Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 365 (2008) 62–68
BBRC
www.elsevier.com/locate/ybbrc

# Mouse nucleolin binds to 4.5S RNAH, a small noncoding RNA

Yutaka Hirose [a,*], Fumio Harada [b,*]

[a] Graduate School of Medical and Pharmaceutical Sciences, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan
[b] Department of Molecular and Cellular Biology, Cancer Research Institute, Kanazawa University, 13-1, Takaramachi, Kanazawa 920-0934, Japan

Received 18 October 2007
Available online 29 October 2007

**Abstract**

4.5S RNAH is a rodent-specific small noncoding RNA that exhibits extensive homology to the B1 short interspersed element. Although 4.5S RNAH is known to associate with cellular poly(A)-terminated RNAs and retroviral genomic RNAs, its function remains unclear. In this study, we analyzed 4.5S RNAH-binding proteins in mouse nuclear extracts using gel mobility shift and RNA–protein UV cross-linking assays. We found that at least nine distinct polypeptides (p170, p110, p93, p70, p48, p40, p34, p20, and p16.5) specifically interacted with 4.5S RNAH *in vitro*. Using anti-La antibody, p48 was identified as mouse La protein. To identify the other 4.5S RNAH-binding proteins, we performed expression cloning from a mouse cDNA library and obtained cDNA clones derived from nucleolin mRNA. We identified p110 as nucleolin using nucleolin-specific antibodies. UV cross-linking analysis using various deletion mutants of nucleolin indicated that the third of four tandem RNA recognition motifs is a major determinant for 4.5S RNAH recognition. Immunoprecipitation of nucleolin from the subcellular fractions of mouse cell extracts revealed that a portion of the endogenous 4.5S RNAH was associated with nucleolin and that this complex was located in both the nucleoplasm and nucleolus.


*Keywords:* Nucleolin; 4.5S RNA; RNA–protein interaction; Small RNA

4.5S RNAH is a small noncoding RNA that is associated with murine retroviral genomic RNAs [1] and with the nuclear and cytoplasmic poly(A)-terminated RNAs of mouse, rat, and hamster cells [2,3]. Its 91–94 nucleotides are transcribed by RNA polymerase III (Pol III) [4,5] and exhibit extensive homology to the rodent major interspersed repeat sequence B1 [6,7]. The rat and mouse genomes contain thousands of copies of 4.5S RNAH genes and pseudogenes [5,8,9], a large number of which reside in several long repeat units [5,8,10]. Although its half-life is short, 4.5S RNAH can be cross-linked *in vivo* by a psoralen derivative to cytoplasmic poly(A)-terminated RNA found in ribonucleoprotein particles (RNPs) [4,8].

The association between 4.5S RNAH and poly(A)-terminated RNA suggests that it might play a role in the processing or transport of particular mRNAs, however, its function remains unknown. Generally, small RNA is associated with specific proteins in cells and such complexes act as functional units. Therefore, characterization of 4.5S RNAH-binding proteins is important for our understanding of the function performed by 4.5S RNAH. To date, the autoantigen La is the only protein known to bind 4.5S RNAH [4]. La is an evolutionally well-conserved ~50 kDa nuclear protein that associates with all nascent Pol III transcripts [11,12]. Association occurs primarily at 3′-terminal uridylate tracts [11,13] and it has been suggested that La acts as a Pol III transcription termination factor [14].

To elucidate the function of 4.5S RNAH, we characterized 4.5S RNAH-binding proteins from mouse nuclear extracts. We found that at least nine distinct nuclear proteins specifically bound to 4.5S RNAH, two of which were identified as La protein and nucleolin. We determined that in nucleolin, the third of four RNA recognition motifs (RRMs) is a major determinant for recognizing 4.5S

* Corresponding authors. Fax: +81 76 434 5171 (Y. Hirose); fax: +81 76 234 4510 (F. Harada).
*E-mail addresses:* yh620@pha.u-toyama.ac.jp (Y. Hirose), harada@kenroku.kanazawa-u.ac.jp (F. Harada).

0006-291X/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2007.10.117

RNAH. In addition, we found that the nucleolin-4.5S RNAH complex exists in both the nucleoplasm and nucleolus in mouse cells.

## Materials and methods

Materials and methods section appears in Supplementary data.

