragment added at the molar excess shown above the lanes. The experiment shown in lane 8 results from a separate migration. Symbols are as in Figure 1.

incubated in the presence of increasing concentrations of unlabeled 5′DI SECIS fragment. Figure 4, lane 3, indicates that adding a 10-fold molar excess of 5′DI SECIS influences moderately the formation of complex C since the intensity of the band is 70% of the value measured in lane 2. Increasing the concentration of the competitor affected more significantly the formation of the complex since a 250-fold molar excess leads to 8% of residual binding. This experiment attests that the 5′DI SECIS element does compete with the GPx homologue for binding to SBP, indicating that this protein can bind one SECIS element or the other. However, and interestingly, it is remarkable that the GPx SECIS shows a competitive advantage over the 5′DI SECIS since a 10-fold excess of GPx SECIS competitor provoked a 60% drop in binding (Fig. 2A, lane 3), while the same excess of 5′DI SECIS competitor induced only a 30% drop (Fig. 4, lane 3).

The GPx SECIS element can form complexes with extracts from different cell types

To determine whether other cell types possess the ability to induce a gel shift, the GPx probe was assayed with Faza and COS-7 cell extracts. The Faza cell line is a 8-azaguanine resistant subclone of the Reuber H-35 rat hepatoma (28). Figure 5 indicates that a retarded band with electrophoretic mobility similar to that in Figure 1A, is observed in lanes 2–4 with Faza extracts. Another band with lower mobility also appears in lanes 3 and 4. To determine whether it contains a specific complex, we undertook a competition assay with the unlabeled GPx SECIS fragment. Figure 5, lanes 5 to 7, shows that the intensity of the band containing the faster migrating complex diminishes progressively and disappears at a 100-fold molar excess of competitor (lane 7), while the intensity of the upper band remains unaffected. This establishes that the faster migrating complex observed in lanes 2–6 is specific, the other one resulting from an unspecific interaction with the GPx SECIS fragment. An assay performed with the labeled GPx RNA probe and COS-7 cell extracts indicated that a bandshift could also be obtained with this type of cell extract (Fig. 5, lane 8). The unspecific band formed in the presence of Faza, but not HeLa and COS-7 extracts. This may be related to the occurrence of a number of RNA-binding proteins in the rat liver, from which the Faza cell line derives.

The GPx SECIS element is thus capable of forming a complex with a protein, very likely SBP, contained in mammalian extracts originating from Faza and COS-7 cells.

DISCUSSION

We have presented in this work evidence that a protein binds specifically to the SECIS element, an RNA motif essential for selenocysteine incorporation (23,24) residing in the 3′-UTR of selenoprotein mRNAs. We have called this protein SBP, for Secis Binding Protein. It has a molecular weight of 60–65 kDa. This is the first report of a protein binding to the 3′-UTR of selenoprotein mRNAs. The RNA–protein interaction was detected with the GPx SECIS element in HeLa, COS-7 and Faza (rat hepatoma cell line) cell extracts. One single specific complex was obtained with the glutathione peroxidase SECIS element and SBP. Surprisingly, binding experiments undertaken with the type I iodothyronine 5′ deiodinase SECIS element led to the isolation of two retarded bands C1 and C2 by mobility shift assay, but we cannot provide any explanation for this differential situation. Competition experiments indicated that both C1 and C2 are specific since they are not challenged by addition of 5S RNA. The fact that complex C2 is displaced by lower concentrations of specific competitor than C1, suggests that each complex might result from the binding of a different protein. The identity of the protein(s) contained in complexes C1 and C2 has not been addressed in this work.

However, the finding that the SECIS element of the 5′DI is able to displace the complex formed with the GPx SECIS element and SBP provides circumstantial evidence that SBP can bind the 5′DI SECIS element, as well, and is contained in C1 or/and C2. Interestingly, comparison of the competition experiments shown in Figures 2A and 4 suggests that SBP possesses a higher affinity for the GPx SECIS element than for the 5′DI counterpart.

The immediate question raised by our finding relates to the function of SBP and the role occupied by the SECIS–SBP interaction. SBP binds specifically to the SECIS element, which in turn is necessary for selenocysteine insertion at the UGA codon. This observation strongly argues in favor of SBP being a genuine component of the eukaryotic machinery. In this respect, it is tempting to draw a parallel between SELB in prokaryotes (7) and SBP in eukaryotes, which both bind an RNA stem–loop structure essential for selenocysteine incorporation. Thus, it might well be that SBP is the eukaryotic homologue to SELB. Obviously, elucidation of the actual role played by SBP awaits its biochemical purification and cDNA cloning, a task being currently underway in our laboratory.

One fundamental difference between prokaryotes and eukaryotes consists in the fact that the RNA stem–loop is located immediately 3′ to the UGA codon in the former, while the SECIS element resides in the 3′-UTR in eukaryotes. Apart from the fact that this dichotomy raises several stimulating mechanistic problems, it also adds another example to the long and bewildering list of the multiple functions achieved by 3′-UTRs in eukaryotes (see refs 30,31 for review).

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