Identification of mRNA that binds to eukaryotic initiation factor 5A by affinity co-purification and differential display

Aiguo XU*, David Li-En JAO* and Kuang Yu CHEN*†[1]

*Department of Chemistry and Chemical Biology, Rutgers The State University of New Jersey, 610 Taylor Road, Piscataway, NJ 08854-8087, U.S.A., and †The Cancer Institute of New Jersey, 195 Little Albany Street, Piscataway, NJ 08854-8087, U.S.A.

Eukaryotic initiation factor 5A (eIF-5A) is the only protein in nature that contains hypusine, an unusual amino acid formed post-translationally by deoxyhypusine synthase and deoxyhypusine hydroxylase. Genetic and pharmacological evidence suggests that eIF-5A is essential for cell survival and proliferation. However, the precise function and interacting partners of eIF-5A remain unclear. We have shown previously that eIF-5A can bind to RRE (Rev-response element) and U6 RNA *in vitro*. Using SELEX (systematic evolution of ligands by exponential enrichment), we have also shown that eIF-5A is capable of binding to RNA in a sequence-specific manner [Xu and Chen (2001) J. Biol. Chem. **276**, 2555–2561]. In the present paper, we show that the identification of mRNA species that bind to eIF-5A can be achieved by affinity co-purification and PCR differential display. Using this approach with three sets of anchoring and arbitrary primers, we have found 20 RNA sequences that co-purified specifically with eIF-5A. Five of them contained AAAUGU, the putative eIF-5A-interacting element that we identified previously using the SELEX method. Direct binding of the cloned RNA to eIF-5A could be demonstrated by electrophoretic mobility-shift assay. BLAST analysis revealed that the eIF-5A-interacting RNAs encode proteins such as ribosomal L35a, plasminogen activation inhibitor mRNA-binding protein, NADH dehydrogenase subunit and ADP-ribose pyrophosphatase. Some, however, encode hypothetical proteins. All the cloned RNAs have the potential to form extensive stem-loop structures.

Key words: eukaryotic initiation factor 5A (eIF-5A), hypusine, RNA, systematic evolution of ligands by exponential enrichment (SELEX).

INTRODUCTION

Eukaryotic initiation factor 5A (eIF-5A) is the only protein in Nature that contains the hypusine amino acid residue. Hypusine is formed post-translationally through the action of deoxyhypusine synthase and deoxyhypusine hydroxylase [1–3]. Disruption of genes encoding either eIF-5A or deoxyhypusine synthase in yeast leads to a lethal phenotype [4,5]. Inhibition of deoxyhypusine synthase activity in mammalian cells causes growth arrest [6,7], cell death [8] or tumour differentiation [9]. In addition, hypusine formation exhibits a striking attenuation in senescent cells [10], but a marked increase in virally transformed cells [11].

Although eIF-5A is essential for cell survival and proliferation, its physiological function remains unclear. Owing to a lack of correlation with general protein synthesis, it has been suggested that eIF-5A may not be a *bona fide* translation initiation factor [12]. Other studies have suggested that eIF-5A may serve as an adaptor protein for the nuclear export of Rev or Rex [13–15]. However, this notion has been questioned because a direct interaction between eIF-5A and Rev or Rex cannot be established *in vitro* [16–18]. Moreover, Rev export can occur at the restrictive temperature in a yeast strain that harbours a non-functional mutant eIF-5A [19], indicating that Rev export does not require eIF-5A.

The archaeal eIF-5A contains two domains connected by a short hinge [20,21]. The N-terminal domain of eIF-5A contains a hypusine residue, which carries two positive charges and closely resembles spermidine and spermine. Abundant evidence in the literature indicates that spermidine and spermine interact specifically with DNA or RNA [22,23]. The C-terminal domain resembles the CSD (cold-shock domain), common in DNA- and RNA-binding proteins [24]. The structural features of eIF-5A suggest that it has the potential to interact with nucleic acids. In this regard, we have demonstrated that eIF-5A binds to RRE (Rev-response element) and U6 RNA, and, importantly, the binding is hypusine-dependent [25]. We have also shown that eIF-5A can bind to SELEX (systematic evolution of ligands by exponential enrichment)-enriched RNA in a hypusine-dependent manner [26]. The SELEX-enriched RNA shares conserved motifs UAACCA and AAAUGU [26].

To search for the physiological RNA targets of eIF-5A, we have now employed an approach similar to SNAAP (specific nucleic acid associated with protein) that has been used previously for identifying the natural mRNA targets for α-CP-1 (α-complex protein 1) [27]. Using both affinity chromatography and immunoaffinity binding, we showed in the present study the feasibility of this approach in the isolation and identification of the potential physiological RNA targets of eIF-5A.

EXPERIMENTAL

Cell culture

HeLa cells, with or without stably transfected plasmids (pFLAG-GFP or pFLAG-GFP-eIF-5A, where GFP is green fluorescent protein), were cultured in Dulbecco's medium supplemented with 10 % (v/v) foetal bovine serum. Cells at late exponential phase of growth were harvested for the isolation of total RNA or cell extract.