## Results

### *4.5S RNAH interacts with multiple proteins in vitro*

To characterize the 4.5S RNAH-binding proteins in mouse nuclear extracts, we performed gel mobility shift analyses. $^{32}$P-labeled 4.5S RNAH was incubated with nuclear extracts prepared from C3H2K/Mo cells, then RNA–protein complexes were analyzed by nondenaturing polyacrylamide gel electrophoresis and five complexes (A–E) were detected (Fig. 1A, lane 2). These complexes were not formed when the nuclear extract was pre-incubated with proteinase K (data not shown), and thus they must comprise both proteins and 4.5S RNAH. To detect proteins that directly interact with 4.5S RNAH, we performed RNA–protein UV cross-linking analyses and detected nine distinct polypeptides named as p170, p110, p93, p70, p48, p40, p34, p20, and p16.5 (Fig. 1B, lane 2). Similar results were obtained using nuclear extracts from other mouse cell lines such as C3H2K, K-1, L1210, and FM3A (data not shown). As 4.5S RNAH has been shown to associate with the La autoantigen [4], we examined whether it could bind to mouse La protein in our assay system. Incubation of nuclear extracts with anti-La serum prior to the addition of 4.5S RNAH specifically inhibited D complex formation (Fig. 1A, lane 3) and reduced the p48 radioactivity level (Fig. 1B, lane 3). Since p48 and La correspond in size, our results suggest that they are the same protein and that most of the D complex consists of 4.5S RNAH and La protein.

The binding specificity of these complexes was examined by competition assay using a variety of unlabeled RNAs. Cross-linking studies indicated that unlabeled 4.5S RNAH efficiently competed labeled with 4.5S RNAH for all nine polypeptides (Fig. 1C, lanes 2–4). In contrast, we observed that 4.5S RNAI, which is a different rodent-specific 4.5S RNA transcribed by Pol III [15], efficiently competed for cross-linking of only p48 (Fig. 1C, lanes 5–7). This result is consistent with the observation that 4.5S RNAI associates with La protein [13] and further supports the idea that p48 is La protein. 4.5S RNAH exhibits extensive homology with the rodent interspersed B1 element. Maraia and co-workers reported that small cytoplasmic B1 (scB1) RNAs, which are 3′-processed Pol III transcripts of B1 elements, were expressed in a variety of mouse cells [16]. Thus, we examined whether scB1 RNA competes with 4.5S RNAH for protein complex formation. We observed that although this RNA did not compete as efficiently as 4.5S RNAH at the highest concentration, competition was detected for interactions with p20, p34, p48, p70, and p110 (Fig. 1C, lane 10).

