The UV-inducible RNA-binding Protein A18 (A18 hnRNP) Plays a Protective Role in the Genotoxic Stress Response*

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We have previously shown that specific RNA-binding proteins (RBP) are activated by genotoxic stress. The role and function of these stress-activated RBPs are, however, poorly understood. The data presented here indicate that the RBP A18 heterogeneous ribonucleoprotein (hnRNP) is induced and translocated from the nuclei to the cytoplasm after exposure to UV radiation. Using a new in vitro system we identified potential cellular targets for A18 hnRNP. Forty-six mRNA transcripts were identified, most of which are stress- or UV-responsive genes. Two important stress-responsive transcripts, the replication protein A (RPA2) and thioredoxin, were studied in more detail. Northwestern analyses indicate that A18 hnRNP binds specifically to the 3′-untranslated region of RPA2 transcript independently of its poly(A) tail, whereas the poly(A) tail of thioredoxin mRNA reinforces binding. Overexpression of A18 hnRNP increases the mRNAs stability and consequently enhances translation in a dose-dependent manner. Moreover, cell lines expressing reduced levels of A18 hnRNP are more sensitive to UV radiation. These data suggest that A18 hnRNP plays a protective role against genotoxic stresses by translocating to the cytosol and stabilizing specific transcripts involved in cell survival.

The cellular response to genotoxic and non-genotoxic stresses is complex. It includes multiple regulatory mechanisms that are generally thought to have protective roles. Cells respond to stress in a limited number of ways by adjusting regulatory components of basic processes such as replication, transcription, and/or translation. Much emphasis has been put on stress responses involving replication or the activation of specific genes in response to DNA damage (1); however, the regulation of post-transcriptional and translational events in response to stress has not been studied extensively. Post-transcriptional regulation can be mediated through interaction of regulatory proteins with an mRNA 3′ end (2). This mechanism, which occurs in several organisms, is not fully understood. Most regulations of this type have been observed during early development of different organisms from Caenorhabditis elegans to mammals (3). One possible mechanism by which regulation of translation initiation can be mediated through the 3′ end of an mRNA transcript has suggested that specific proteins bound to this region could contact the basal translation apparatus and influence translational activation or repression (2). A recent review (4) described the possibility for RNA-binding proteins to shuttle between cellular compartments either constitutively or in response to stress and regulate the localization, translation, or turnover of mRNAs. Post-transcriptional regulation can also occur through mRNAs stabilization. Recent studies describe the stabilization of mRNAs by specific RBPs1 in response to hypoxia (5) or extracellular signals (6).

In a previous study (7) we have shown that RNA binding activity of specific proteins can be induced by DNA-damaging agents. Induction of an RBP at the mRNA levels was first reported for the A18 hnRNP after UV radiation (8). The hnRNPs are a sub-group of ribonucleoproteins (RNP)s found in the nucleus and involved in RNA processing (9). The A18 hnRNP was originally cloned by hybridization subtraction on the basis of rapid induction in UV-irradiated Chinese hamster ovary cells (8). Since then, the human A18 hnRNP was cloned and characterized (10). The human A18 hnRNP is a rather unique RNP. In addition to containing a conserved RNA binding domain, it also contains several repeats of an RGG box in its auxiliary domain (10). The RGG boxes were first identified as single-stranded nucleic acid binding motifs in an hnRNP that does not contain the conserved RNA binding domain (11). The auxiliary domains of RNPs are associated with protein-protein interaction (9); therefore, the presence of single-stranded nucleotide binding domains in this location is unusual.

In this report we show that the nuclear A18 hnRNP is not only induced but also translocated to the cytoplasm in response to UV radiation. In addition, we established an in vitro system to isolate and identify A18 hnRNP most probable mRNA targets. Forty-six mRNAs transcripts have been identified, a large proportion of which are UV- or stress-responsive. Our data indicate that A18 hnRNP binds specifically to the 3′-UTR of the replication protein A (RPA2) and the human thioredoxin mRNAs. Co-transfection of A18 hnRNP with a CAT expression vector resulted in increased message stability and CAT activity. Moreover, cells expressing reduced levels of A18 hnRNP are more sensitive to UV radiation. Taken together these data suggest that stabilization of stress-responsive transcripts by A18 hnRNP may protect the cells against genotoxic insults.

