Cloning and Characterization of a Human Genotoxic and Endoplasmic Reticulum Stress-inducible cDNA That Encodes Translation Initiation Factor 1(eIF1A121/SUI1)*

(Received for publication, October 19, 1998, and in revised form, March 12, 1999)

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We report the cloning and characterization of a DNA damage-inducible (DDI) transcript DDI A121. The full-length human DDI A121 cDNA contains an open reading frame of 113 amino acids, corresponding to a protein of 12.7 kDa. The deduced amino acid sequence of A121 shows high homology to the yeast translation initiation factor (eIF) sui1 and also exhibits perfect identity to the partial sequence of recently purified human eIF1. Expression of human A121 corrected the mutant sui1 phenotype in yeast, demonstrating that human A121 encodes a bona fide translation initiation factor that is equivalent to yeast sui1p. The mammalian A121/SUI1 gene exhibits two transcripts (1.35 kilobases and 0.65 kilobases) containing a common coding region but differing in their 3'-untranslated region. The long and short A121/SUI1 mRNAs are differentially regulated by genotoxic and endoplasmic reticulum stress. The genotoxic stress induction of A121/SUI1 mRNA is conserved in both humans and rodents and occurs in a p53-independent manner. Our identification of a stress-inducible cDNA that encodes eIF1 suggests that modulation of translation initiation appears to occur during cellular stress and may represent an important adaptive response to genotoxic as well as endoplasmic reticulum stress.

DNA damage (genotoxic stress) can be induced by exogenous agents including radiation and chemicals or by endogenous processes involving the generation of reactive oxygen species (reviewed in Ref. 1). Cellular response to genotoxic stress is complex and affects a variety of cellular processes including cell cycle progression, replication, transcription, signal transduction, DNA repair, mutagenesis, and apoptosis (reviewed in Refs. 1 and 2). A number of different genes have been identified which are believed to affect these cellular processes in response to genotoxic stress (reviewed in Ref. 1). Low ratio hybridization subtraction has been previously used in this laboratory to isolate and clone a number of genotoxic stress-inducible genes including five GADD (growth arrest and DNA damage-inducible) genes (3, 4). GADD45 is a p53-regulated gene that appears to play a role in genomic stability5; GADD153 encodes a small protein of the C/EBP family of transcription factors (5); GADD34 codes for a protein that shows homology to a herpes simplex viral protein and appears to play a role in apoptosis (6). The hamster GADD83 gene is equivalent to the human cornifin gene that is involved in keratinocyte differentiation (1), whereas GADD7 does not appear to code for a protein product (7). The expression of the GADD genes has been shown to be regulated by a number of genotoxic and nongenotoxic stresses (Ref. 8 and references therein).

In addition to GADD genes, the cDNAs representing p21WAF1, proliferating cell nuclear antigen, initiation factor 5 (eIF5), and thrombomodulin genes were also detected as genotoxic stress-inducible transcripts in the same enriched library (8). In an attempt to identify and clone the genes corresponding to the remaining novel genotoxic stress-inducible transcripts present within the same library, we have recently cloned and characterized a novel genotoxic stress-inducible gene (8). This gene, named A18 heteronuclear ribonucleoprotein (18hnRNP), is specifically regulated by UV and UV-mimetic agents and encodes a putative glycine-rich RNA and DNA-binding protein (8). In the current study, we report the cloning and characterization of another human gene that corresponds to one of the previously uncharacterized genotoxic stress-inducible transcripts named A121. The full-length cDNA of human A121 encodes translation eIF1, showing a high homology to the yeast translation initiation factor sui1.

EXPERIMENTAL PROCEDURES

Cell Lines—The following cell lines were used in this study: MCF-7, breast carcinoma cells; RKO, colon carcinoma cells; A549 and H1299 lung carcinoma cells; Sk-N-SH, neuroblastoma cells; OVCAR, ovarian carcinoma cells; HL60, promyelocytic leukemia cells; ML-1, myeloid leukemia cells; WMN, Burkitt’s lymphoma cells; GM536, lymphoblastoma cells.