Abbreviations used: α-CP-1, α-complex protein 1; eIF-5A, eukaryotic initiation factor 5A; GFP, green fluorescent protein; Ni-NTA, Ni^{2+}-nitrilotriacetate; RB buffer, RNA-binding buffer; RRE, Rev-response element; SELEX, systematic evolution of ligands by exponential enrichment; SNAAP, specific nucleic acid associated with protein.

[1] To whom correspondence should be addressed (email KYCHEN@rutchem.rutgers.edu).

Reagents and enzymes

For the differential display, the primers and reagents were purchased from GenHunter (Nashville, TN, U.S.A.). Molecular biological supplies were from Amersham Biosciences (Piscataway, NJ, U.S.A.). All other chemicals were of reagent grade.

Expression and purification of modified and unmodified 6 × His–eIF-5A

The unmodified histidine-tagged eIF-5A precursor, designated as 6 × His-18K^0, was purified from *Escherichia coli* strain BL 21 (DE3) harbouring pQEh18K [26]. The hypusine-containing histidine-tagged eIF-5A, designated as 6 × His-18K^{hy}, was purified from yeast strain 2602 that harbours pYER18K as described previously [26].

Preparation of S130 extract and total RNA from HeLa cells

The cytoplasmic S130 extract was prepared according to the procedure described by Trifillis et al. [27]. Briefly, cells were harvested and washed, and the cell pellets were frozen at − 70 °C. The frozen cell pellets were thawed in the presence of RNA-binding buffer (RB buffer: 10 mM Tris/HCl, pH 7.5, 1.5 mM $MgCl_2$, 10 mM 2-mercaptoethanol, 150 mM NaCl, 0.1 mM EDTA, 1 mM PMSF and 1 unit of RNase inhibitor) at about 0.5 ml per 100-mm-diameter dish of cells. The thawed cells were homogenized with a Dounce homogenizer. The cell debris and nuclei were removed by a brief centrifugation (10 000 ***g*** for 5 min), and the supernatant was layered over a 30 % sucrose cushion and centrifuged at 130 000 ***g*** for 2 h. The supernatant collected was designated as the S130 fraction. The total RNA was prepared as described previously [10].

Affinity isolation of RNA bound to FLAG–GFP–eIF-5A

The S130 fractions obtained from transfected HeLa cells were mixed with equal volumes of anti-FLAG M2 agarose resin for 30 min at 4 °C, washed six times with RB buffer, followed by elution with FLAG peptide in RB buffer (5 μg/ml). The eluate was extracted by phenol/chloroform (1:1, v/v), followed by ethanol precipitation (with 20 μg of glycogen added), and washed by 70 % ethanol.

Affinity isolation of RNAs with 6 × His–eIF-5A on Ni-NTA (Ni^{2+}-nitrilotriacetate) resins

Approx. 20 μg of 6 × His–18K^{hy} or 6 × His–18^0 was bound to 50 μl of Ni-NTA-resin. After an incubation at 4 °C for 30 min, the resin was washed six times with 100 μl of RB buffer containing 0.5 % (v/v) Triton X-100, 2 μg/ml leupeptin and 0.5 % (v/v) aprotinin. The washed affinity resin was suspended in 100 μl of RB buffer. Total RNA (5 μg) was incubated with affinity resin at 4 °C for 1 h. The resin was subsequently washed six times in RB buffer containing 0.1 % (v/v) Triton X-100, followed by an elution with 0.4 M imidazole in RB buffer. The bound RNAs were extracted by phenol/chloroform (1:1, v/v), ethanol-precipitated, and washed with 70 % ethanol. The dried RNA was resuspended in 10 μl of DEPC (diethyl pyrocarbonate)-treated water.

Identification of co-purified RNA

RNA species isolated using either of the above methods were subjected to the differential display as described by Liang and Pardee [28]. The co-purified RNAs isolated were reverse transcribed with one of three different 3′-primers containing a stretch of 11 T nucleotides followed by an A, C or G nucleotide. The first strand was then used in a PCR amplification with the same 3′-primer and a set of random 5′-primers. The radiolabelled PCR products were resolved on 6 % (w/v) polyacrylamide gel, yielding a profile of the RNA population that interacted with the affinity column. The radiolabelled bands specific for resins containing eIF-5A were excised from the gel, eluted by soaking in water for 10 min and boiling for 15 min. The cDNAs eluted were re-amplified and cloned into the pCRII vector (Invitrogen, Carlsbad, CA, U.S.A.) and sequenced. The identity of the RNA species was then determined by using BLAST analysis.