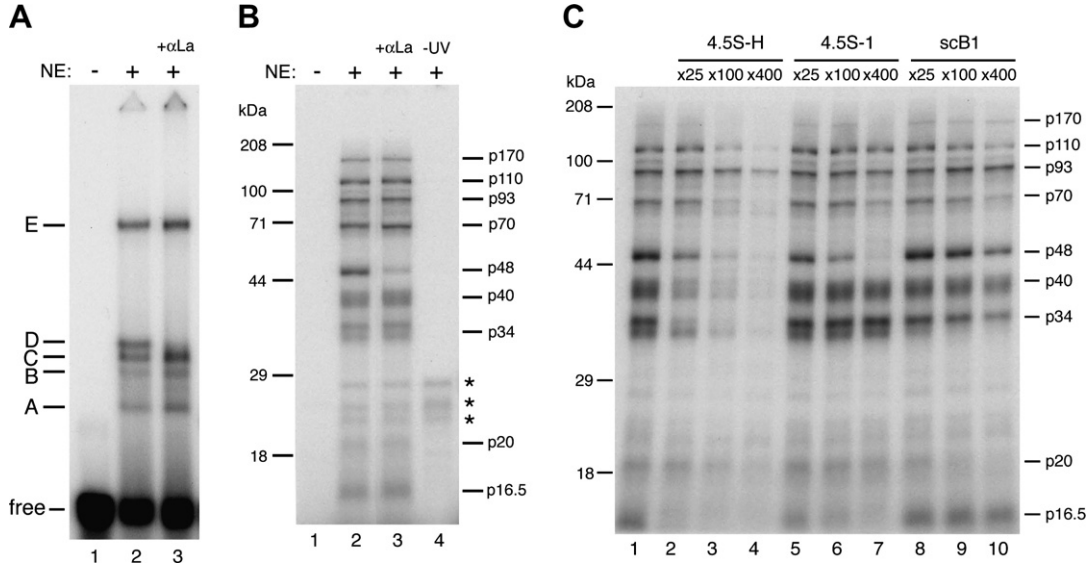


Fig. 1. 4.5S RNAH interacts with multiple nuclear proteins *in vitro*. (A) Gel mobility shift analyses of 4.5S RNAH-binding proteins. $^{32}$P-labeled 4.5S RNAH was incubated with no protein (lane 1) or nuclear extract prepared from C3H2K/Mo cells (lanes 2 and 3). RNA–protein complexes were analyzed by electrophoresis on 5% polyacrylamide native gels. Nuclear extract was pre-incubated with anti-La serum (lane 3). Complexes A–E and free 4.5S RNAH are indicated. (B) UV cross-linking analyses of 4.5S RNAH-binding proteins. The same reaction mixtures as in (A) were irradiated with UV light, digested with RNases A and T1, then analyzed by SDS–PAGE and autoradiography. An unirradiated reaction mixture was included (lane 4). Asterisks denote partial RNase digestion products of 4.5S RNAH. The major cross-linked polypeptides (right) and molecular weight standards (left) are indicated. (C) The specificities of various 4.5S RNAH–protein interactions were analyzed by competition with the unlabeled RNAs indicated. $^{32}$P-4.5S RNAH (2 ng) was incubated under standard binding conditions in the absence (lane 1) or presence of a 25- (lanes 2, 5, and 8), 100- (lanes 3, 6, and 9) or 400-fold (lanes 4, 7, and 10) molar excess of unlabeled RNAs, then analyzed by UV cross-linking.

*The 4.5S RNAH-binding protein p110 is nucleolin*

To obtain cDNAs encoding 4.5S RNAH-binding proteins, we screened a λgt 11 mouse cDNA expression library using $^{32}$P-labeled 4.5S RNAH as a probe. Three positive clones were isolated from $1 \times 10^6$ independent clones. Partial nucleotide sequences of these cDNAs were determined and a BLAST search of the NCBI database revealed that all three sequences matched mouse nucleolin cDNA. The clone containing a 1.5 kb cDNA insert is illustrated in Fig. 3A as λHBP1.

Nucleolin is a major nucleolar phosphoprotein that has an apparent molecular mass of 110 kDa, as well as an evolutionally conserved domain structure (Fig. 3A) [17]. The amino acid sequence encoded by λHBP1 contained four RNA recognition motifs (RRMs) and an RGG box (RGG). To examine whether p110 is mouse nucleolin, we prepared two types of nucleolin-specific polyclonal antibodies. These antisera were raised against the N-terminal 72 amino acids or the C-terminal 148 amino acids of mouse nucleolin and were referred to as anti-Nu$^n$ or anti-Nu$^c$ antibodies, respectively. Total protein from mouse C3H2K/Mo cells was examined by Western blot analysis and both anti-Nu$^n$ and anti-Nu$^c$ antibodies detected a predominant 110 kDa band (Fig. 2A, lanes 3 and 5), whereas the pre-immune sera did not (Fig. 2A, lanes 2 and 4). Both the anti-Nu$^n$ and anti-Nu$^c$ antibodies recognized recombinant mouse nucleolin (data not shown), and these results suggest that they also recognize the nonrecombinant endogenous form.

To test whether mouse nucleolin specifically binds to 4.5S RNAH *in vitro*, we pre-incubated mouse nuclear extracts with pre-immune serum, anti-Nu$^n$, anti-Nu$^c$ or anti-La antibodies and then performed a gel mobility shift assay. With both anti-nucleolin antibodies we observed a significant reduction in complex C formation and several super-shifted bands appeared in the higher molecular weight region (Fig. 2B, lanes 4 and 5). In contrast, these changes did not occur following pre-incubation with pre-immune or anti-La sera (Fig. 2B, lanes 3 and 6). Furthermore, the UV cross-linked p110 was specifically precipitated by anti-nucleolin antibody, whereas it was not precipitated by pre-immune sera (Fig. 2C). These results suggest that p110 is mouse nucleolin and that complex C contains nucleolin.

*The third RRM of nucleolin is a major determinant for binding 4.5S RNAH*

To investigate the region of nucleolin responsible for specific recognition of 4.5S RNAH, we performed UV cross-linking analyses using various deletion mutants of mouse nucleolin expressed as GST fusion proteins in *Escherichia coli* (Fig. 3A). Proteins were affinity purified with glutathione-Sepharose resin (Fig. 3B, lower panel) and equimolar amounts (40 pmol) of each mutant were