Cell Treatment—Cellular exposure to UV or ionizing radiation and treatment with MMS were as described previously (4, 9). For treatment with thapsigargin (LC Laboratories) and 2,5-di-tert-butylhydroquinone.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AF100737 and F100738.

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** The abbreviations used are: eIF, eukaryotic translation initiation factor; MMS, methylmethane sulfonate; DDI, DNA damage-inducible; EST, expressed sequence-tagged; DBHQ, 2, 5-di-tert-butylhydroquinone; ER, endoplasmic reticulum.
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**Fig. 1.** The cDNA and deduced amino acid sequences of the human A121 gene. The two polyadenylation signals are underlined.

none (DBHQ) (Sigma), exponentially growing cells were treated with 2 μM thapsigargin or 50 μM DBHQ for various time points, and cells were harvested for RNA extraction.

DDI Library Construction and Sequencing—The low ratio hybridization subtraction procedure and construction of the DDI A121 cDNA clone corresponding to the 3′ untranslated region of the human eIF1A gene have been described previously (4). In brief, Chinese hamster ovary cells in logarithmic phase of growth were exposed to 14 J/m² UV, and 4 h later, poly (A)+ RNA was extracted. Poly(A)+ RNA from UV-irradiated cells was reverse-transcribed into single-stranded cDNAs, which were then hybridized at a high Rot with poly(A)+ RNA from unirradiated cells. The single-stranded cDNA was obtained from cDNA:RNA hybrids after alkali treatment and hybridized to the original irradiated poly(A)+ RNA, and the resulting DNA:RNA duplexes were obtained using a hydroxyapatite column. Single-stranded DNA was isolated from the cDNA:RNA duplex and subsequently used for second strand synthesis. The double-stranded cDNAs were subcloned into a plasmid pXF3 using a GC-tailing method (3, 4). The partial-length clones in the DDI A121 library were sequenced by the dideoxy chain termination method as described previously (4).

cDNA Cloning of Human A121—The hamster DDI A121 clone was 298 base pairs long; a human EST clone 117124 (accession number T87717) showing high homology to the nucleotide sequence of hamster DDI A121 was obtained from IMAGE consortium, LLNL (Livermore National Laboratory) and sequenced in its entirety. The sequence information from the EST clone revealed the presence of a polyadenylation signal and a poly(A) tail, suggesting that the EST clone 117124 corresponded to the 3′-end of the gene. Based on the EST sequence information, we synthesized primers corresponding to the most 5′-end of the EST sequence to amplify the remaining 5′-end of the cDNA from a human placenta Marathon-Ready cDNA library (CLONTECH, Palo Alto, CA). This library contains adaptor-ligated double-stranded cDNA and the adaptor sequence serves as the 5′- and 3′-ends of the inserts (5). The adaptor sequence specific primer 5′-CCATCTAATACGACTCAG-3′ was used as 3′-primer in the amplification reactions. The amplification conditions were as follows: one cycle of 1 min at 94 °C; 25 cycles of 1 min at 94 °C, 1 min at 60 °C, and 4 min at 68 °C, and then 1 cycle of 4 min at 68 °C. The amplified products were gel-purified and cloned into a TA-cloning vector (CLONTECH, Palo Alto, CA). Several independent clones were analyzed for the presence of the amplified inserts, and the two largest cDNA clones representing the 5′-end of human A121 were sequenced in their entirety. Both of these clones, which were otherwise identical, differed in length from each other at their 5′-ends by 107 nucleotides and were collinear at their 3′-ends with the EST clone 117124. Homology searches using GenBank®/EBI and EST data banks identified a number of EST clones exhibiting nucleotide sequence corresponding to the coding region of our human A121 cDNA. These EST sequences have also been aligned to obtain a computer-generated open reading frame (10).

Northern and Dot Blot Hybridizations and cDNA Probes—RNA extraction, β-scope, and a light densitometer were used to quantitate the signals. The cDNA probes used in this study include a hamster 298-base pair A121 cDNA clone corresponding to the 3′-untranslated region and a human A121 cDNA clones corresponding to the complete open reading frame and the 3′-untranslated region. For human eIF1A cDNA probe, a human EST clone (380394) carrying the human eIF1A cDNA was obtained from IMAGE consortium, LLNL.