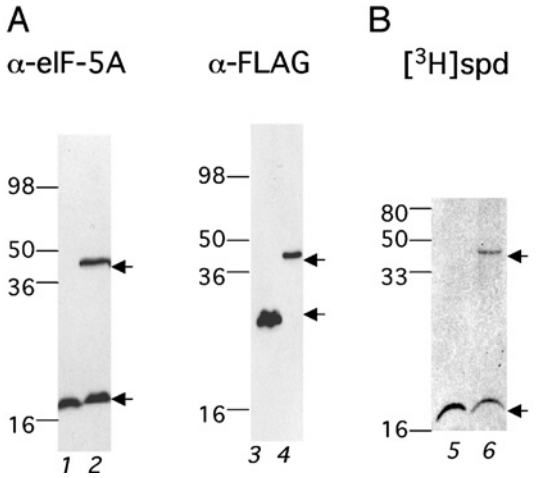

Figure 1 Expression of FLAG-tagged eIF-5A

(**A**) Western blot analysis of the eIF-5A fusion protein. Cell extracts from pFLAG-GFP- (lanes 1 and 3) and pFLAG-GFP-eIF5A- (lanes 2 and 4) transfected cells were subjected to SDS/12 % PAGE. Each lane contained 20 μg of proteins. In lanes 1 and 2, chicken anti-eIF-5A antibody (1:1000 dilution) was used. Endogenous eIF-5A and the FLAG–GFP–eIF-5A migrated at approx. 18 kDa and 45 kDa respectively, and are indicated with arrows. In lanes 3 and 4, mouse anti-FLAG M2 antibody (1:800 dilution) was used to detect FLAG–GFP (lane 3) and FLAG–GFP–eIF-5A (lane 4) fusion proteins. (**B**) Metabolic labelling of eIF-5A. pFLAG-GFP- and pFLAG-GFP-eIF-5A-transfected HeLa cells were incubated with [^{3}H]spermidine ([^{3}H]spd) (2 μCi/ml) for 48 h. Cell extracts were prepared and analysed by SDS/PAGE and fluorography. Lane 5, endogenous eIF-5A was radiolabelled. Lane 6, both endogenous eIF-5A and FLAG–GFP–eIF-5A fusion protein were radiolabelled. Molecular-mass standards are expressed in kDa to the left of the gels.

Electrophoretic mobility-shift assay

Electrophoretic mobility-shift assays were performed as described previously [25,26]. The cloned cDNA for the selected RNA was used as the template for *in vitro* transcription in the presence of [^{32}P]CTP to produce labelled RNA probes. Approx. 10 fmol of [^{32}P]RNA was incubated with various amounts of eIF-5A proteins for 15 min on ice in 15 μl of RB buffer. The mixture was electrophoresed on an 8 % (w/v) polyacrylamide gel containing 5 % (v/v) glycerol at 4 °C. The gel was dried on a membrane and visualized by autoradiography.

RESULTS

Stably transfected HeLa cells expressing FLAG–GFP–eIF-5A

In order to isolate the eIF-5A-interacting RNA using affinity tag, we have generated a stably transfected cell line that expresses a GFP–eIF-5A fusion protein containing a FLAG tag. We also generated a cell line expressing FLAG–GFP as a control. The inclusion of a GFP moiety facilitated the visualization and selection of the transfected cells. Figure 1 shows that the expressed level of eIF-5A fusion protein (the 45 kDa band) was equivalent to that of the endogenous eIF-5A (the 18 kDa band) (Figure 1,

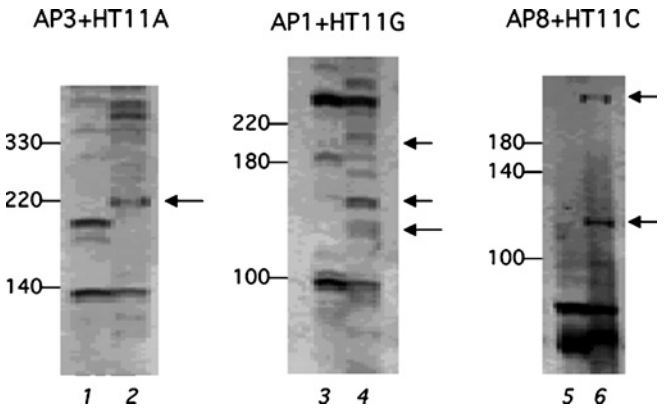

Figure 2 Differential display of specific nucleic acids associated with FLAG-tagged eIF-5A in cell extracts

pFLAG-GFP- and pFLAG-GFP-eIF-5A-transfected cells were used in immunoprecipitation assays for differential display as described in the Experimental section. The primer pairs used were AP3 + HT11A (lanes 1 and 2), AP1 + HT11G (lanes 3 and 4) and AP8 + HT11C (lanes 5 and 6). Lanes 1, 3 and 5, samples from pFLAG-GFP cells; lanes 2, 4 and 6, samples from pFLAG-GFP-eIF-5A cells. Arrows indicate the RNA species that specifically interacted with FLAG–GFP–eIF-5A. The sequences of the primers were: HT11A, 5′-AAGCTTTTTTTTTTA-3′; HT11G, 5′-AAGCTTTTTTTTTTG-3′; HT11C, 5′-AAGCTTTTTTTTTTC-3′; AP3, 5′-AAGCTTTGGTCAG-3′; AP1, 5′-AAGCTTGATTGCC-3′ and AP8, 5′-AAGCTTTTACCGC-3′. The size markers to the left of the gels are in bp.

lane 2 compared with lane 1). Only the tagged eIF-5A, but not endogenous eIF-5A, can be recognized by anti-FLAG M2 monoclonal antibody (Figure 1, lane 4 compared with lane 3), indicating that anti-FLAG M2 immunoaffinity resin may be used to isolate the RNA bound to the FLAG-tagged eIF-5A. To determine whether the FLAG fusion protein still retains biological activity, we performed a metabolic labelling experiment. As shown in Figure 1 (lane 6 compared with lane 5), when incubated with [^{3}H]spermidine in culture medium, the fusion protein was radiolabelled to the same extent as the endogenous eIF-5A, suggesting that the FLAG–GFP tag on the fusion protein did not interfere with its biological activity.