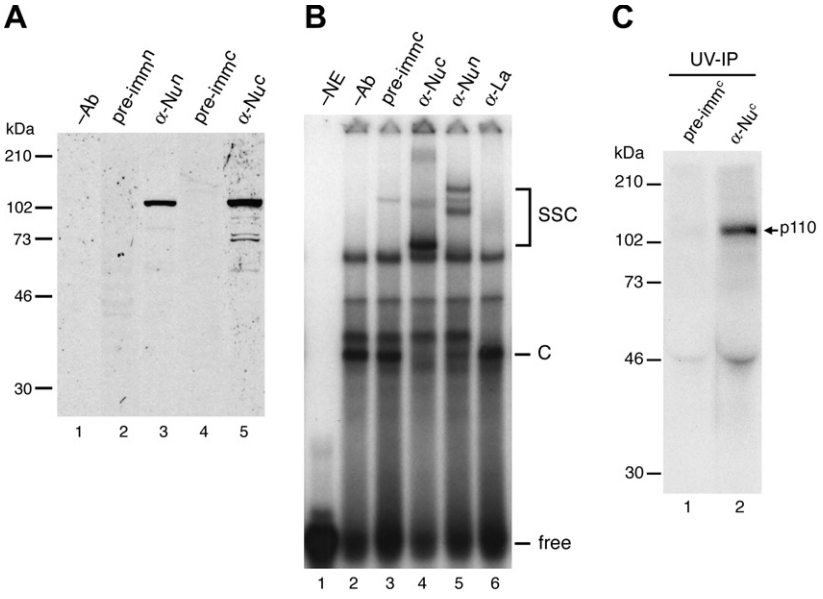


Fig. 2. p110 is recognized by nucleolin-specific antibodies. (A) Characterization of antibodies raised against the purified recombinant proteins encoding N-terminal (Nu$^n$) or C-terminal (Nu$^c$) portions of mouse nucleolin. Western blot analyses of whole cell extracts from C3H2K/Mo cells were performed with no antibody (lane 1), pre-immune serum from the rabbit immunized against Nu$^n$ protein (lane 2), anti-Nu$^n$ antibodies (lane 3), pre-immune serum from the rabbit immunized against Nu$^c$ protein (lane 4), and anti-Nu$^c$ antibodies (lane 5). Positions of molecular weight markers are shown on the left. (B) Nucleolin-specific antibodies super-shifted complex C in a gel mobility shift analysis. Mouse nuclear extracts were pre-incubated with no antibody (lane 2), pre-immune serum (lane 3), anti-Nu$^c$ antibodies (lane 4), anti-Nu$^n$ antibodies (lane 5), or anti-La serum (lane 6), then analyzed using a gel mobility shift assay. Free 4.5S RNAH probe (free), complex C, and super-shifted complexes (SSC) are indicated. (C) 4.5S RNAH–protein complexes were irradiated with UV light, digested with RNases A and T1, then immunoprecipitated with pre-immune serum (lane 1) or anti-Nu$^c$ antibodies (lane 2). Complexes were analyzed using SDS–PAGE and autoradiography. Positions of molecular weight protein standards (left) and p110 (right) are indicated.