**EXPERIMENTAL PROCEDURES**

**Plasmids**—To express A18 hnRNP in mammalian cells, the open reading frame (ORF) of A18 hnRNP cDNA was cloned into the HindIII/...
Expression and Purification of Recombinant A18 hnRNP. The hnRNP binding DNA region (516 base pairs) was amplified by PCR and cloned into the NdeI and XhoI sites of pET21a (Novagen, Madison, WI). Expression was achieved in Escherichia coli BL21(DE3) as recommended by the manufacturer (Novagen) except that the bacteria were grown at 30 °C. Soluble proteins were loaded on a nickel-nitrioltriacetic acid column (Novagen) and stepwise eluted with 100–350 mM imidazole. The imidazole was removed by gel filtration on a PBS. Nonspecific sites were blocked with 2% milk in PBS containing 2% Tween 20 and washed two more times with PBS. Conjugation of the indicated dose of UV. Treatments were performed in triplicate for each dose. Two weeks later, colonies containing more than 50 cells were counted.

CAT Assay—The CAT activity was measured essentially as described before (12). Cellular extracts containing either 50 or 25 μg of protein were incubated at 37 °C for 1–3 h and separated on TLC plates. The conversion of chloramphenicol was quantified on a PhosphorImager (Molecular Dynamics STORM) with the ImageQuant software.

Fluorescence—The A18 hnRNP-GFP-overexpressing cells were grown on coverslips and treated with UV radiation at a dose of 20 J/m². Cells were put back into culture for 3 h, and fluorescence was observed with a fluorescence microscope (Zeiss, Axioskop, objective 10×, HB100-W mercury lamp).

Ribonucleic Acid Protection Assay—RNAse protection was performed using the ribonuclease protection kit RPAII (Ambion). Briefly, total RNA was prepared from the cells co-transfected with A18 hnRNP and the CAT reporter gene constructs. A 100-base pair fragment upstream of the stop codon of the CAT open reading frame was in vitro transcribed into the complement RNA probe and purified. For each reaction, 20 μg of total RNA was co-transcribed with the RNA probe (2 × 10⁶ cpm for 1 h) and hybridized overnight at 42 °C. For control, 20 μg of yeast RNA was co-precipitated with the RNA probes. The hybridized RNA probe was digested with RNase A/T and protected. Protected RNA was separated on a 6% polyacrylamide gel.

RESULTS

Stabilization of Stress Responsive Transcripts by A18 hnRNP

Determining the RNA-Protein Interaction in Nunc-Immuno Tubes—Recombinant A18 hnRNP and nucleolin were coated in Nunc-Immuno tubes (Nalgen Nunc International, Rochester, NY). The A18 hnRNP-GFP fusion expression vector was constructed by amplifying the coding region of GFP from pEGFP (Invitrogen) and cloning it downstream of A18 hnRNPyt into pCDNA3.1 (Invitrogen). For constitutive expression of the CAT protein, the ORF of CAT was cloned into the NotI/BamHI sites of pcDNA3.1-ORF to generate the plasmid pCDNA3.1-CAT (CAT). The 3′-UTR of RNA was amplified by PCR from the full-length cDNA of RNA and cloned into the BamHI/XhoI sites of pcDNA3.1-CAT to generate the plasmid pcDNA3.1-CAT/3′-UTR (CAT-UTR). To generate the antisense A18 hnRNP vector, the ORF of A18 hnRNP was cloned in antisense orientation into the HindIII/XhoI sites of pcDNA3.1. CAT Assays—Probes (25 μg) were synthesized by transcribing with MAXI-scripts (Ambion Inc., Austin, TX). The probes for Thioredoxin were 5′-UTR (30), 3′-UTR (Ambion Inc., Austin, CA) and superscript reverse transcriptase (Life Technologies, Inc.). The reaction was performed in 20 μl at 42 °C for 1 h. Five μl of the reaction mixture was used to amplify the double strand cDNA by long distance PCR using SmartII cDNA PCR amplification oligo (CLONTECH). PCR was performed for 30 cycles at 95 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min. The PCR product was cloned in antisense orientation into the pGEM-T vector (Promega, Madison, WI), and transformed into E. coli (DH5a). Positively colonies were picked for further analysis by PCR fingerprinting and sequencing. Sequencing was performed on an Applied Biosystems 373 (ABI, Foster City, CA) automated sequencer.