**RESULTS**

Molecular Cloning of Human A121—As recently described (8), the cDNAs representing the DDI transcripts in the original hamster library were only partial length and ranged in size from 0.2–0.5 kilobases. These cDNAs were sequenced, and the sequence information was used to search the sequence data banks. The partial-length sequence of the original hamster DDI clone named A121 exhibited high homology (84.3%) to several human EST sequences. These EST clones did not show homology to the sequences of known genes deposited in the public data banks, suggesting that they represented a potentially novel gene. The sequence information from EST clone 117124 (accession number T87717) was used to screen a human placenta cDNA library as described under “Experimental Procedures.” The complete nucleotide sequence of the full-length cDNA of human DDI A121 is shown in Fig. 1. The 1312-nucleotide-long human A121 cDNA contains an open reading frame predicted to code for a 113-amino acid-long protein of 12.7 kDa. The BLASTX alignment of the predicted protein sequence of the human A121 cDNA with other protein sequences in the data banks revealed that human A121 is homologous to a less well characterized yeast translation initiation factor named sui1 (16). In Fig. 2, the deduced amino acid sequences of human A121 is aligned with the predicted amino acid sequences from various species. As shown, human A121 (hereafter referred to as A121/SUI1) shows a high degree of homology to the corresponding proteins from other species including mouse, chick, mosquito, flower, and yeast.

Molecular Characterization of Human A121/SUI1 Transcripts—A121/SUI1 gene displayed two major transcripts of ~1.35 and ~0.65 kilobases in size, with some heterogeneity occurring in their expression in various tissues (Fig. 3). The two
A121/SUI1 transcripts could result from alternative splicing or from the differential usage of polyadenylation signals. Sequence analysis of the 3'-untranslated region of the human A121/SUI1 cDNA revealed the presence of two polyadenylation signals, suggesting that the latter possibility was more likely.

To further investigate this issue, we performed Northern analyses using two cDNA probes corresponding to the different regions of human A121/SUI1 cDNA. Probe A (Fig. 3) corresponds to the entire open reading frame and part of the 3'-untranslated region up to the first polyadenylation signal (upper panels). The same blot was also probed with the human A121/SUI1 probe (Probe B), corresponding to the 3'-untranslated region downstream of the first polyadenylation signal (lower panels). S. Muscle, skeletal muscle; kb, kilobases; ORF, open reading frame; UTR, untranslated region.

FIG. 2. Amino acid sequence alignment of human A121/SUI1 with the corresponding amino acid sequences from other species. GenBank accession number and reference for each sequence are as follows: chick, P51971 (23); mouse, P48024 (direct submission); Anopheles gambiae, P42678 (24); Arabidopsis P41568 (direct submission); yeast, P32911 (16). Chick and mouse sequences are partial-length, and the dashes represent gaps that were introduced to achieve better sequence alignment.

FIG. 3. Molecular characterization of the two A121/SUI1 transcripts of A121/SUI1 in various human tissues. A Northern blot (CLONTECH) containing poly(A)+ RNA from various human organs was hybridized with human A121/SUI1 probe (Probe A), corresponding to the coding region and part of the 3'-untranslated region up to the first polyadenylation signal (upper panels). The same blot was also probed with the human A121/SUI1 probe (Probe B), corresponding to the 3'-untranslated region downstream of the first polyadenylation signal (lower panels). S. Muscle, skeletal muscle; kb, kilobases; ORF, open reading frame; UTR, untranslated region.

transcript. Because the human EST sequences correspond to the 3'-untranslated region of human A121/SUI1 and not to the coding region, the above findings may also explain why the former did not display homology to sui1 from yeast and other species.

