Identification of Several Human Homologs of Hamster DNA Damage-inducible Transcripts

CLONING AND CHARACTERIZATION OF A NOVEL UV-INDUCIBLE cDNA THAT CODES FOR A PUTATIVE RNA-BINDING PROTEIN

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Low ratio hybridization subtraction technique was previously used in this laboratory to enrich and isolate a number of low abundance UV-inducible hamster transcripts (Fornace, A. J., Jr., Alamo, I. J., and Hollander, M. C. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 8800–8804) that led to the identification and cloning of five important hamster and human GADD genes (Fornace, A. J., Jr., Nebert, D. W., Hollander, M. C., Luethy, J. D., Papathanasiou, M., Fargnoli, J., and Holbrook, N. J. (1989) Mol. Cell. Biol. 9, 4196–4203). In this study we have characterized the remaining DNA damage-inducible (DDI) transcripts. Of the 24 DDI clones, 3 clones (A13, A20, and A113) representing different regions of the same hamster cDNA exhibited near perfect homology to human p21WAF1/CIP1 cDNA. The DDI clones A26, A88, and A99 displayed very high sequence homologies with the human proliferating nuclear antigen, rat translation initiation factor-5 (eIF-5), and human thrombomodulin, respectively, whereas clones A29 and A121 were different isolates of the same hamster cDNA (hereafter referred to as A18) and displayed high sequence homology with the members in the heterogeneous ribonucleoprotein (hnRNP) family. Using the hamster A18 partial-length cDNA as a probe, we screened human fibroblast cDNA library and isolated the corresponding full-length human cDNA. The deduced amino acid sequence revealed that the putative protein contains all the canonical features of a novel glycine-rich hnRNP. The A18 mRNA levels were specifically increased in response to DNA damage induced by UV irradiation or UV mimetic agents. Thus the putative A18 hnRNP is the first hnRNP whose mRNA is specifically regulated in response to UV-induced DNA damage; accordingly, it may play some role in repair of UV-type DNA damage.

Both prokaryotic and eukaryotic cells are constantly exposed to endogenous as well as exogenous DNA damaging agents. DNA damage (genotoxic stress) is implicated not only in enhancing the rate of mutagenesis but has also been shown to invoke replication and transcription blocks (reviewed in Refs. 1 and 2). UV irradiation and UV mimetic agents inflict DNA damage that is predominantly repaired via NER pathway (reviewed in Ref. 2). DNA damage induced by base damaging agents such as MMS and H2O2, on the other hand, is repaired primarily by base excision repair pathway (reviewed in Ref. 1 and 2). The cellular response to genotoxic stress is mediated via important genes that control a multitude of complex regulatory pathways. Although a number of mammalian genotoxic stress-inducible genes have been identified in recent years, the actual number of these genes is probably far greater (reviewed in Ref. 1). These genes have been implicated to play roles in a number of important cellular processes such as control of cell cycle progression, replication, transcription, signal transduction, DNA repair, and mutagenesis (reviewed in Ref. 1).

Low ratio hybridization subtraction technique is a highly sensitive method that allows for the enrichment of cDNAs of low abundance transcripts that are increased only a few-fold over the uninduced levels (3). This technique was used previously in this laboratory to enrich and isolate low abundance transcripts that were rapidly induced (within 4 h) by UV irradiation in CHO cells (4). The cDNAs representing those UV-regulated, enriched transcripts were used to construct a DDI library (4). Based on their response to genotoxic stress, these cDNA clones were later divided into two classes. Class I contained clones that were induced solely by UV irradiation and UV mimetic agents, whereas class II contained clones that were regulated by UV radiation and a host of other genotoxic agents producing base damage in DNA, such as MMS (4). Upon further characterization of the isolated clones, five important GADD (growth arrest and DNA damage-inducible) genes including GADD45, GADD153, GADD34, GADD33, and GADD7 were identified (5, 6).