Affinity isolation of RNA species bound to FLAG–GFP–eIF-5A

Immunoaffinity resins constructed with anti-FLAG M2 antibody was employed to enrich RNA species that are bound to FLAG–GFP–eIF-5A in cell extracts. HeLa cells transfected with pFLAG-GFP were used as a control to eliminate the RNA species that may target only to the FLAG–GFP tag, but not to eIF-5A. The RNAs isolated from the control and the cells containing eIF-5A fusion proteins were displayed side by side on a polyacrylamide gel after reverse transcription and PCR amplification. Figure 2 shows a representative differential display profile using three different random primers and universal anchoring primers. The bands unique only for FLAG–GFP–eIF-5A were isolated and processed for cloning for characterization (Figure 2, lanes 2, 4 and 6 compared with lanes 1, 3 and 5). In total, we have obtained ten RNA clones, which were used for sequencing and BLAST analysis. The results are summarized in Table 1. Among the ten RNA clones, one did not match to any sequence in GenBank®; three encoded known proteins (L35a ribosomal protein, plasminogen activator inhibitor mRNA-binding protein and PRO3113 protein) and one encoded a hypothetical protein. The other four clones contained sequences corresponding to that in BAC or DNA clones in GenBank®, and thus the identity was unclear. Three out of ten sequences contained AAAUGU, the consensus sequence previously identified as the eIF-5A-interacting sequence in the post-SELEX RNA [26].

Table 1 RNA sequences identified by co-purification with FLAG–GFP–eIF-5A

Clone*	Accession no.	Protein encoded†
1	NM_000996	Ribosomal L35a protein
2	AL365332	RP11-212L9
3‡	BC020555	PAI-1 mRNA-binding protein
4	AC096656	RP11-20F18
5‡	AL355112	R-159D23
7‡	AL138689	RP11 272L14
8	AC000353	18h3
9	XM_088688	FLJ20378 protein
10	AF305828	PRO3113 protein

* Clone 6 did not match any sequence in GenBank®.

† Clones 2, 4, 5, 7 and 8 matched sequences found in the DNA clones listed in GenBank® with the accession number as indicated. Whether these sequences encode mRNA or not remains to be investigated.

‡ mRNA containing the eIF-5A-interacting sequence AAAGUG.

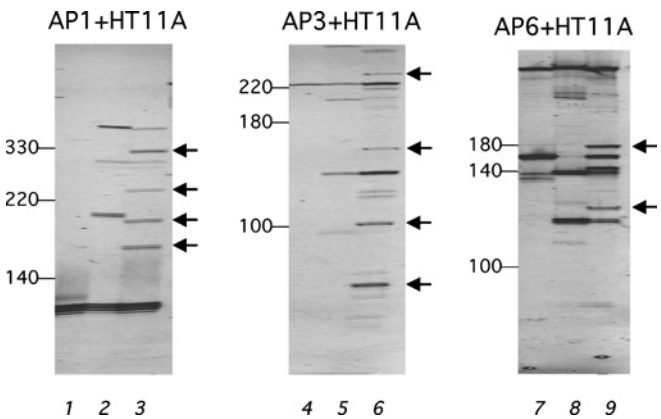

Figure 3 Differential display of specific nucleic acids associated with 6 × His–18K^{hy} by gel affinity chromatography

RNA species specifically bound to 6 × His–18K^{hy} were displayed on a polyacrylamide gel and are indicated by arrows. PCR was carried out using primer pairs AP1 + HT11A (lanes 1–3), AP3 + HT11A (lanes 4–6) and AP6 + HT11A (lanes 7–9). Lanes 1, 4 and 7, PCR products interacting with Ni-NTA; lanes 2, 5 and 8, PCR products interacting with 6 × His–18K^0–Ni-NTA resin; lanes 3, 6 and 9, PCR products interacting with 6 × His–18K^{hy}–Ni-NTA resin. HT11A, 5′-AAGCTTTTTTTTTTTA-3′; AP3, 5′-AAGCTTTGGTCAG-3′; AP1, 5′-AAGCTTGATTGCC-3′; AP6, 5′-AAGCTTGCACCAT-3′. The size markers are indicated to the left of the gels in bp.

Affinity isolation of RNA species directly bound by 6 × His–eIF-5A

Since S130 cell extracts were used as the source for the immunoaffinity co-purification as described above, the RNA isolated could interact with eIF-5A directly or indirectly via other proteins. We therefore tested another type of affinity co-purification using total RNA as the source for affinity chromatography. Total RNA isolated from HeLa cells was loaded on to an eIF-5A-affinity column, which was constructed by attaching 6 × His–18K^{hy} to Ni-NTA resin. The Ni-NTA alone or Ni-NTA coupled with 6 × His–18K^0, the unmodified version of eIF-5A, was employed as controls. The RNA specifically retained in the 6 × His–18K^{hy} affinity column was isolated and differentially displayed. Figure 3 shows the differential display result. The bands specific only to eIF-5A (6 × His–18K^{hy}) were identified visually, amplified, and cloned for sequencing. We obtained ten different RNA sequences for BLAST analysis and Table 2 summarizes the results. Three of these RNA clones contained the AAAUGU consensus sequence. The proteins encoded by these RNAs include mitochondrial NADH