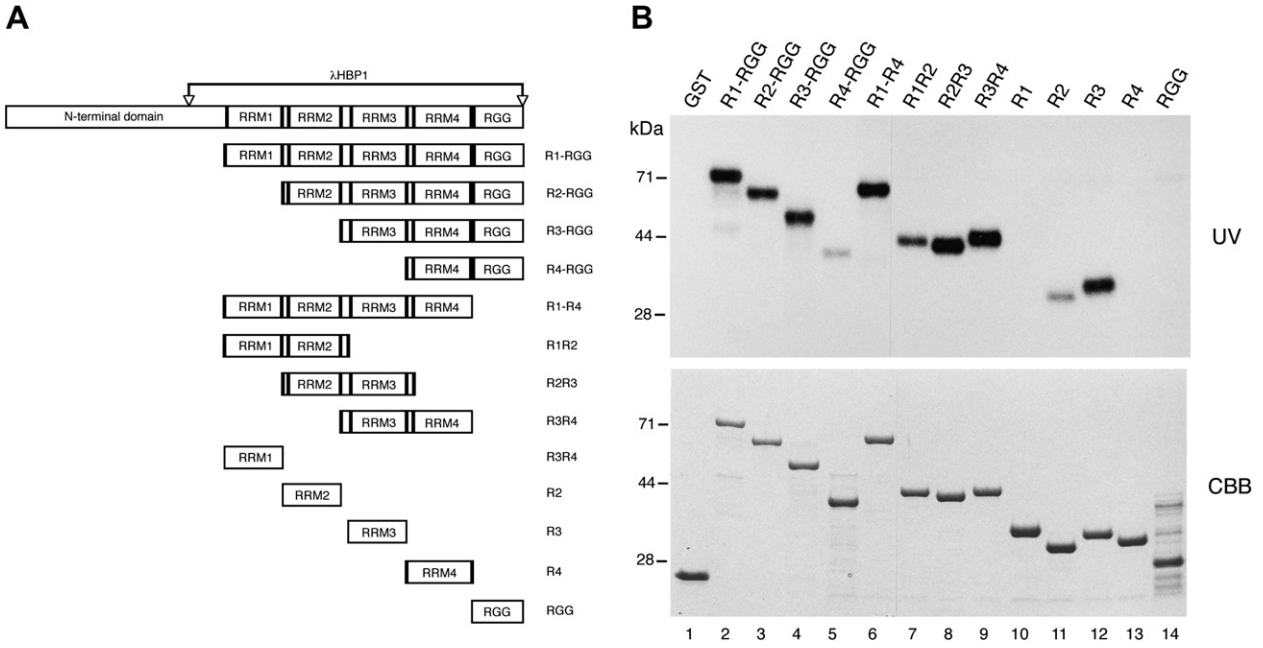


Fig. 3. The third RRM of mouse nucleolin is a major determinant for 4.5S RNAH binding. (A) Schematic representation of mouse nucleolin and its deletion mutants used for UV cross-linking analyses. Mouse nucleolin comprises the following specific structural domains: N-terminal domain, four RNA recognition motifs (RRM1 to RRM4), and a C-terminal glycine/arginine-rich region (RGG). The λHBP1 cDNA clone was isolated from a mouse cDNA expression library and the region that it encodes is indicated. (B) Various GST-fusions of mouse nucleolin mutants were constructed and expressed in *E. coli*, then purified using glutathione-Sepharose resin. Equimolar (40 pmol) amounts of each protein were incubated with excess (~100 pmol) $^{32}$P-labeled 4.5S RNAH, then subjected to UV cross-linking. Upper panel shows autoradiogram of cross-linked samples separated by SDS–PAGE. Lower panel shows Coomassie blue staining of purified deletion mutants used for UV cross-linking analysis. Positions of the molecular weight markers are shown on the left.

incubated with excess $^{32}$P-labeled 4.5S RNAH (~100 pmol) (Fig. 3B, upper panel). R1-RGG could be efficiently cross-linked to 4.5S RNAH (lane 2), whereas GST alone did not bind (lane 1). Removing the first RRM (R2-RGG) and both the first and second RRMs (R3-RGG) from R1-RGG did not have a significant affect on binding (lanes 3 and 4). However, removing the third RRM from the N-terminus (R4-RGG) significantly reduced binding activity (lane 5). We examined mutant proteins containing a combination of two RRMs and observed that R2R3 and R3R4 exhibited almost full binding activity, whereas the binding of R1R2 was lower than R3-containing mutants (compare lane 7 to lanes 8 and 9). Binding was unaffected by removal of the RGG box (R1–R4, lane 6). We investigated the binding capacity of mutant proteins containing individual motifs (Fig. 3B, upper panel, lanes 10–14). Quantitation of radioactivity from the UV cross-linked bands revealed that third RRM exhibited ca. 90% of the binding activity (lane 12), whereas that of the second RRM was ca. 20% (lane 11) and other motifs were <5% (lanes 10, 13, and 14) of the R1-RGG protein (lane 2). In a competition assay using cold 4.5S RNAH and 4.5S RNAI, we determined that the interaction between the third RRM and 4.5S RNAH was specific (data not shown). From these results, we concluded that mouse nucleolin can directly bind to 4.5S RNAH *in vitro* and that its third RRM is the major determinant for recognizing 4.5S RNAH.

### *A portion of nucleolin exists as a complex with 4.5S RNAH in mouse cells*

To investigate whether nucleolin forms complexes with the endogenous 4.5S RNAH in mouse cells, we immunoprecipitated C3H2K/Mo cell extracts using anti-nucleolin antibodies. Northern blot analyses indicate that 4.5S RNAH precipitated specifically with both anti-nucleolin antibodies (Fig. 4A, lanes 4 and 6), whereas it did not precipitate in the absence of antibody or with pre-immune sera (lanes 2, 3, and 5). Consistent with reports that La binds most Pol III transcript precursors such as 4.5S RNAH and 4.5S RNAI [4,13], we observed that anti-La antibody precipitated large amounts of these RNAs but not the U3 small nucleolar RNA (Fig. 4A, lane 7). These results supported the idea that nucleolin directly binds to endogenous 4.5S RNAH and a portion of 4.5S RNAH exists as an RNP with nucleolin in mouse cells. Consistent with the report that a portion of U3 RNA associates with nucleolin [18], we observed that U3 RNA was specifically precipitated by anti-nucleolin antibodies (Fig. 4A, lanes 4 and 6).