GADD45 is directly regulated by p53 at transcriptional level (7) and appears to play a role in controlling cell proliferation (8). Recently, the human GADD45 protein was also found to physically interact with proliferating cell nuclear antigen (PCNA) and p21WAF1/CIP1 (9, 10). The GADD153 gene codes for a protein that belongs to the C/EBP family of transcription factors (11, reviewed in Ref. 1), whereas the GADD34 gene product appears to play a role in cell differentiation and perhaps in regulation of apoptosis (8). The GADD33 is a hamster equivalent of a human cornifin gene and appears to regulate
keratinocyte differentiation (1). Although the protein product of the GADD7 gene has not been detected and its transcript is probably not translated (12), enforced overexpression of exogenous GADD7 gene was sufficient to reduce the colony-forming efficiency of Chinese hamster ovary and RKO human colon carcinoma cells (12). The GADD genes are induced in response to various types of genotoxic and nongenotoxic stresses that elicit growth arrest (reviewed in Refs. 1 and 2). Unlike the GADD genes, a number of other transcripts were variably induced only by genotoxic agents and were not modulated in response to nongenotoxic growth-arresting conditions (4). The identity of these transcripts remains unknown. We undertook this study to further characterize the remaining DNA damage-inducible clones and to identify their human homologs.

### EXPERIMENTAL PROCEDURES

#### Cell Lines and Cell Treatment—UV irradiation and/or MMS regulation of human homologs of hamster DDI transcripts were investigated in the following human cell lines: MCF-7, breast carcinoma cells; BKO, colon carcinoma cells; A549, lung carcinoma cells; H1299, lung carcinoma cells; HeLa, cervical carcinoma cells; Sk-N-SH, neuroblastoma cells; OVCAR, ovarian carcinoma cells; ML-1, myeloid leukemia cells; WMN, Burkitt’s lymphoma cells; GM536, lymphoblastoma cells.

Cells were irradiated with UV or γ-irradiation or treated with chemical agents as described previously (4–6).

#### cDNA Library Construction and Screening—The low ratio hybridization subtraction procedure has been described in detail elsewhere (3, 4). Briefly, poly(A)^+ RNA was extracted from cells 4 h after UV irradiation and subjected to cDNA synthesis. The cDNAs from irradiated cells were hybridized at a high R, with poly(A)^+ RNA from unirradiated cells. The single-stranded cDNA thus obtained was then hybridized to original poly(A)^+ RNA obtained from irradiated cells, and the cDNA/RNA duplex was separated by hydroxylapatite column. The RNA was removed from the cDNA by alkaline treatment, and the remaining single-stranded cDNAs were utilized as templates to synthesize second strand cDNAs using a mixture of random primers. The resulting double-stranded cDNAs were cloned into a plasmid pX9 via GC-tailing (3, 4). For cloning of the human homolog of hamster A18, the Okayama-Berg GM637 human fibroblast cDNA library (13) (kindly provided by H. Okayama) was screened using a partial-length hamster A18 cDNA.

DNA Sequence Analysis—The DDI clones and the cDNAs representing human homologs of hamster A18 were sequenced by dideoxy chain termination method as described previously (4, 5).

#### RNA Isolation, Northern and Dot Blot Hybridization—Poly(A)^+ RNA preparation, Northern blotting, and quantitative dot blot hybridization were performed essentially as described previously (14). For probe labeling, the appropriate cDNA inserts were excised from the plasmids and labeled using random primer methods (14, 15). The relative poly(A) content of each RNA sample was determined using a labeled poly-lysylate probe, and the values were used to correct for variations in sample loading as described (14). For quantitation, the radioactive signals were either counted directly using a Betascope (Betagen), or the signals on the autoradiograms were measured by a densitometer.

#### Southern Blot Hybridization—DNA extraction and Southern blot hybridization analysis were performed using standard protocols (16). cDNA Probes—The following cDNA probes were used in this study: (a) cDNA inserts of DDI clones, (b) human equivalent of hamster A18 cloned in this study, (c) human EST sequences homologous to hamster DDI A29, A88, and A121 (obtained from IMAGE Consortium, LLNL), (d) human β-actin cDNA probe, as previously described (5).

#### In Vitro Transcription and Translation—The A18 cDNA containing the ORF and 3′-untranslated region was polymerase chain reaction-amplified. The TT promoter sequence was incorporated into the 5′ primer so that it was collinear with the A18 cDNA sequence starting with ATG. The polymerase chain reaction primer sequences are as follows: 5′ primer, 5′-GGATGAGGAATCATACACACCAATGGCATCAGATGAAGGCA-3′, 3′ primer, 5′-CACGACGACAGATGGACGCA-3′.