Northwestern Blot—Northwestern blots were performed essentially as previously described (7).

Determining the RNA-Protein Interaction in Nunc-Immuno Tubes—Recombinant A18 hnRNP and nucleolin were coated in Nunc-Immuno tubes (Nalgen Nunc International, Rochester, NY). The A18 hnRNP-GFP fusion expression vector was constructed by amplifying the coding region of GFP from pEGFP (Invitrogen) and cloning it downstream of A18 hnRNPyt into pCDNA3.1 (Invitrogen). For constitutive expression of the CAT protein, the ORF of CAT was cloned into the NotI/BamHI sites of pcDNA3.1-ORF to generate the plasmid pCDNA3.1-CAT (CAT). The 3′-UTR of RNA was amplified by PCR from the full-length cDNA of RNA and cloned into the BamHI/XhoI sites of pcDNA3.1-CAT to generate the plasmid pcDNA3.1-CAT/3′-UTR (CAT-UTR). To generate the antisense A18 hnRNP vector, the ORF of A18 hnRNP was cloned in antisense orientation into the HindIII/XhoI sites of pcDNA3.1. CAT Assays—Probes (25 μg) were synthesized by transcribing with MAXI-scripts (Ambion Inc., Austin, TX). The probes for Thioredoxin were 5′-UTR (30), 3′-UTR (Ambion Inc., Austin, CA) and superscript reverse transcriptase (Life Technologies, Inc.). The reaction was performed in 20 μl at 42 °C for 1 h. Five μl of the reaction mixture was used to amplify the double strand cDNA by long distance PCR using SmartII cDNA PCR amplification oligo (CLONTECH). PCR was performed for 30 cycles at 95 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min. The PCR product was cloned in antisense orientation into the pGEM-T vector (Promega, Madison, WI), and transformed into E. coli (DH5a). Positively colonies were picked for further analysis by PCR fingerprinting and sequencing. Sequencing was performed on an Applied Biosystems 373 (ABI, Foster City, CA) automated sequencer.

Northwestern Blot—Northwestern blots were performed essentially as previously described (7).
AhnRNP-GFP is shown.

were compared with GenBankTM and EST data bases through
dant clones by an automated sequencer. The partial sequences
library. Sequencing was performed randomly on non-redun-
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precipitated, reverse-transcribed, and amplified by PCR. The
A18 hnRNP-coated tube, the specifically bound mRNAs were
RKO cells could be a good source of mRNA targets for A18
vated by genotoxic stress (7), we reasoned that UV-irradiated
Because the RKO cells contain several RBP that can be acti-

Fig. 1. A18 hnRNP translocates to the cytosol after UV radia-
tion. Fluorescence microscopy in RKO cells stably transfected with A18
hnRNP-GFP is shown. A, untreated, light microscope. B, untreated
fluorescence. C, 3 h after UV radiation (20 J/m2), light microscope. D, 3 h after UV radiation (20 J/m2), fluorescence. Cytoplasmic proteins are indicated by white arrows.

determine the specificity of the RNA-protein interaction. The last two tubes were coated with either the recombinant A18
hnRNP protein or nucleolin, another RNA-binding protein
known for its specific binding to stem loop RNA structure (16).
The data presented in Fig. 2 indicate that, under all the elution
conditions used, the amount of RNA bound nonspecifically to
BSA or the tube walls was less than 20% of the RNA bound to
either A18 hnRNP or nucleolin. To make sure that we had
eluted all the protein-bound RNA, we treated the tubes with
proteinase K. As shown in Fig. 2 (lanes 17–20), proteinase K
treatment released some RNA in all the tubes but in much
smaller amounts than the salt treatment. These data indicate
that under the conditions used most RNAs that are specifically
bound to an RNA-binding protein can be eluted. The immuno-
tube technique is thus a suitable and selective technique to
identify the potential targets of a given RNA-binding protein.