To examine the subcellular localization of the 4.5S RNAH–nucleolin complex, we separated C3H2K/Mo cells into cytoplasmic and nuclear fractions. The nuclear fraction was further separated into nucleoplasmic and nucleolar fractions. Nucleolin was detected in all subcellular fractions (Fig. 4B upper panel, lanes 1–4). Total RNAs were extracted from each fraction and Northern blot anal-

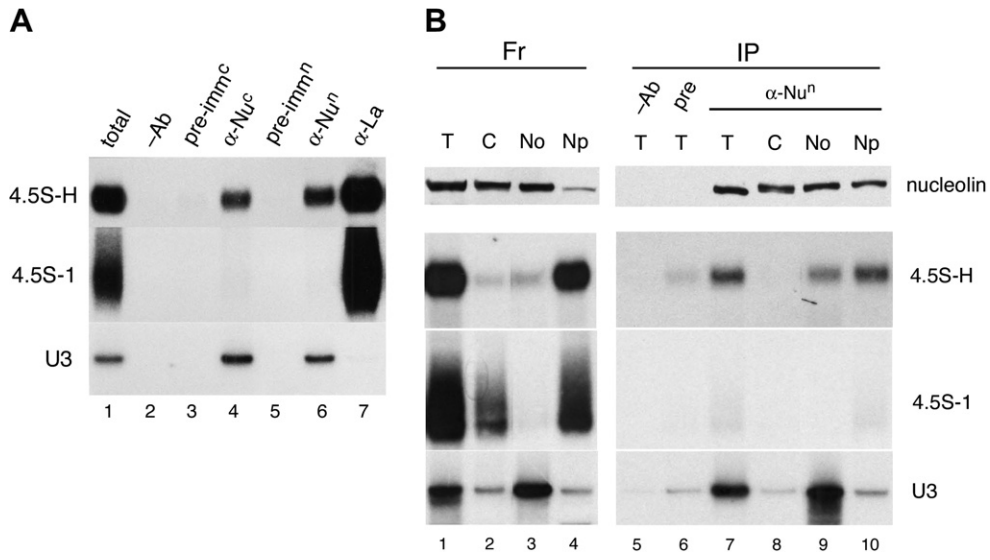


Fig. 4. Nucleolin interacts with a portion of the endogenous 4.5S RNAH in mouse cells. (A) Total cell lysates from mouse C3H2K/Mo cells were immunoprecipitated with the antibodies indicated. RNAs extracted from these precipitates were analyzed by Northern blotting using oligonucleotides specific for the RNAs indicated (left). We analyzed total RNA from a 20% input of cell lysate (lane 1), as well as RNAs precipitated with no antibodies (lane 2), pre-immune sera (lanes 3 and 5), anti-C- and N-terminal mouse nucleolin antibodies (lanes 4 and 6, respectively), and anti-La serum (lane 7). (B) C3H2K/Mo cells were fractionated as described in Materials and methods. Proteins and RNA in total (T), cytoplasmic (C), nucleolar (No), and nucleoplasmic (Np) fractions (lanes 1–4, respectively), as well as immunoprecipitates with no antibodies (lane 5), pre-immune serum (lane 6), and anti-Nu$^{c}$ antibodies (lanes 7–10) were analyzed from the fractions indicated. Western blotting was performed using anti-Nu$^{c}$ antibodies (upper panel). RNAs extracted from each fraction (lanes 1–4) and immunoprecipitate (lanes 5–10) were analyzed by Northern blotting using oligonucleotide probes specific to the RNAs indicated (lower panel).

ysis was performed using oligonucleotides specific for 4.5S RNAH, 4.5S RNAI, and U3 RNA. Whereas 4.5S RNAH has been reported in both the cytoplasm and nucleus [8], we detected only a small amount in the cytoplasm (Fig. 4B lower panel, lane 2). While U3 RNA predominantly localized in the nucleolus (Fig. 4B lower panel, lane 3), 4.5S RNAH and 4.5S RNAI primarily localized in the nucleoplasm (lane 4). Subsequently, each subcellular fraction was immunoprecipitated with anti-nucleolin antibodies and precipitated RNA was subjected to Northern blot analysis (Fig. 4B lower panel, lanes 5–10). Whereas 4.5S RNAH was predominantly precipitated from the nucleoplasmic fraction (lane 10), lower but significant levels of 4.5S RNAH were also precipitated from the nucleolar fraction (lane 9). These results suggest that the 4.5S RNAH–nucleolin complexes localize to both the nucleoplasm and nucleolus in cultured mouse cells.

## Discussion

In this study, we characterized mouse nuclear proteins that bind to 4.5S RNAH by using gel mobility shift and RNA–protein UV cross-linking assays. We found that nine polypeptides (including La) specifically bound to the RNA *in vitro*. Although 4.5S RNAH exhibits extensive homology to B1 elements, the RNA lacks the typical cruciform structure shared by the family of 7SL-like RNAs including scB1 RNA but has a specific 20 nt sequence. Since most of the binding polypeptides were not efficiently competed by scB1 RNA for binding 4.5S RNAH (Fig. 1), they may recognize the 4.5S RNAH-specific sequences and/or structure. We speculate that these two evolutionary related small RNAs exist as distinct RNPs and perform different functions in mouse cells.