Aliquots ranging from 0.2 μg to 1 μg of the polymerase chain reaction-amplified A18 cDNA were subjected to in vitro transcription/translation using TNT-coupled reticulocyte lysate system (Promega) and [35S]methionine (Amersham Life Science, Inc.) per manufacturer’s instructions. Briefly, the reactions were performed in 50 μl for 90 min at 30 °C. Luciferase cDNA was used as a positive control, and reactions without DNA template were performed to determine the background incorporation of [35S]methionine. Five μl of each sample were analyzed on 4–20% SDS-polyacrylamide gel. Gel was fixed, soaked for 30 min in an enhancer solution (Enlightening; Dupont), dried, and exposed to x-ray film.

#### RESULTS

As described previously (3, 4), cDNA clones isolated after our hybridization subtraction procedure were only partial length, with an average insert size of 0.2–0.3 kb. In addition, many of these hamster cDNA sequences were from nontranslated portions of the UV-inducible transcripts and cross-hybridized poorly with human RNA. These partial-length cDNA clones were sequenced in their entirety and compared with all published sequences in the nucleotide and peptide sequence data bases using the BLAST network service at the National Center for Biotechnology Information. Table I illustrates the results of
the homology searches. As reported previously, these clones can be divided into two classes; class I comprises the clones that are induced by UV irradiation, whereas the class II clones are UV irradiation- and MMS-inducible. Among the DDI clones shown in Table I, the clone A26 exhibited 96, 96, and 94% nucleotide sequence homology, respectively, with mouse, rat, and human cDNAs encoding PCNA (17). The nucleotide sequence of A88 was 97% homologous to the rat cDNA encoding eukaryotic translation initiation factor-5 (eIF-5) and 91% homologous to human EST sequence bearing homology to rat eIF-5 cDNA (18). The DDI clone A99 was 86 and 72% homologous to mouse and human thrombomodulin clones, respectively (19). The DDI clones A113 as well as A8 and A20 each displayed 80, 85, and 80% homologies to mouse, rat, and human p21<sup>WAF1/CIP1</sup> cDNA sequences, respectively (20, 21). A18, A106, and A107 exhibited homology to nucleotide sequences encoding hnRNPs of diverse origin (22-24) as well as a number of unpublished EST sequences. The DDI clones A29 and A121 also displayed homology to EST sequences of unknown identity. An automated BLASTX search for the possible translation products of A29 and A121 from all reading frames did not reveal significant identities to any of the known amino acid sequences in the databases. None of the remaining clones exhibited homology to any of the nucleotide or amino acid sequences deposited in these databases. Fig. 1 shows the nucleotide sequence alignment of various hamster DDI clones to their respective human homologs.

PCNA, p21<sup>WAF1/CIP1</sup>, and thrombomodulin are among the known stress-inducible genes (25-27). No information is available on the regulation of eIF-5 (A88 homolog), since only rat (18) and yeast (28) eIF-5 cDNAs are cloned and sequenced, whereas the corresponding human cDNA remains to be cloned and sequenced. We next sought to investigate whether human homologs of hamster A88, A29, and A121 genes were also stress-inducible. In CHO cells, A88 is regulated by UV treatment, whereas the A29 and A121 are induced by UV and MMS. We exposed various human cell lines to UV irradiation, MMS, and γ-irradiation; poly(A)<sup>+</sup> RNA was isolated and subjected to quantitative RNA blot hybridization using corresponding human cDNA probes. Our results (summarized in Table I) demonstrated that MMS induced the mRNA levels of human A29 and A121 in all the cell lines tested, whereas it had no effect on the expression of A88 (eIF-5 homolog). γ-Irradiation by contrast had no appreciable effect on the levels of these transcripts.
in any of the human cell lines tested (data not shown). The UV effects were, however, cell-type specific. Of the cell lines tested (mentioned under “Experimental Procedures”), UV treatment modestly enhanced the mRNA levels of human A88 (eIF-5) in H1299 human lung carcinoma and in GM536 human lymphoblastoma cells, whereas the A29 and A121 regulation was noted in ML-1 myeloid leukemia cell line and GM536 human lymphoblastoma cells. Both A29 and A121 were also regulated by UV irradiation and by MMS in CHO cells, whereas A88 was regulated only by UV irradiation and not by MMS in CHO cells. These results, therefore, demonstrate that the regulation of these genes in response to genotoxic stress is conserved in rodent and human (Table I). Northern blot analyses were performed to determine the sizes of these transcripts, and the overall results are summarized in Table I.