Table 2 RNA identified by 6 × His–eIF-5A affinity chromatography

Clone*	Accession no.	Protein encoded†
11	AAL54514	NADH dehydrogenase subunit 1
12	NM_014673	KIAA0103 gene product
13‡	BC020555	PAI-1 mRNA-binding protein
14	AK021675	cDNA clone FLJ11613
15	BC000666	CGI-31 protein
16‡	AL353774	Clone RP11-267011
17	AY026252	ADP-ribose pyrosphosphatase NUDT9
19‡	Z84466	Clone RP1-230G1
20	AC005365	Clone 79-2A

* Clone 18 did not match any sequence in GenBank®.

† Clones 16, 19 and 20 matched sequences in DNA clones listed in GenBank® with the accession number as indicated. Whether these sequences encode mRNA or not remains to be investigated.

‡ mRNA containing the eIF-5A-interacting sequence AAAGUG.

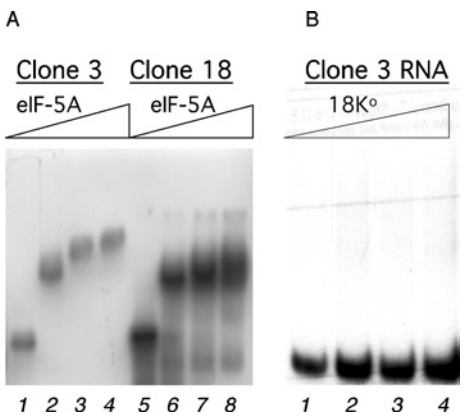

Figure 4 Electrophoretic mobility-shift analysis of the cloned RNA

(**A**) Radioactively labelled RNA obtained by *in vitro* transcription of RNA clones was incubated with 6 × His–eIF-5A ($18K^{hy}$) at 0 (lanes 1 and 5), 0.25 (lanes 2 and 6), 0.75 (lanes 3 and 7) and 2.5 (lanes 4 and 8) μg for 15 min. Lanes 1–4: Clone 3 RNA; lanes 5–8: Clone 18 RNA. The electrophoretic mobility-shift assay was performed on an 8 % polyacrylamide gel as described in the Experimental section. (**B**) A similar binding experiment was performed with Clone 3 RNA using unmodified eIF-5A ($18K^0$) at 0, 0.25, 0.75 and 2.5 μg (from lane 1 to lane 4 respectively). The electrophoretic mobility-shift assay was performed as described in the Experimental section.

dehydrogenase subunit 1, PAI-1 mRNA-binding protein, ADP-ribose pyrophosphatase, CGI-31 protein, KIAA0103 protein and a hypothetical protein (FLJ11613). The other clones contained sequences corresponding to certain DNA clones in GenBank®.

The binding of eIF-5A to cloned RNA

To confirm that the RNA species obtained by co-purification do interact with eIF-5A (hypusine-containing), but not with the unmodified eIF-5A precursor, we have performed electrophoretic mobility-shift experiments using radiolabelled RNA transcribed from the RNA clones, one isolated using anti-FLAG column (Clone 3), the other with the eIF-5A column (Clone 18). Clone 3 RNA, but not that of Clone 18, contains the AAAUGU consensus sequence. The modified eIF-5A (hypusine-containing) and the unmodified version (denoted as $18K^0$) were prepared from yeast and *E. coli* respectively as described previously [26]. Both Clone 3 RNA and Clone 18 RNA exhibited binding to eIF-5A with similar affinity. We noticed a slight decrease in the mobility of the binding complex of eIF-5A and Clone 3 RNA as the amount of eIF-5A increased in the binding mixture (Figure 4A, lane 4 compared with lane 2). This phenomenon could reflect additional binding sites for eIF-5A on this RNA. As a control, we showed in Figure 4(B) that the unmodified eIF-5A precursor did not bind to Clone 3 RNA at comparable concentrations, indicating that the presence of hypusine on eIF-5A was needed for binding at the dosage tested.

Secondary structure of cloned RNAs

It is well known that the recognition of proteins to RNA and DNA differs in many respects, primarily due to the structural diversity of RNA compared with that of DNA [29]. Unlike DNA-binding proteins, many RNA-binding proteins target structural elements, such as hairpins, bulges and stem-loops, instead of defined sequence [29]. For example, the high-affinity binding of HIV-1 Rev to RRE strictly depends on the presence of extensive stem-loop structure in domain II of RRE [30]. Since not all of the RNA clones that we identified contain the consensus AAAUGU, we speculate that some structural elements in RNA are likely to be involved in the eIF-5A–RNA interaction. To explore this possibility, we asked whether the cloned RNAs do possess certain common structural elements, which may serve as the eIF-5A-recognition sites. We have employed the RNA-folding programs of Zuker et al. [31] to analyse the RNA secondary structure of all the cloned RNAs using the energy-minimalization method. The predicted structures are illustrated in Figure 5. We found that all the cloned RNAs exhibit extensive secondary structures containing structural elements, such as hairpins and internal loops. Although further work is needed to determine the molecular nature of the binding sites, it seems reasonable to assume that, if these structural elements do occur in cloned RNAs under physiological conditions, some of them may serve as the recognition sites for eIF-5A.