Identifying A18 hnRNP mRNAs Targets—We then repeated
this technique with a newly coated A18 hnRNP immunotube
and 10 μg of mRNAs isolated from UV-irradiated RKO cells.
Because the RKO cells contain several RBP that can be acti-
vated by genotoxic stress (7), we reasoned that UV-irradiated
RKO cells could be a good source of mRNA targets for A18
hnRNP. After incubation of the UV-treated mRNAs with the
A18 hnRNP-coated tube, the specifically bound mRNAs were
precipitated, reverse-transcribed, and amplified by PCR. The
resulting amplicons were cloned and digested with the four-
base cutter HaeIII (New England Biolabs, Beverly, MA) to
evaluate redundancy. Based on this analysis we have deter-
mined that several genes were present more than once in the
library. Sequencing was performed randomly on non-redund-
ant clones by an automated sequencer. The partial sequences
were compared with GenBankTM and EST data bases through
the BLAST search engine. As shown in Table I, three main
classes of transcripts representing 46 different clones have
been identified. A large proportion (~40%) of these genes are
UV- or stress-responsive. This indicates that the technique is
highly selective.

Four of these genes, RPA2, thioredoxin, ferritin, and nucleo-
ophosmin, are either UV-inducible or UV-responsive (17). We
also verified the specificity of the mRNAs selected by attempt-
ing to amplify by reverse transcription-PCR other transcripts
known to be UV-inducible. Transcripts for the growth arrest
and DNA damage-inducible gene GADD45 and the cyclin-
dependent kinase inhibitor p21 (1) were not found in the pool of
mRNAs selected by A18 hnRNP under these conditions.

Another major class of transcripts bound by A18 hnRNP
encodes ribosomal proteins. This large number of ribosomal
proteins is in good agreement with the increasing body of
evidence indicating that translation is an important component
of the cellular stress response (18). Several type of stress,
including heat shock stress and several chemicals, can induce
the synthesis of stress proteins while inhibiting the rate of
protein synthesis (Ref. 19 and references within). Stress-in-
duced changes in the stoichiometry of ribosomal proteins may
trigger an adaptive response by allowing the translation of a
specific set of proteins (20). Three of the ribosomal proteins
identified in our screen, L10, S13, and L19, have been associ-
ated with the oxidative stress response (21, 22). L5 is involved
in cellular resistance to general stress stimulus (20), and S6 is
induced by cold shock (23). L13 has been suggested as a medi-
ator of the stress induction of the sigma (B) factor in bacteria
(24). These data support the idea that our technique is highly
selective and that A18 hnRNP specifically targets transcripts
involved in the stress response. We do not know whether the
other ribosomal proteins identified in our screen play a direct
or indirect role in the stress response. Nevertheless, A18
hnRNP may contribute to modulate an adaptive response to
cellular stress by targeting specific sets of ribosomal protein
transcripts.

Other genes encoding a variety of transcripts were also iden-
tified. Among these, two encode new sequences, one of un-
known function and one that encodes a hypothetical protein.
Binding of A18 hnRNP to the 18 S rRNA is also of interest since
this rRNA is part of the 40 S ribosomal small subunit. Binding
to this rRNA may thus indicate a potential role for A18 hnRNP
in translation. Based on sequence analysis we have determined
that the 18 S rRNA sequence bound to A18 hnRNP corresponds
to nucleotides 706–1385 of the 18 S rRNA (data not shown).
This sequence is located in the central domain of the 18 S rRNA
and is thought to be involved in eIF3 interaction, which pre-
vents premature association of the large and small ribosomal
subunits (25). A potential role in translation regulation is also
supported by the binding of A18 hnRNP to the transcripts of
two translational elongation factors (1α and 1β).