A18, A106, and A107 were the three other DDI clones that belonged to class I and displayed nucleotide sequence homology to known sequences in the data bases. A comparison (using BLASTX search) of the predicted amino acids of the partial sequences of A18, A106, and A107 in all reading frames with known sequences in the data bases revealed high similarity with the hnRNPs of the RNP family (22–24). Further analysis revealed that these were three different isolates of the same cDNA and hereafter referred to as A18. The hnRNPs belong to a sub-group in a large family of the RNPs and exist in the nucleus in association with other proteins (22–24). These proteins form hnRNP complexes and are responsible for hnRNA processing (22–24). Isolation of three clones representing different regions of the same cDNA that did not cross-hybridize with any of the other isolated DDI clones coupled to the fact that none of the other clones exhibited homology to RNP family members suggested that the A18 mRNA might encode a novel hnRNP that is exclusively regulated by UV irradiation. The nucleotide and deduced amino acid sequences of A18 displayed a high degree of homology with the corresponding sequences of human and rat A1 hnRNP (22). However, in hamster cells the rat A1 hnRNP cDNA hybridized to a more abundant transcript of approximately 2 kb, which differs in size from that of A18 mRNA (1.4 kb), suggesting that the hamster DDI A18 clone does not represent hamster A1 hnRNP cDNA (22) but rather codes for a different hnRNP. Using hamster A18 and rat A1 hnRNP cDNAs as probes, we next investigated the effect of UV irradiation on the mRNA levels of A18 and A1 hnRNP in CHO cells. The results in Table II show that UV, near UV, and the UV mimetic agent AAAF up-regulated only A18 message and had no effect on A1 mRNA levels. Furthermore, only UV and UV mimetic agents enhanced the A18 transcript levels, whereas a number of other stress-inducing agents did not alter the A18 mRNA levels in these cells (Table II). It is interesting that the UV irradiation and AAAF-induced DNA damage is predominantly repaired via NER pathway (Ref. 4; see Refs. 1 and 2 for review). The specific induction of A18 mRNA exclusively by DNA damage that is repaired by NER is quite intriguing. To investigate the significance of the A18 gene in response to DNA damage that is repaired by NER and to investigate whether the A18 gene is also regulated in human cells, we sought to isolate and sequence the full-length human equivalent of hamster A18.

A human fibroblast cDNA library was screened using a partial-length 258-base pair A18 CHO cDNA as probe, and nine individual cDNA clones were isolated. Two of the nine cDNA clones were found to be identical in size. The complete nucleotide sequence of the human DDI A18 clone is shown in Fig. 2. The human A18 cDNA is composed of 1272 base pairs and contains an open reading frame with the first in-frame ATG triplet 82 nucleotides from the 5’-end and an in-frame termination codon 516 nucleotides farther downstream. The first termination codon is also followed by two additional in-frame stop codons in the 3’-untranslated region. Thus, based on the predicted amino acid sequence, the ORF could potentially encode a protein product of 172 amino acids with predicted molecular mass of 18 kDa. The A18 cDNA was subjected to in vitro transcription/translation to confirm that A18 cDNA can indeed be translated to a protein of 18 kDa. As shown in Fig. 3, the in vitro translated A18 hnRNP has a molecular mass of approximately 18 kDa.

The BLASTX alignment of the deduced amino acid sequence of the human A18 cDNA with the known amino acid sequences in the data bases revealed that the human A18 sequence is unique and bears good homology with the hnRNPs of diverse origin. The structural features of the members in the RNP family is that they contain 1–4 RNA binding domains (RBDs) of approximately 80–90 amino acids as well as a carboxy-terminal auxiliary domain that is believed to exhibit protein-protein interactions (29, 30, 23). Each RBD contains two ribonucleoprotein consensus sequences known as RNP1 and RNP2.