Genotoxic and Endoplasmic Reticulum (ER) Stress Regulation of Human A121/SUI1—The expression of hamster A121 transcripts was induced by the genotoxic agents UV, UV mimetic agents, and MMS in Chinese hamster ovary cells; a number of other agents, by contrast, did not modulate A121 expression in hamster cells. Table I summarizes the overall effects of various stress-inducing agents on A121 mRNA regulation in Chinese hamster ovary cells. We next sought to investigate the regulation of human A121/SUI1 in response to genotoxic and nongenotoxic stress in human cells. Our results demonstrated that human A121/SUI1 mRNA was induced by MMS in all the cell lines tested, whereas its induction in response to UV was cell type-specific. Ionizing radiation (γ-rays, 5 to 20 gray), on the other hand, did not regulate human A121/SUI1 expression in any of the human cell lines tested. The overall effects of MMS and UV on A121/SUI1 mRNA expression in the various cell lines are outlined in Table II.

MMS up-regulates only the larger A121 transcript and not the smaller transcript (see below), suggesting that MMS appears to mediate A121 regulation via elements that reside in the 3'-untranslated region. We next investigated the effects of the transcription inhibitor actinomycin D and the protein synthesis inhibitor cycloheximide on the MMS induction of human A121/SUI1 mRNA. As shown in Fig. 4, actinomycin D blocked the MMS induction of A121/SUI1 mRNA expression in MCF-7 cells; cycloheximide alone, by contrast, enhanced the mRNA levels of A121/SUI1 and further potentiated the MMS effect on A121/SUI1 mRNA levels. These results suggest that new protein synthesis is not required for the MMS-mediated increase in the A121/SUI1 mRNA levels. These results also suggest that the constitutive A121/SUI1 mRNA levels appear to be negatively regulated by certain labile protein factors. Similar experiments were also performed in Chinese hamster ovary cells, and identical results were obtained (data not shown).

We next sought to determine whether the expression of A121/SUI1 can also be modulated by conditions that primarily cause ER stress. Thapsigargin and DBHQ are specific inhibitors of sarcoplasmic/endoplasmic reticulum Ca2+ transport ATPases. By inhibiting sarcoplasmic/endoplasmic reticulum Ca2+ transport ATPases, these agents block the re-uptake of cytosolic Ca2+ into the ER, resulting in depletion of Ca2+ from the ER Ca2+ storage compartments. Stress inflicted upon cells by depletion of these Ca2+ pools can have profound effects on cell growth (18) and can lead to certain adaptive responses (17–19). Treatment of MCF-7 cells with either thapsigargin or DBHQ for 4 and 24 h results in induction of A121/SUI1 mRNA.
TABLE I
A121 mRNA regulation by different stress-inducing agents in Chinese hamster ovary cells

Cells were treated with the indicated agents and harvested for poly(A)+ RNA extraction and quantitative dot blot hybridization. A partial-length hamster cDNA probe was used in the hybridization reactions; values were normalized with respect to the corresponding poly(U) signals. The cut-off value for A121 mRNA induction ("+" sign) was considered 2-fold and higher.

| Agent and UV mimetic | Dose | Time | A121 mRNA induction |
|----------------------|------|------|---------------------|
| UV                   | 14 J/m² | 4 h | +                   |
| Near UV              | 300 J/m² | 4 h | +                   |
| N-acetoxy-2-acetylaminofluorine | 20 µM | 4 h | +                   |
| Cisplatin            | 45 µg/ml | 4 h | +                   |
| Nitrogen mustard     | 40 µM | 4 h | +                   |
| Base damaging        | 100 µg/ml | 4 h | +                   |
| MMS                  | 0.4 mM | 4 h | No induction        |
| IR and radiomimetic | 5 gray | 4 h | No induction        |
| Adriamycin           | 0.4 µg/ml | 4 h | No induction        |
| Bleomycin            | 50 µg/ml | 4 h | No induction        |
| Others               | Serum starvation | 48 h | No induction |
|                      | Heat shock | 45.5 °C | No induction |
|                      | H₂O₂      | 0.4 mM | 1 h | No induction       |

TABLE II
MMS and UV regulation of human A121/SUI1 mRNA in various human cancer cell lines