To identify 4.5S RNAH-binding proteins, we performed expression cloning and isolated cDNA clones encoding mouse nucleolin. Several experiments using nucleolin-specific antibodies and various deletion mutants of nucleolin demonstrated that mouse nucleolin directly and specifically bound to 4.5S RNAH *in vitro* (Figs. 2 and 3). Nucleolin binds various RNA targets including pre-rRNA, virus RNAs, and some mRNAs through the central region composed of four RRMs [17]. The first two RRMs are responsible for recognition of a hexanucleotide motif U/GCCCGA within a short stem-loop structure found in pre-rRNA [19] but the individual RRM is inactive. In this study, we found that the third RRM of mouse nucleolin is the major determinant for recognizing 4.5S RNAH. These suggest that mouse nucleolin can interact with multiple target RNAs through a combination of RRMs as well as individual RRMs.

Subcellular fractionation and immunoprecipitation experiments demonstrated that a portion of endogenous 4.5S RNAH was associated with nucleolin in both the nucleoplasm and nucleolus while 4.5S RNAH was predominately found in the nucleoplasmic fraction (Fig. 4). Nucleolin is a multifunctional protein that has been implicated in several steps of ribosome biogenesis, mRNA metabolism, and cell proliferation [17]. Although nucleolin is predominately localized to the nucleolus, it is also found in the

other cell compartments such as nucleoplasm and cytoplasm [17]. The dual localization of 4.5S RNAH–nucleolin complex implies that the RNP may transiently translocate from one compartment to the other or may have distinct function in each compartment. In nucleolus, 4.5S RNAH may modulate one of the nucleolar activities of nucleolin such as rDNA transcription and pre-rRNA processing. Alternatively, since the nucleolus has recently been implicated in a variety of cellular processes besides ribosome biogenesis including cell-cycle control, stress responses, mRNA transport, biogenesis of RNPs, and RNA editing [20], 4.5S RNAH–nucleolin complex in the nucleolus may associate with one of these processes.

4.5S RNAH was originally described as a short-lived small RNA base-paired with polyadenylated RNAs in the nucleus and cytoplasm and has been implicated in transport of specific mRNAs [2,3]. Notably, recent studies have reported that nucleolin exchanges rapidly between the nucleoplasm and nucleolus [21] and that it is capable of accelerating nucleic acid annealing through its C-terminal domain (RRM3-4-RGG) [22]. Thus, we speculate that 4.5S RNAH is hybridized to its target mRNAs in the nucleoplasm with the help of nucleolin and then the RNPs are transiently localized to the nucleolus for regulated transport or editing of the particular mRNAs.

Kramerov and co-workers recently reported that 4.5S RNAH gene is distributed among only six rodent families whereas B1 element is present in all rodent families [10]. Considering the nucleotide sequence conservation and relatively recent origin of 4.5S RNAH, they predicted that the RNA may be functional and play some additional roles in stress response or defenses against parasitic agents [10]. Although at present we do not know whether the expression level or subcellular localization of 4.5S RNAH is affected by some stresses, a large amount of the RNAs may be produced from multiple copies of 4.5S RNAH genes (~800 copies in mouse) [5,8] under some specific condition, which may then participate in some cellular processes together with the 4.5S RNAH-binding proteins. Interestingly, several kinds of stresses, such as heat shock or DNA damage, cause nucleolin to interact with the tumor suppressor protein p53 and to relocalize from the nucleolus to the nucleoplasm where nucleolin binds replication protein A and inhibits DNA replication [23]. 4.5S RNAH might be involved in this stress response through affecting mobility or binding partner of nucleolin in both the nucleolus and nucleoplasm. We are currently investigating the dynamics of subcellular localization of 4.5S RNAH in relation to the cell proliferation and stresses.

## Acknowledgments

We thank Drs. S. Nishimura, K. Shimotohno, and N. Okada for encouragement. We also thank Dr. N. Okada for providing the anti-La serum and M. Takamatsu, W. Nagatani, K. Terada, and T. Nishikawa for technical assistance. This work was supported in part by a Grant-in-Aid for Scientific Research on Priority Areas from the Ministry of Education, Science, Sports, and Culture of Japan.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2007.10.117.