### Table II

Specific induction of DDI A18 mRNA by UV and UV mimetic AAAF in Chinese hamster ovary cells

| Agent                        | Dose          | DDI A18 | cDNA probe |
|------------------------------|---------------|---------|------------|
|                              |               | A1 hnRNP | β-actin    |
| UV                           | 14 J/m²       | 3.1     | 1.0        | 0.9        |
| Near UV                      | 300 J/m²      | 2.9     | 1.1        | 0.9        |
| AAAF                         | 20 μM         | 3.0     | 1.0        | 1.1        |
| MMS                          | 100 μg/ml     | 1.1     | 1.1        | 1.3        |
| H2O2                         | 200 μg/ml     | 1.0     | ND         | 1.1        |
| N-methyl-N′-nitro-N-nitrosoguanidine | 10 μM     | 1.1     | 0.8        | 1.0        |
|                              | 30 μM         | 1.0     | 0.9        | 0.9        |
| x-ray                        | 5 gray        | 0.9     | ND         | 0.9        |
| Nitrogen mustard             | 40 gray       | 1.1     | ND         | 1.1        |
| Bleomycin                    | 4 μM          | 1.0     | ND         | 0.6        |
| Adriamycin                   | 8 μM          | 1.0     | 0.9        | 1.0        |
| H2O2                         | 40 μM         | 1.4     | 1.0        | 1.0        |
| H2O2                         | 0.4 mM        | 1.3     | 0.9        | 1.1        |
| Bleomycin                    | 50 μg/ml      | 1.2     | ND         | 0.9        |
| Adriamycin                   | 0.4 μg/ml     | 1.4     | 1.1        | 0.8        |
| Heat shock                   | 45 °C         | 0.5     | 1.2        | 0.7        |
| 12-O-tetradecanoyl-phorbol-13-acetate | 20 ng/ml | 1.2     | 1.1        | 0.7        |

*ND, not determined.*
A shows the structural features of the putative A18 hnRNP protein. Thus, from the deduced amino acid sequence, the putative A18 hnRNP contains one RNA binding domain and a carboxyl-terminal auxiliary domain. The RBD contains all the consensus sequences of the conserved features in the RNA binding domain; the highly conserved RNP1 and RNP2 motifs perfectly align with those of other RBDs of various RNPs. It is intriguing that as opposed to most other RNPs that generally contain several RBDs (29, 30, 23), the A18 hnRNP contains only one RBD. Few hnRNPs of diverse origin with only one RBD have been reported (23). These one-RBD-containing hnRNPs contain additional features of homology in their RBDs such as the highly conserved sequence motifs DRET and MNGKSLDG, whereas the multiple RBD-containing RNPs do not exhibit these structural features (23, 31) (Fig. 4B). As shown in Fig. 4B, the putative A18 hnRNP contains the additional conserved motifs DRET and MNGKSVLDG, which can be perfectly aligned with one-RBD-containing hnRNPs. The carboxyl-terminal auxiliary domain of the putative A18 hnRNP, like most other hnRNPs, is highly rich in glycine residues (23, 31–34). However, the carboxyl-terminal auxiliary domain of A18 hnRNP contains several repeats of the RGG sequence, the so-called “RGG box” (35). The RGG boxes are novel single-stranded nucleic acid binding motifs that were initially identified in hnRNP U protein and were also found to be present in a few other proteins (35) (Fig. 5). The hnRNP U protein does not contain the canonical RNA binding RNP consensus sequences and was shown to bind single-stranded DNA and RNA via several repeats of RGG boxes (35). Thus, the putative A18 hnRNP is unique in that it may use its auxiliary domain to not only interact with other proteins but to also bind single-stranded nucleotide sequences.

Southern blot analysis performed on the human genomic DNA digested with various restriction enzymes revealed a distinct band pattern (data not shown), suggesting that human A18 hnRNP is a single copy gene. Fig. 6 shows that the hamster and human A18 transcripts are similar in size (1.4 kb), and just as in hamster cells, the A18 mRNA in human cells is also regulated by UV irradiation. Thus the mRNA size, the nucleotide, and amino acid sequence as well as UV regulation of DDI A18 is conserved from rodent to human.