A18 hnRNP Binds Specifically to RPA2 and Thioredoxin
3′-UTRs—Our immunotube technique revealed that A18
hnRNP binds to a large number of stress-responsive trans-
scripts (Table I). To determine the binding specificity of A18
hnRNP, we performed Northwestern analyses with RPA2 and
thioredoxin, two stress-responsive transcripts identified in our
screen (Table I). RPA2 and thioredoxin were selected for this
assay based on their UV responsiveness and their well estab-
lished role in the stress response. The RPA2 protein is im-
portant for DNA replication and nucleotide excision repair and is
specifically phosphorylated after exposure to UV radiation (17).
RPA2 is also implicated in UV-induced replication arrest (17).
Thioredoxin is a UV-inducible protein involved in transcrip-
tional processes such as induction of AP-1 activity and the
inhibition of NF-κB activation (26). Thioredoxin has also been
associated with tumor growth and is now becoming a new
target for anti-cancer drugs (26).

We first constructed four overlapping probes with the RPA2
transcript (Fig. 3A) and hybridized them to increasing amounts
of recombinant A18 hnRNP protein (Fig. 3B). Our data indicate
that A18 hnRNP does not bind to RPA2 ORF or the 5′-UTR. On
the other hand, A18 hnRNP binds very strongly to RPA2 3′-
UTR irrespective of the presence of the poly(A) tail. Binding is
detectable with as little as 200 ng of recombinant protein (Fig. 3B, lanes 2). We also repeated a similar experiment with the thioredoxin transcript (Fig. 4). Six different overlapping probes were generated (Fig. 4A). Our data (Fig. 4B) indicate that again A18 hnRNP does not bind to the ORF of the transcript and binds only weakly to the 5'/H11032-UTR. However, in this case, binding is reinforced by the presence of the transcript poly(A) tail. Interestingly, binding appears to be more sensitive in the presence of the ORF even though A18 hnRNP does not bind to it. The presence of the ORF may affect the overall structure of the RNA and increase binding. These data confirm the binding specificity of A18 hnRNP to two transcripts selected by our immunotube technique.

### Effects of A18 hnRNP on Translation and mRNAs Stability—
Binding of proteins to the 3'-UTR of a transcript can affect both the rate of translation initiation and the stability of a transcript. To evaluate the overall effect of A18 hnRNP on translation, we performed transient co-transfection of A18 hnRNP with a constitutive expression vector for the CAT protein. We used two different CAT expression vectors to determine whether A18 hnRNPs required the RPA2 3'-UTR to mediate an effect on translation. The first construct (CAT) contains the CAT cDNA under the control of the pCMV promoter and a poly(A) tail from the bovine growth hormone transcript (Fig. 5, A and B). The second construct (CAT-UTR, Fig. 5, C and D) is identical to the CAT construct except that we have inserted the RPA2 3'-UTR without its poly(A) tail upstream of the bovine growth hormone poly(A)s. Our data (Fig. 5) indicate that co-transfection of A18 hnRNP with either construct produced a dose-dependent increase of the CAT activity (Fig. 5, A and C). The addition of the RPA2 3'-UTR to the CAT construct resulted in a marked (60%) decrease of the basal CAT activity (Fig. 5, A and B, lanes 0). However, co-transfection of increasing amounts of A18 hnRNP with either construct generated similar levels of CAT activity (Fig. 5, A and C). These data suggest that A18 hnRNP can relieve the repressive effect of the RPA2 3'-UTR.
The overall effect of A18 hnRNP on the stimulation of the CAT activity is thus more pronounced when the RPA2 3′-UTR is present. This is better illustrated by the fold conversion of chloramphenicol measured with the two constructs (Fig. 5). The overall effect of A18 hnRNP on the stimulation of the CAT mRNA transcripts. Mechanisms other than mRNAs stabilization are also probably involved in the overall effect of A18 hnRNP on translation since A18 hnRNP can stimulate translation at a greater rate than it stabilizes mRNAs (Fig. 5, B−D, versus Fig. 6).