## References

[1] F. Harada, Y. Ikawa, A new series of RNAs associated with the genome of spleen focus forming virus (SFFV) and poly(A)-containing RNA from SFFV-infected cells, Nucleic Acids Res. 7 (1979) 895–908.
[2] W. Jelinek, L. Leinwand, Low molecular weight RNAs hydrogen-bonded to nuclear and cytoplasmic poly(A)-terminated RNA from cultured Chinese hamster ovary cells, Cell 15 (1978) 205–214.
[3] F. Harada, N. Kato, H. Hoshino, Series of 4.5S RNAs associated with poly(A)-containing RNAs of rodent cells, Nucleic Acids Res. 7 (1979) 909–917.
[4] L.A. Leinwand, R.M. Wydro, B. Nadal-Ginard, Small RNA molecules related to the Alu family of repetitive DNA sequences, Mol. Cell. Biol. 2 (1982) 1320–1330.
[5] F. Harada, Y. Takeuchi, N. Kato, Molecular cloning and in vitro transcription of rat 4.5S RNAH genes, Nucleic Acids Res. 14 (1986) 1629–1642.
[6] F. Harada, N. Kato, Nucleotide sequences of 4.5S RNAs associated with poly(A)-containing RNAs of mouse and hamster cells, Nucleic Acids Res. 8 (1980) 1273–1285.
[7] A.S. Krayev, D.A. Kramerov, K.G. Skryabin, A.P. Ryskov, A.A. Bayev, G.P. Georgiev, The nucleotide sequence of the ubiquitous repetitive DNA sequence B1 complementary to the most abundant class of mouse fold-back RNA, Nucleic Acids Res. 8 (1980) 1201–1215.
[8] L.O. Schoeniger, W.R. Jelinek, 4.5S RNA is encoded by hundreds of tandemly linked genes, has a short half-life, and is hydrogen bonded in vivo to poly(A)-terminated RNAs in the cytoplasm of cultured mouse cells, Mol. Cell. Biol. 6 (1986) 1508–1519.
[9] R. Kraft, L. Kadyk, L.A. Leinwand, Sequence organization of variant mouse 4.5S RNA genes and pseudogenes, Genomics 12 (1992) 555–566.
[10] I.K. Gogolevskaya, A.P. Koval, D.A. Kramerov, Evolutionary history of 4.5SH RNA, Mol. Biol. Evol. 22 (2005) 1546–1554.
[11] A.M. Francoeur, M.B. Mathews, Interaction between VA RNA and the lupus antigen La: formation of a ribonucleoprotein particle in vitro, Proc. Natl. Acad. Sci. USA 79 (1982) 6772–6776.
[12] J. Rinke, J.A. Steitz, Precursor molecules of both human 5S ribosomal RNA and transfer RNAs are bound by a cellular protein reactive with anti-La lupus antibodies, Cell 29 (1982) 149–159.
[13] R. Reddy, D. Henning, E. Tan, H. Busch, Identification of a La protein binding site in a RNA polymerase III transcript (4.5 I RNA), J. Biol. Chem. 258 (1983) 8352–8356.
[14] E. Gottlieb, J.A. Steitz, Function of the mammalian La protein: evidence for its action in transcription termination by RNA polymerase III, EMBO J. 8 (1989) 851–861.
[15] T.S. Ro-Choi, R. Reddy, D. Henning, T. Takano, C.W. Taylor, H. Busch, Nucleotide sequence of 4.5S ribonucleic acid of Novikoff hepatoma cell nuclei, J. Biol. Chem. 247 (1972) 3205–3222.
[16] D.Y. Chang, R.J. Maraia, A cellular protein binds B1 and Alu small cytoplasmic RNAs in vitro, J. Biol. Chem. 268 (1993) 6423–6428.
[17] H. Ginisty, H. Sicard, B. Roger, P. Bouvet, Structure and functions of nucleolin, J. Cell Sci. 112 (Pt 6) (1999) 761–772.
[18] H. Ginisty, F. Amalric, P. Bouvet, Nucleolin functions in the first step of ribosomal RNA processing, EMBO J. 17 (1998) 1476–1486.
[19] G. Serin, G. Joseph, L. Ghisolfi, M. Bauzan, M. Erard, F. Amalric, P. Bouvet, Two RNA-binding domains determine the RNA-binding specificity of nucleolin, J. Biol. Chem. 272 (1997) 13109–13116.

[20] F.M. Boisvert, S. van Koningsbruggen, J. Navascues, A.I. Lamond, The multifunctional nucleolus, Nat. Rev. Mol. Cell. Biol. 8 (2007) 574–585.
[21] D. Chen, S. Huang, Nucleolar components involved in ribosome biogenesis cycle between the nucleolus and nucleoplasm in interphase cells, J. Cell Biol. 153 (2001) 169–176.
[22] L.A. Hanakahi, Z. Bu, N. Maizels, The C-terminal domain of nucleolin accelerates nucleic acid annealing, Biochemistry 39 (2000) 15493–15499.
[23] Y. Daniely, D.D. Dimitrova, J.A. Borowiec, Stress-dependent nucleolin mobilization mediated by p53-nucleolin complex formation, Mol. Cell. Biol. 22 (2002) 6014–6022.