**DISCUSSION**

In this study we have identified and characterized several DNA damage-inducible hamster cDNAs. Low ratio hybridization subtraction technique was used to isolate and clone these low abundance DNA-damage-inducible cDNAs on the basis of their rapid induction in response to UV irradiation (4). Homology searches in the data bases revealed that three of these clones exhibited near perfect matches with the different regions of rat, mouse, and human p21WAF1/CIP1 cDNAs (20, 21). The BLASTX search for the possible translation products of these three clones from all six reading frames also revealed very high amino acid identities with p21WAF1/CIP1 protein (20, 21). Since the cDNA library was made with a mixture of random primers and all three clones hybridized to the same size transcript (2 kb), it is therefore likely that the A20, A113, and A13 were three different isolates of the hamster p21WAF1/CIP1 cDNA. The finding that the DDI clones representing hamster p21WAF1/CIP1 are class II transcripts, i.e. they are induced in...
response to a variety of genotoxic stresses, is in line with the well established fact that p21 \(\text{WAF1/CIP1}\) is a stress-regulated gene (25, 26). As subsequently shown (36), the genotoxic responses of p21 \(\text{WAF1/CIP1}\) and \(\text{GADD45}\) are qualitatively identical when mediated by either p53-dependent or -independent mechanisms. Our p21 \(\text{WAF1/CIP1}\) clones were also found to be strongly stress-responsive in the original study (4) but were excluded from the \(\text{GADD}\) group because they were not induced by our medium starvation protocol as the \(\text{GADD}\) genes were. Subsequent studies have revealed that the responses of these two genes to nongenotoxic growth-arrest conditions are frequently dissimilar (37–40).

Sequence of A26 exhibited 96, 96, and 94% homology, respectively, with rat, mouse, and human cDNAs encoding PCNA. The PCNA transcript has been shown to be induced in response to DNA damage (25). The hamster A26 mRNA (1.3 kb) is also similar in size with that of 1.4-kb mRNA of PCNA from human origin (17, 25). The DDI A26, therefore, represents the hamster equivalent of human PCNA transcript. The A99 sequence exhibited near perfect match with the murine and human thrombomodulin cDNAs (19). The DDI A99 clone hybridizes with a transcript of approximately 3.8 kb that is similar in size to that of the human thrombomodulin transcript (3.8 kb) (19, 27).

The human thrombomodulin gene has been shown to be regulated by a diverse set of stresses (27). Our identification of DDI clone A99 as hamster thrombomodulin cDNA demonstrates that the stress regulation of thrombomodulin gene is conserved in rodent and human species. It is of note that this DDI library was constructed nearly a decade ago; the presence of several cDNAs of important genes such as the \(\text{GADD}\)s, p21 \(\text{WAF1/CIP1}\), PCNA, and the like in its repertoire demonstrates that low ratio hybridization subtraction is a powerful approach to isolate and identify the cDNAs of differentially expressed important genes. Although the identity of A29 and A121 remains unknown, the availability of their corresponding human EST sequences enabled us to investigate whether the transcripts of A29 and A121 were regulated in response to DNA damage in human cells. Our results demonstrated that both were indeed regulated by UV in several cell lines, whereas UV regulation occurred in a cell-type-specific manner. Further studies are in progress to clone and sequence the full-length cDNAs containing the complete open reading frames of these clones.

![DNA Damage-inducible Transcripts](image-url)
same hamster cDNA. Human cDNA corresponding to these clones was isolated and sequenced. The deduced amino acid sequence revealed that the putative protein is a novel glycine-rich hnRNP. This A18 hnRNP contains one RBD and shares sequences of homology with other hnRNPs of one RBD category (23, 31, 32). The biological significance of A18 mRNA regulation by UV irradiation and UV mimetic agents in rodent and in human remains unclear at this time. The regulation of gene expression is controlled at several levels and involves modulation of gene transcription, mRNA stability, and hnRNA processing. It is thus possible that UV may regulate the expression of important cellular genes by modulating the processing of their hnRNA via putative A18 hnRNP.