A18 hnRNP Has a Protective Role—Our data show that A18 hnRNP binds specifically to the 3′-UTRs of RPA2 and thioredoxin and may influence their translation (Fig. 5). Both of these proteins have been associated with increased survival after stress. RPA protein can reverse the replication arrest induced by UV radiation (27), whereas expression of thioredoxin is associated with UV resistance (28). To evaluate the general effect of A18 hnRNP on cell survival after genotoxic stress, we first established a cell line expressing reduced levels of A18 hnRNP by stably transfecting an antisense vector for A18 hnRNP in RKO cells. After selection, the protein levels of A18 hnRNP were measured by Western blot. The data presented in Fig. 7A indicate that in cells transfected with an empty vector (RKO), A18 hnRNP was induced 4 h (lanes 3) after exposure to UV radiation (20 J/m²). The levels remain consistently high, up to 8 h after radiation (lane 4). This is in good agreement with early reports of the induction of A18 hnRNP mRNA by UV radiation (8). However, in the antisense cell line, the basal levels of A18 hnRNP were reduced (lanes 1 and 5), and no induction of the protein was detected even 8 h after radiation (lanes 5−8). Consistency of the protein loading was measured by analyzing the amount of the actin protein in all the samples (lanes 1−8). Similar results were also obtained with another A18 hnRNP antisense cell line (RKO AS-2, data not shown).

We then proceeded to evaluate the antisense cell line (RKO AS-1) capacity to survive UV radiation. Our data (Fig. 7B) indicate that the RKO AS-1 cells are much more sensitive to UV radiation. A difference in sensitivity can be observed at every dose, even with doses as low as 2 and 5 J/m². An increase in sensitivity of almost 6-fold is achieved at 7 J/m² in the antisense cell line. We have also performed survival analyses with RKO cells stably transfected with an A18 hnRNP vector in the sense orientation. Our data (not shown) indicate that the
cells were slightly more resistant than the parent cells, but the difference was not significant. Taken together these data suggest that the translocation of A18 hnRNP to the cytosol after UV radiation (Fig. 1) stimulates the translation of specific transcripts (Fig. 5) that increase cell survival after stress (Fig. 7). A protective role for A18 hnRNP in the genotoxic stress response seems likely.

**DISCUSSION**

To ascertain the role of A18 hnRNP in the genotoxic stress response, we first aimed to identify its potential mRNA targets. Several techniques exist to isolate mRNAs bound to a specific RBP, but most of them have a high degree of nonspecific mRNAs binding. We took advantage of the Nunc Immuno tube inert matrix to design a technique that would allow specific binding of mRNAs binding. We took advantage of the Nunc Immuno tube inert matrix to design a technique that would allow specific binding of mRNAs to A18 hnRNP. Several techniques exist to isolate mRNAs bound to a specific RBP, but most of them have a high degree of nonspecific mRNAs binding. We took advantage of the Nunc Immuno tube inert matrix to design a technique that would allow specific binding of mRNAs to A18 hnRNP.

Using Northwestern analyses (Figs. 3–4) we have confirmed the binding specificity of A18 hnRNP to two of the transcripts identified in our screen (Table I). Regulation of RPA2 at the post-transcriptional or translational level is for the most part unexplored. Recent studies indicate that RPA2 is phosphorylated by the DNA-dependent protein kinase and probably the ataxia telangiectasia-mutated protein kinase in response to DNA damage (17). However, the functional significance of this phosphorylation is still unclear. It is believed that phosphorylation of RPA2 may alter the conformation of RPA heterotrimer and modulate its interaction with other proteins such as p53 (29). A18 hnRNP binds specifically to RPA2 3'-UTR (Fig. 3) and stimulates translation (Fig. 5). Translation initiation can be regulated by specific binding of proteins to the 3'-UTR of a transcript. It has been suggested that proteins bound to this region could contact the basal translation apparatus and influence translational activation or repression (2). Alternatively, as described in a recent review (4), RNA-binding proteins that shuttle between cellular compartments either constitutively or in response to stress may regulate the localization, translation, or turnover on mRNAs. Our data (Fig. 1) indicate that A18 hnRNP is translocated in response to UV radiation and increases mRNAs stability (Fig. 6). However, we cannot rule out the possibility that A18 hnRNP could also increase translation through direct interaction with the translational machinery. Recent evidence has shown (30) that interaction of the polyadenylate-binding protein with the eukaryotic initiation factor 4G, which interacts with the 5' cap binding initiation factor 4E (31), enhances translation. Furthermore, a human homologue of eukaryotic initiation factor 4G, PAIP-1, can also stimulate translation by bridging the polyadenylate-binding protein to the initiation factor 4A (32). These mechanisms explain how