Cells were exposed to MMS (100 µg/ml) or UV irradiation (14 J/m²) and 4 h later were harvested for RNA extraction and quantitative dot blot hybridization. Values of one representative experiment are given, which were normalized with respect to the corresponding poly(U) signals. ND, not determined.

| Cell line | Cell type | p53 status | MMS effect (fold-induction) | UV effect (fold-induction) |
|-----------|-----------|------------|-----------------------------|---------------------------|
| A549      | Lung      | Wild type  | 6.6 (fold-induction)        | 1.0 (fold-induction)      |
| ML-1      | Leukemia  | Wild type  | 6.1 (fold-induction)        | 2.1 (fold-induction)      |
| MCF-7     | Breast    | Wild type  | 5.2 (fold-induction)        | 1.2 (fold-induction)      |
| RKO       | Colon     | Wild type  | 3.2 (fold-induction)        | 1.1 (fold-induction)      |
| OVCAR     | Ovarian   | Wild type  | 2.6 (fold-induction)        | 0.8 (fold-induction)      |
| WMN       | Burkitt’s lymphoma | Wild type | 3.1                           | 0.8                          |
| GM 536    | Lymphoblasts | Wild type | 3.4 (fold-induction)        | 3.6 (fold-induction)      |
| H1299     | Lung      | Null       | ND                           | 2.3 (fold-induction)      |
| HL60      | Leukemia  | Null       | 2.5 (fold-induction)        | 1.0 (fold-induction)      |
| Sk-N-SH   | Neuroblastoma | ND       | 2.4                           | 1.0                          |

levels when compared with untreated cells (Fig. 5), and the degree of increase in the A121/SUI1 mRNA levels appear to parallel that seen with MMS (Fig. 5). These data suggest that the induction of A121/SUI1 may represent a stress response that occurs when cells are exposed to injury (genotoxic or otherwise). It is also of note that unlike MMS and UV irradiation (see below), which up-regulate only the larger transcript, the ER stress-inducing agents up-regulate both transcripts (Fig 5), suggesting that the potential mechanisms of A121/SUI1 mRNA regulation by genotoxic stress and ER stress might be different.

Genotoxic Stress Induction of A121/SUI1 Is Conserved in Mouse and Occurs in a p53-independent Manner—The levels of wild-type p53 protein are enhanced by genotoxic agents such as ionizing radiation, UV, and MMS (Ref. 11 and references therein). p53 in turn transcriptionally up-regulates the expression of its downstream effector genes (11 and references therein). To ascertain whether A121/SUI1 is a p53-regulated gene, we investigated the genotoxic stress regulation of A121 in p53 wild-type and p53 knock-out cells. Primary keratinocytes obtained from p53 wild-type and p53-knock-out mice were exposed to MMS and UV irradiation, and the effect on A121/SUI1 expression was investigated. As shown in Fig. 6, MMS and UV induction of A121/SUI1 mRNA was noted in both genotypes, and the lack of p53 did not considerably affect the extent of A121/SUI1 mRNA induction. Again, the data in Fig. 6 show that both MMS and UV irradiation predominantly up-regulate the larger A121/SUI1 transcript. These results demonstrate that the genotoxic stress regulation of A121/SUI1 mRNA is conserved in humans and rodents and that it occurs in a p53-independent manner.