Recent studies have demonstrated that the RNPIs are not just passive piggybacks bound to RNAs during RNA processing, but rather, they play active roles in modulating important processes such as transcription, DNA replication, and apoptosis (Ref. 23 and references therein). For example, A1 hnRNP binds to single-stranded DNA and RNA with similar affinity and has been shown to shuttle between nucleus and cytoplasm during transcription (22, 41). The A1 hnRNP has also been reported to function as a helix destabilizing protein to modulate DNA replication (22). In a recent study, the hnRNP K has been characterized as a transcription factor that binds to a cis element in a sequence-specific manner and interacts with basic transcription machinery to stimulate transcription (42). Specific cleavage of a 70-kDa U1 snRNP was recently shown to be required during the induction of apoptosis (43). Overexpression of the protein interaction domain of a poly(A) binding RNP, namely TIA1, was also found to induce apoptosis in cytoplasmic T lymphocytes (44). In this study we have identified a novel cDNA that is only regulated by UV and UV mimetic agents and codes for a putative hnRNP, which we have named A18 hnRNP. UV and UV mimetic agent-mediated regulation of A18 hnRNP mRNA appears specific and novel since these agents were unable to alter the A1 hnRNP mRNA levels in the same cells, none of the other DD1 clones displayed homology to the RNP family members, and the literature search did not reveal any report documenting DNA damage-inducible regulation of any of the known RNPIs.

UV-inflicted DNA damage is repaired by NER, which is an important DNA repair pathway in mammalian cells (reviewed in Refs. 2 and 45). The inability to remove UV-induced lesions from DNA can lead to pathological conditions such as the cancer prone disease xeroderma pigmentosum (Ref. 46 and references therein). The possibility exists that the putative A18 hnRNP whose transcript is specifically induced only by UV and UV mimetic agents may play some role in modulating the process of NER. Furthermore, the putative A18 hnRNP contains unique features such as the presence of RGG boxes (35) in its auxiliary domain. The auxiliary domains of RNPIs are thought to be responsible for protein-protein interactions, whereas the RBDs serve to bind the single-stranded nucleic acids (Ref. 23 and references therein). Thus the UV regulation of putative A18 hnRNP mRNA coupled with the presence of several RGG boxes in the auxiliary domain that are responsible for binding to single-stranded DNA as well as RNA would suggest a unique function for putative A18 hnRNP. Further studies are in progress to elucidate the biological function(s) of A18 hnRNP and its role, if any, in modulation of NER pathway.

Addendum.—While our manuscript was being revised Nishiyama et al. (47) reported the cloning and characterization of the mouse homolog of our human A18 hnRNP, which they named cirp (cold-inducible RNA-binding protein). The mouse homolog of A18 hnRNP shows 86% nucleotide sequence homology and 90% amino acid identity to our A18 hnRNP. Furthermore, the purified recombinant mouse cirp exhibits strong binding to RNA.