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2 C. Yang and F. Carrier, unpublished observation.
regulation through the 3′-UTR of a transcript can influence translation initiation at the 5′ end. Our data indicate that A18 hnRNP binding to mRNAs is reinforced by poly(A) tails (Figs. 3–4). Hence, A18 hnRNP could potentially affect translation through similar mechanisms.

As mentioned earlier, regulation of protein translation is apparently an important component of the cellular stress response (18). Modification of protein synthesis patterns was among the first phenomena observed after cellular stress (33). Typically, an immediate arrest followed by an increased rate of protein synthesis was observed after UV radiation (33). Down-regulation of protein synthesis in response to stress is thought to be an adaptive response triggered to protect the cells and conserve the resources required to survive (34). On the other hand, induction of specific ribosomal proteins in response to stress may indicate the involvement of the translational machinery in the sensing and response to cellular stress (20). The idea that ribosomes could be sensitive to stress is supported by the finding that bacterial strains lacking the ribosomal protein L11 can not activate the transcription factor sigma B in response to environmental stress (35). Moreover, the association of several ribosomal proteins with the oxidative stress response (36) is additional evidence that translation regulation is a significant component of the cellular stress response. Our data (Table I) indicate that several transcripts targeted by A18 hnRNP code for ribosomal proteins, some of which are stress-responsive. Regulation of ribosomal protein expression could thus represent an alternative mechanism by which A18 hnRNP may regulate the translation of a specific set of proteins in response to stress.

As RPA2, very little is known on the post-transcriptional or translational regulation of thioredoxin in response to stress. Thioredoxin expression is increased in several tumors, specially gastric tumors (37), and in response to a variety of stress such as x-ray irradiation, UV radiation, and other types of oxidative stress (38). Increased levels of thioredoxin are associated with resistance to chemotherapy and cellular proliferation, probably through inhibition of apoptosis (26). In cultured human retinal epithelial cells, thioredoxin induction by prostaglandin E1 and H2O2 has been shown to be dependent on cAMP levels (39). However, the mechanism for the increased levels of endogenous thioredoxin in cancer cells is still unknown (26). Thioredoxin is a key regulator of cells signaling events that involve transcriptional processes such as induction of AP-1 activity and the inhibition of NF-κB (26). Translocation of thioredoxin to the nucleus in response to UV radiation (40) and other types of stress may contribute to this activity. The cytoprotective effect of thioredoxin against oxidative stress was best illustrated in experiments where the levels of thioredoxin had been reduced with an antisense expression vector (28). These cells became more sensitive to H2O2, a variety of anticancer drugs, and UV radiation. Our data indicate that cells expressing reduced levels of A18 hnRNP are also more sensitive to UV light (Fig. 7). A18 hnRNP could thus contribute to increase cell survival by stimulating the translation of RPA2, thioredoxin, and other stress-activated transcripts such as ferritin (Table I). As mentioned earlier, ferritin is induced by UV light (41) and protects DNA against UV-induced damage (42). Other RBPs have been shown to translocate to the cytoplasm in response to stress (15). The uniqueness of A18 hnRNP is that it is also induced (Ref. 8 and Fig. 6) by UV radiation and targets stress-activated transcripts (Table I). Moreover, A18 hnRNP can stimulate translation (Fig. 5) and increase survival after genotoxic stress (Fig. 7). In the absence of stress, A18 hnRNP is
nuclear (Fig. 1) and does not affect cell proliferation (data not shown). The capacity of A18 hnRNP to stimulate translation and increase survival is apparently directly related to its translocation to the cytosol in response to stress. This translocation may allow A18 hnRNP to play a protective role by stabilizing the transcripts of genes involved in cell survival.

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