DISCUSSION

It has been known for more than a decade that eIF-5A is an essential protein for cell survival and proliferation [1–3]. However, its function has been difficult to define, primarily due to lack of information on its interacting partners. *In vitro* study has shown that eIF-5A binds to RRE and U6 RNA [25], and that hypusine is required for a sequence-specific interaction of eIF-5A with post-SELEX RNA [26]. Based on these findings, we have proposed that eIF-5A may function as an RNA-binding protein. The combined use of affinity co-purification and differential display, termed SNAAP, has been shown to be versatile in identifying target RNA of putative RNA-binding proteins [27]. For example, α-globulin mRNA, but not the closely related γ-globulin mRNA, was identified as the target RNA of GST (glutathione S-transferase)-tagged α-CP-1 by using this method [27]. In the present study, we have adopted a similar approach in order to search for and to identify the physiological mRNA targets of eIF-5A.

We showed that a selected group of mRNA co-purified with eIF-5A, either by co-immunoprecipitation with FLAG-tagged eIF-5A (Table 1) or by binding directly to the His-tagged eIF-5A (Table 2). The identification of RNA sequences that could bind to eIF-5A *in vivo* allows further study of the significance of these interactions. Although different starting materials were used for the two affinity isolation methods, it is interesting to note that both methods yielded the same RNA clone encoding PAI-1 mRNA-binding protein.

Since our current goal is to demonstrate that eIF-5A could interact with physiological RNA, we only used three sets of primer pairs to perform differential display. Previous calculation suggests that 80 arbitrary primers and three anchor primers will be required in differential display to cover all the expressed genes [32]. Thus, based on the number of primer sets that we used in this study,

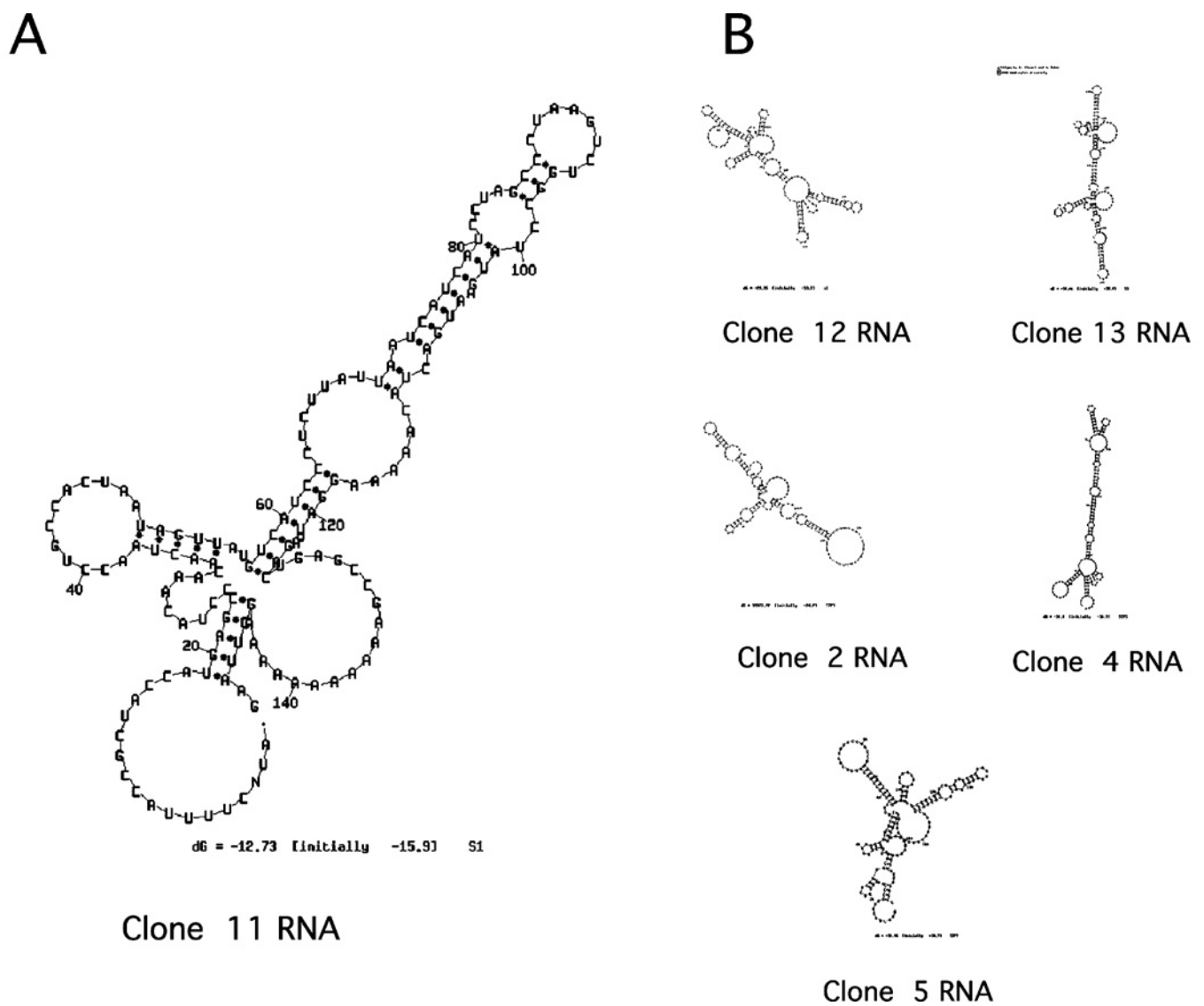

Figure 5 Secondary structures of cloned RNA

The secondary structures of various cloned RNAs were predicted by energy minimalization using the Program Mfold, version 3.1 [31].