Human A121 Appears to Encode Human eIF1 and Can Correct the Mutant sui1 Phenotype in Yeast—Pestova et al. (20) recently purified and partially sequenced the human eIF1 and eIF1A proteins. The sequence (GDDLLPAGT and TLTTVQ-GIA) of two tryptic peptides representing human eIF1 show exact identity to the corresponding amino acid sequence predicted for A121/SUI1. The temperature-sensitive sui1 mutations also allow the mutant strains to grow at 37 °C (13, 16, 21). The molecular mass (13 kDa) of purified eIF1 is also similar to the predicted molecular mass (12.7 kDa) of the A121 protein product. Human eIF1 and eIF1A were reported to have distinct yet synergistic activities that were required for recognition of the initiation codon (20). Human eIF1 alone has been reported to be necessary for ensuring the fidelity of translation initiation by recognizing and destabilizing the incorrectly assembled ribosomal complexes at the initiation codon. Similar function has been reported for yeast sui1p. Deletion of the sui1 gene is lethal (16, 21); temperature-sensitive mutations within the sui1 gene have been identified that do not allow the mutant strains to grow at 37 °C (13, 16, 21). The temperature-sensitive sui1 mutations also allow translation to initiate at UUG start codon (16, 21, 13). Introduction of the exogenous wild-type sui1 gene has been shown to inhibit translation of his4<sup>UUG</sup>-lacZ in sui1 mutant background (13, 16, 21). Furthermore, sui1 mutant strains carrying the exogenous wild-type sui1 gene were able to grow at both permissive and nonpermissive temperatures (13, 16, 21).

We reasoned that if A121/SUI1 cDNA encodes a bona fide
translation initiation factor equivalent to yeast sui1p, then human A121/SUI1 should function in yeast and correct mutant sui1 phenotype. To test this contention, we subcloned the human A121 cDNA into a yeast single copy expression vector pG-1 (13, 14) and tested its ability to suppress translation of his4\textsuperscript{UUG}-lacZ gene. The reporter vector his4\textsuperscript{UUG}-lacZ contains the His4 gene fused in-frame with the lacZ gene, and the AUG start codon of the His4 gene is replaced with UUG as a start codon (16, 21, 13). Various yeast strains carrying the plasmid borne human A121/pJD176F) or expression plasmid without A121 insert (pG-1) were transformed with the reporter vector his4\textsuperscript{UUG}-lacZ, and the \( \beta \)-galactosidase activities were determined. From the results summarized in Table III, it is clear that human A121/SUI1 suppresses his4\textsuperscript{UUG}-lacZ \( \beta \)-galactosidase activity only in the sui1 strain and not in the sui2 and SUI3 mutant strains. sui2 and SUI3 encode the \( \alpha \) and \( \beta \) subunits of
eIF2, respectively (22), and mutations in both sui2 and SU3 also allow translation to initiate at the UUG codon, although only sui1 and sui2 mutations confer growth related temperature sensitivity (16, 21, 22). The plasmid expressing human A121/SUI1 only allowed the sui1–1 strain and not the sui2–1 to grow at both permissive and nonpermissive temperatures (Table IV). The ability of human A121/SUI1 to correct only the sui1 phenotype and not the sui2 and SU3 phenotypes not only highlights the specificity of A121/SUI1 function but also demonstrates that human A121/SUI1 can function in yeast.

**DISCUSSION**

Previously, we have reported the cloning and characterization of several important genotoxic stress-inducible genes from the hamster DDI library originally made using a low ratio hybridization subtraction technique (Refs. 3, 4, and 8 and references therein). The clones in this library represent transcripts that are induced within 4 h after exposure to UV irradiation (4). Here we report the identification and molecular cloning of the full-length cDNA of a previously uncharacterized DDI A121 transcript from the same library. The deduced amino acid sequence of human A121 shows high homology to the yeast translation initiation factor sui1 (16) and the corresponding proteins from other species including mouse (direct submission, accession number P48024), chick (23), mosquito (24), and *Arabidopsis thaliana* (direct submission, accession number P41568). The tissue distribution of A121/SUI1 mRNA in human and mouse tissues revealed that A121/SUI1 is expressed in most tissues; whether A121/SUI1 is absolutely required for protein translation in mammalian cells or is dispensable must await the results of A121/SUI1 gene knock-out studies. Yeast sui1 has been shown to be an essential gene (16, 21) and was originally identified as a translation initiation suppressor locus (21). In addition to sui1, two other genes, namely sui2 and SU3, have also been identified in yeast (21, 22). Mutations at all these three unlinked loci restored expression of the HIS4 allele lacking the canonical AUG translation initiation start site (21), a finding that implicates their role in controlling the fidelity of translation initiation (21).