REFERENCES

1. Fornace, A., Jr. (1992) Annu. Rev. Genet. 26, 507–526
2. Hollander, M. C., and Fornace, A., Jr. (1994) DNA Repair Mechanisms: Impact on Human Diseases and Cancer, pp. 221–237, Landes Co., Georgetown, TX
3. Fargnoli, J., Holbrook, N. J., and Fornace, A., Jr. (1990) Anal. Biochem. 187, 364–373
4. Fornace, A., Jr., Alamo, I. J., and Hollander, M. C. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 8800–8804
5. Fornace, A., Jr., Nebert, D. W., Hollander, M. C., Luthy, J. D., Fupathannasirikul, M., Fargnoli, J., and Holbrook, N. J. (1989) Mol. Cell. Biol. 9, 4196–4203
6. Papathannasirikul, M., Kerr, N. C., Robbins, J. H., McBride, O. W., Alamo, I. J., Barrett, S. F., Hickson, I. D., and Fornace, A., J. Jr. (1991) Mol. Cell. Biol. 11, 1009–1016
7. Kastan, M. B., Zhan, Q., El-Deiry, W. S., Carrier, F., Jacks, T., Walsh, W. V., Plunkett, B. S., Vogelstein, B., and Fornace, A., Jr. (1992) Cell 71, 587–597
8. Zhan, Q., Lord, K. A., Alamo, I. J., Hollander, M. C., Carrier, F., Ron, D., Kohn, K. W., Hoffman, B., Lieberman, D. A., and Fornace, A., Jr. (1994) Mol. Cell. Biol. 14, 2361–2371
9. Smith, M. L., Chen, I., Zhan, Q., Bae, I., Chen, C., Gilmer, T., Kastan, M. B., O’Connor, P. M., and Fornace, A., Jr. (1994) Science 266, 1376–1380
10. Kearsy, J. M., Coates, P. J., Prescott, A. R., Warbrick, E., and Hall, P. A. (1995) Cell 81, 1731–1739
11. Luethy, J. D., Fargnoli, J., Park, J. S., Fornace, A. J., Jr. and Holbrook, N. J. (1990) J. Biol. Chem. 265, 16521–16526
12. Hollander, M. C., Alamo, I., Fornace, A., Jr. (1990) Nucleic Acids Res. 18, 387–395
13. Chiu, D. J., Gil, G., Russell, D. W., Loscum, L., Lusky, K. L., Basu, S. K., Okayama, H., Berg, P., Goldstein, J. L., and Brown, M. S. (1984) Nature 310, 613–617
14. Hollander, M. C., Fornace, A., Jr. (1990) BioTechniques 9, 174–179
15. Feinberg, A. P., and Vogelstein, B. (1983) Anal. Biochem. 132, 6–13
16. Cobianchi, F., SenGupta, D. N., Zmudzka, B. Z., and Wilson, S. H. (1986) J. Biochem. 105, 281–285
17. El-Deiry, W. S., Tokino, T., Velculescu, V. E., Levy, D. B., Parsons, R., Trent, J. M., Lin, D., Mercer, W. E., Kinzler, K. W., and Vogelstein, B. (1993) Cell 75, 817–825
18. Harper, J. W., Adami, G. R., Wei, N., Keyomarsi, K., and Elledge, S. J. (1993) Cell 75, 805–816
19. Cobianchi, F., SenGupta, D. N., Zmudzka, B. Z., and Wilson, S. H. (1986) J. Biol. Chem. 261, 3536–3543
20. Bae, I., Fan, S., Bhatia, K., Kohn, K. W., Hoffman, B., Lieberman, D. A., and Fornace, A., Jr. (1994) J. Biol. Chem. 269, 2361–2371
21. Kumar, A., Williams, K. R., and Szer, W. (1996) J. Biol. Chem. 271, 11266–11273
22. Brud, C. G., Swanson, M. S., Gorlach, M., and Dreyfuss, K. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 9788–9792
23. Bae, I., Fan, S., Bhatia, K., Kohn, K. W., Fornace, A., Jr., and O’Connor, P. M. (1995) Cancer Res. 55, 2387–2393
24. Conway, E. M., Lui, L., Nowakowski, B., Steiner-Mosonyi, M., and Jackman, R. W. (1994) J. Biol. Chem. 269, 22864–22870
25. Chakravarti, D., and Maitra, U. (1993) J. Biol. Chem. 268, 10524–10533
26. Jiang, H., Lin, J., Su, Z., Collart, F. R., Huberman, E., and Fisher, P. B. (1994) Oncogene 9, 3399–3406
27. Michaloff, E. F., Michelot, G. A., Aronson, I. A., and Levens, D. (1996) Mol. Cell. Biol. 16, 2350–2360
28. Casciola-Rosen, L. A., Miller, D. K., Anhalt, G. J., and Rosen, A. (1995) J. Biol. Chem. 269, 30757–30760
29. Tian, Q., Steruli, M., Saito, H., Schlossman, S. F., and Anderson, P. (1991) Cell 67, 629–639
30. Sancar, A. (1995) J. Biol. Chem. 270, 15915–15918
31. Burns, J. L., Guer, S. N., Song, P., Prakash, S., and Prakash, L. (1996) J. Biol. Chem. 271, 11607–11610
32. Nishiyama, H., Itoh, K., Kaneko, Y., Kashitsha, M., Yoshida, O., and Fujita, J. (1997) J. Cell Biol. 137, 899–908