we estimate that eIF-5A may interact with at least 800 different mRNAs, representing approx. 5% of total possible sequences *in vivo*. This estimation is consistent with a previous proposal that eIF-5A may be required for translation of a small set of mRNAs [12]. Since eIF-5A shares a structural similarity with CspA, a putative RNA chaperone [10,21], it may function as an RNA chaperone during translation of a small class of mRNA with extensive secondary structures. In this regard, it can be noted that all the isolated RNA clones have the potential to form extensive secondary structures (Figure 5). Alternatively, the interaction of eIF-5A with selected groups of RNA may be related to the regulation of the metabolism of these RNAs. This notion is consistent with findings that link eIF-5A to mRNA stability and degradation [19,33]. To delineate further the physiological function of eIF-5A, we can now focus our attention on the role of eIF-5A in the metabolism and regulation of these eIF-5A-interacting RNA species.

We thank Dr Herbert Tabor and Dr Celia Tabor for pYES2, and Dr Rashmi Gupta for constructing pYER18K. The work was supported in part by United States Public Health Services Grant RO1 CA49695 awarded by the National Cancer Institute, National Institutes of Health.

REFERENCES

1 Park, M. H., Lee, Y. B. and Joe, Y. A. (1997) Hypusine is essential for eukaryotic cell proliferation. Biol. Signals **6**, 115–123
2 Chen, K. Y. and Liu, A. Y. (1997) Biochemistry and function of hypusine formation on eukaryotic initiation factor 5A. Biol. Signals **6**, 105–109
3 Chen, K. Y. and Jao, D. L. (1999) Chemistry of hypusine formation on eukaryotic initiation factor 5A in biological systems. J. Chin. Chem. Soc. **46**, 727–734
4 Schnier, J., Schwelberger, H. G., Smit-McBride, Z., Kang, H. A. and Hershey, J. W. B. (1991) Translation initiation factor 5A and its hypusine modification are essential for cell viability in the yeast *Saccharomyces cerevisiae*. Mol. Cell. Biol. **11**, 3105–3114
5 Sasaki, K., Abid, M. R. and Miyazaki, M. (1996) Deoxyhypusine synthase gene is essential for cell viability in the yeast *Saccharomyces cerevisiae*. FEBS Lett. **384**, 151–154
6 Jakus, J., Wolff, E. C., Park, M. H. and Folk, J. E. (1993) Features of the spermidine-binding site of deoxyhypusine synthase as derived from inhibition studies: effective inhibition by bis- and mono-guanylated diamines and polyamines. J. Biol. Chem. **268**, 13151–13159