Unlike sui1, the sui2 and SU3 genes have been reported to encode the α and β subunits of eIF2, respectively (22). Although sui1p has been reported to encode the function of eIF3, sui1p may not be a bona fide subunit of eIF3 (25). Recently Cai et al. (13) reported the characterization of a novel allele of sui1 named mó2–1. The sequence of the wild-type mó2 gene was identical to that of sui1, and both were able to independently correct mó2–1 or sui1 phenotypes and ensure translation fidelity (13). The fact that the partial amino acid sequence of the recently purified human eIF1 (20) is identical to the predicted amino acid sequence of A121/SUI1 suggests that the latter is likely to encode human eIF1. It has recently been reported that human eIF1 and eIF1A act in concert to promote 48 S ribosomal complex formation at the initiation codon (20); 43 S complex lacking eIF1 and eIF1A does not reach the initiation codon (20). Furthermore, eIF1 alone was able to recognize and destabilize the aberrantly formed complexes at the initiation codon, a function that is consistent with that reported for yeast sui1 (16, 13). Our findings that expression of human A121/SUI1 in yeast was able to correct the sui1 but not the sui2 or SU3 phenotypes demonstrate that A121/SUI1 encodes the human equivalent of yeast sui1p.

The A121/SUI1 gene exhibits two transcripts of varying abundance in different tissues. Using cDNA probes corresponding to different regions of A121/SUI1, we have demonstrated that these transcripts result from alternative usage of two poly(A) signals present in the 3′-untranslated region. It is interesting that both transcripts are differentially regulated by genotoxic and ER stress. For example, the genotoxic agents MMS and UV induced expression of only the larger transcript, whereas ER stress-inducing agents up-regulate both transcripts. These results would suggest that genotoxic stress either exerts a transcriptional influence or enhances the A121/SUI1 mRNA stability via elements residing within the 3′-untranslated region. ER stress-inducing agents, on the other hand, may transcriptionally up-regulate the levels of A121/SUI1 transcripts from the common 5′-end. Future studies will illuminate the molecular basis for the differential regulation of A121/SUI1 transcripts by genotoxic and ER stress-inducing agents.

Although the transcripts encoding human eIF5 were also present in the original DDI library, eIF5 mRNA expression was not induced by MMS and only minimally induced by UV in some human cell lines (8). eIF1A is the interacting partner of human eIF1; however, eIF1A expression was not regulated by genotoxic or ER stress (data not shown). From the results presented in this study, it is evident that (a) only the expression of A121/SUI1 is regulated by genotoxic and ER stress, whereas that of eIF1A is not, (b) the A121/SUI1 gene expression is regulated in an agent-specific manner, and (c) its induction is not a general cellular response to all types of stresses. Because of the lack of the availability of antibodies against eIF1, we were unable to test its regulation at the protein levels. Conceivably, genotoxic and ER stress induction of A121/SUI1 mRNA and protein is coupled and may represent part of the defense mechanism of a cell to provide a translational checkpoint control to modulate the expression of certain proteins at the translational level. Further studies are needed to test this contention and should lead to a better understanding of the process of translation initiation during various cellular stresses.

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| Strain       | Y157 (sui1–1) | Y158 (sui2–2) | Y244 (SU3–3) | Y159 (wild type) |
|--------------|----------------|----------------|---------------|-----------------|
|              | pg1            | pJD176F        |               |                 |
| Y157 (sui1–1)| 0.191          | 0.026          |               |                 |
| Y158 (sui2–2)| 0.188          | 0.189          |               |                 |
| Y244 (SU3–3) | 0.104          | 0.109          |               |                 |
| Y159 (wild type) | 0.028          | 0.029          |               |                 |

**TABLE IV**

When expressed in yeast, the A121 cDNA clone (pJD176F) allows growth at both permissive and nonpermissive temperature only in sui1–1 background and not in sui2–1 background.

| Strains     | Growth at 30 °C | Growth at 37 °C |
|-------------|-----------------|-----------------|
| Y157 sui1–1 | +               | +               |
| Y158 sui2–2 | +               | –               |
| Y159 wild type | +           | +               |
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