7 Park, M. H., Wolff, E. C., Lee, Y. B. and Folk, J. E. (1994) Antiproliferative effects of inhibitors of deoxyhypusine synthase. J. Biol. Chem. **269**, 27827–27832
8 Tome, M. E., Fiser, S. M., Payne, C. M. and Gerner, E. W. (1997) Excess putrescine accumulation inhibits the formation of modified eukaryotic initiation factor 5A (eIF-5A) and induces apoptosis. Biochem J. **328**, 847–854
9 Chen, Z. P., Yan, Y. P., Ding, Q. J., Knapp, S., Potenza, J. A., Schugar, H. J. and Chen, K. Y. (1996) Effects of inhibitors of deoxyhypusine synthase on the differentiation of mouse neuroblastoma and erythroleukemia cells. Cancer Lett. **105**, 233–239
10 Chen, Z. P. and Chen, K. Y. (1997) Dramatic attenuation of hypusine formation on eukaryotic initiation factor 5A during senescence of IMR-90 human diploid fibroblasts. J. Cell. Physiol. **170**, 248–254
11 Chen, Z. P. and Chen, K. Y. (1997) Marked elevation of hypusine formation activity on eukaryotic initiation factor 5A in v-HA-RAS transformed mouse NIH3T3 cells. Cancer Lett. **115**, 235–241
12 Kang, H. A. and Hershey, J. W. (1994) Effect of initiation factor eIF-5A depletion on protein synthesis and proliferation of *Saccharomyces cerevisiae*. J. Biol. Chem. **269**, 3934–3940
13 Ruhl, M., Himmelspach, M., Bahr, G. M., Hammerschmid, F., Jaksche, H., Wolff, B., Aschauer, H., Farrington, G. K., Probst, H., Bevec, D. and Hauber, J. (1993) Eukaryotic initiation factor 5A is a cellular target of the human immunodeficiency virus type 1 Rev activation domain mediating trans-activation. J. Cell Biol. **123**, 1309–1320
14 Katahira, J., Ishizaki, T., Sakai, H., Adachi, A., Yamamoto, K. and Shida, H. (1995) Effects of translation initiation factor eIF-5A on the functioning of human T-cell leukemia virus type I Rex and human immunodeficiency virus Rev inhibited trans dominantly by a Rex mutant deficient in RNA binding. J. Virol. **69**, 3125–3133
15 Bevec, D., Jaksche, H., Oft, M., Wohl, T., Himmelspach, M., Pacher, A., Schebesta, M., Koettnitz, K., Dobrovnik, M., Csonga, R. et al. (1996) Inhibition of HIV-1 replication in lymphocytes by mutants of the Rev cofactor eIF-5A. Science **271**, 1858–1860
16 Henderson, B. R. and Percipalle, P. (1997) Interactions between HIV Rev and nuclear import and export factors: the Rev nuclear localization signal mediates specific binding to human importin-b. J. Mol. Biol. **274**, 693–707
17 Mattaj, I. W. and Englmeier, L. (1998) Nucleocytoplasmic transport: the soluble phase. Annu. Rev. Biochem. **67**, 265–306
18 Lipowsky, G., Bischoff, F. R., Schwarzmaier, P., Kraft, R., Kostka, S., Hartmann, E., Kutay, U. and Gorlich, D. (2000) Exportin 4: a mediator of a novel nuclear export pathway in higher eukaryotes. EMBO J. **19**, 4362–4371
19 Valentini, S. R., Casolari, J. M., Oliveira, C. C., Silver, P. A. and McBride, A. E. (2002) Genetic Interactions of yeast eukaryotic translation initiation factor 5A (eIF5A) reveal connections to poly(A)-binding protein and protein kinase C signaling. Genetics **160**, 393–405
20 Peat, T. S., Newman, J., Waldo, G. S., Berendzen, J. and Terwilliger, T. C. S. (1998) Structure of translation initiation factor 5A from *Pyrobaculum aerophilum* at 1.75 Å resolution. Structure **6**, 1207–1214
21 Kim, K. K., Hung, L. W., Yokota, H., Kim, R. and Kim, S. H. (1998) Crystal structures of eukaryotic translation initiation factor 5A from *Methanococcus jannaschii* at 1.8 Å resolution. Proc. Natl. Acad. Sci. U.S.A. **95**, 10419–10424
22 Frydman, L., Rossomando, P. C., Frydman, V., Fernandez, C. O., Frydman, B. and Samejima, K. (1992) Interactions between natural polyamines and tRNA: an ^{15}N NMR analysis. Proc. Natl. Acad. Sci. U.S.A. **89**, 9186–9190
23 Bancroft, D., Williams, L. D., Rich, A. and Egli, M. (1994) The low-temperature crystal structure of the pure-spermine form of Z-DNA reveals binding of a spermine molecule in the minor groove. Biochemistry **33**, 1073–1086
24 Wolffe, A. (1994) Structural and functional properties of the evolutionarily ancient Y-box family of nucleic acid binding proteins. BioEssays **16**, 245–251
25 Liu, Y. P., Nemeroff, M., Yan, Y. P. and Chen, K. Y. (1997) Interaction of eukaryotic initiation factor 5A with the human immunodeficiency virus type 1 Rev response element RNA and U6 snRNA requires deoxyhypusine or hypusine modification. Biol. Signals **6**, 166–174
26 Xu, A. and Chen, K. Y. (2001) Hypusine is required for a sequence-specific interaction of eukaryotic initiation factor 5A with postsystematic evolution of ligands by exponential enrichment RNA. J. Biol. Chem. **276**, 2555–2561
27 Trifillis, P., Day, N. and Kiledjian, M. (1999) Finding the right RNA: identification of cellular mRNA substrates for RNA-binding proteins. RNA **5**, 1071–1082
28 Liang, P. and Pardee, A. B. (1992) Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. Science **257**, 967–971
29 Varani, G. (1998) RNA–protein recognition during RNA processing and maturation. In The Many Faces of RNA (Eggleston, D. S., Prescott, C. D. and Pearson, N. D., eds.), pp. 67–82, Academic Press, London
30 Heaphy, S., Finch, J. T., Gait, M. J., Karen, J. and Singh, M. (1991) Human immunodeficiency virus type 1 regulator of virion expression, rev, forms nucleoprotein filaments after binding to a purine-rich "bubble" located within the rev-responsive region of viral mRNAs. Proc. Natl. Acad. Sci. U.S.A. **88**, 7366–7370
31 Zuker, M., Mathews, D. H. and Turner, D. H. (1999) Algorithms and thermodynamics for RNA secondary structure prediction: a practical guide. In RNA Biochemistry and Biotechnology (Barciszewski, J. and Clark, B. F. C., eds.), pp. 11–43, Kluwer Academic Publishers, Dordrecht
32 Liang, P. and Pardee, A. B. (1994) Methods of differential display. Methods Mol. Genet. **5**, 3–16
33 Zuk, D. and Jacobson, A. (1998) A single amino acid substitution in yeast eIF-5A results in mRNA stabilization. EMBO J. **17**, 2914–2925

Received 21 July 2004/accepted 10 August 2004
Published as BJ Immediate Publication 10 August 2004, DOI 10.1042/BJ20041232