CHOP/GADD153 Gene Expression Response to Cellular Stresses Inhibited by Prior Exposure to Ultraviolet Light Wavelength Band C (UVC)

INHIBITORY SEQUENCE MEDIATING THE UVC RESPONSE LOCALIZED TO EXON 1*

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CHOP/GADD153 is both an activating and repressing transcription factor that is markedly induced in response to a variety of cellular stresses. The CHOP/GADD153 gene was originally cloned because of its inducibility by ultraviolet light wavelength band C (UVC) and has since been found to be activated in response to many different cellular stresses. Some of the recent studies have questioned the UVC responsiveness of the CHOP gene. Contradiction in our own data led us to reexamine the UVC effects on CHOP expression. UVC is capable of strongly activating the mouse CHOP promoter in stably transfected NIH 3T3 cells but has only a modest and transient effect on the level of the CHOP messenger RNA. In addition to its positive effect on CHOP promoter activity, we show that UVC negatively affects CHOP mRNA and protein expression. Pretreatment of NIH 3T3 cells with UVC markedly attenuates the subsequent induction of CHOP mRNA by the cellular stress activators methylmethane sulfonate, tunicamycin, glucose deprivation, and methionine deprivation for as long as at least 16 h. This inhibitory effect of UVC on CHOP expression in response to stress is independent of the presence or absence of p53 and does not involve mRNA degradation as opposed to the UVC effect that inhibits p21 expression seen only in the absence of p53. The target of the inhibitory effect of UVC on CHOP expression is located in the first exon of the gene, a 5′-untranslated region that is unusually conserved between different species. These findings suggest that an unknown function encoded by the 5′-untranslated region somehow modifies the response of CHOP gene transcription to UVC.

Cells of unicellular or multicellular organisms have adapted to live and propagate in ways determined by their genetic makeup and by the environmental cues received. Perturbations or adverse conditions that place cells in danger trigger the stress response: an ensemble of changes in cellular physiology including the rapid alteration of expression of specific genes. Although many of these changes have not been functionally characterized, they most probably participate in mechanisms such as adaptation, protection, damage assessment, repair, and cellular death if the damage places the rest of the organism at risk.

CHOP (C/EBP homology protein, also called GADD 153 (growth arrest DNA damage 153)) is a basic region leucine zipper transcription factor and heterodimerizes with members of the C/EBP family of transcription factors (1). The expression of CHOP is markedly induced by a variety of cellular stresses. The dimerization of CHOP with C/EBPβ inhibits the function of the latter by preventing DNA binding to a C/EBP site in the promoters of a subset of genes. In addition, CHOP/C/EBP heterodimer binds to a different DNA sequence of another subset of genes (2) and stimulates the transcription of these genes (3). CHOP also heterodimerizes with other non-C/EBP subfamilies of basic region leucine zipper proteins, e.g. activating transcription factor 3 (4) and Jun/Fos (5). Several functions have been proposed for CHOP. Heterotopic overexpression of CHOP induces growth arrest in fibroblasts (6), inhibits adipocyte differentiation (7), and induces apoptosis in vitro (8). Studies in CHOP knockout mice also suggest a role for CHOP in apoptosis during the endoplasmic reticulum stress response in kidney cells (9).

CHOP (GADD 153) was first identified based on the finding that it was expressed in response to growth arrest and DNA damage (10). The expression of CHOP has subsequently been shown to be induced by many other cellular stresses such as amino acid deprivation (11, 12), growth inhibition by prosta
glandin A2 (13), hypoxia (14), acute phase response (15), endoplasmic reticulum stress (16), glucose deprivation (17), cysteine conjugates (18), tunicamycin (2, 14), and calcium ionophore treatment (19). These findings have also questioned the initially observed growth arrest and DNA damage inducibility of CHOP. Glucose or amino acid limitation could have been the inducers in the initial growth arrest studies (10, 20) in which cells were grown at high densities so that cell culture media could have become nutrient-deprived. Also, DNA damaging agents are known to not only target DNA but other molecules in the cell as well. Therefore, the signals that promote CHOP gene expression may not be DNA damage per se (16). The response of CHOP expression to UVC irradiation is representative of the contradictions in the literature. CHOP was first isolated as GADD153 because of its inducibility by UVC radiation (10). Further studies showed that the gadd153 gene

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1 The abbreviations used are: C/EBP, CCAAT/enhancer-binding protein; CAT, chloramphenicol acetyltransferase; MAP, mitogen-activated protein; MMS, methylmethane sulfonate; UVC, ultraviolet light wavelength band C; HIV, human immunodeficiency virus.
promoter mediates this response (21–23). In contradiction to these results, Wang et al. (16) showed no increase in CHOP mRNA or protein levels following the treatment of human fibroblasts with UVC, confirming the data obtained in keratinocytes (24, 25). These discrepancies could reflect differences in cell type, mode of treatment, or culture conditions.

We have cloned the promoter region of the mouse CHOP gene. Promoter-CAT constructs stably integrated in the genome show strong UV responsiveness, whereas endogenous CHOP mRNA or protein do not. A closer analysis reveals that UVC acts in a dual and antagonistic way on CHOP expression. Here we describe studies that implicate the first exon of the CHOP gene as a novel and critical UVC-responsive modulator of the responsibility of the expression of CHOP to cellular stress.

MATERIALS AND METHODS

Cell Lines and Their Treatment—NIH 3T3 fibroblasts were grown in Dulbecco's modified Eagle's medium (Life Technologies, Inc.) supplemented with 10% calf serum, penicillin (100 units/ml), and streptomycin (100 µg/ml). Cells were maintained at 37 °C and 5% CO₂. The p53+/− and p53−/− mouse embryonic fibroblasts (26) were cultured under the same conditions except that serum was 10% fetal bovine. For induction experiments cells were split the day before at a density of about 2–4×10⁵ cells/5-cm dish. Cells were always subconfluent during the course of the experiments. Tunicamycin (Sigma) was resuspended in the depleted medium before incubation. NIH 3T3 cells were irradiated with 20 or 25 J/m² using a UVB125 TUV-2B lamp (UVP, Upland, CA) at 18 J/m²/cm². Cells were exposed to 50–70% of the maximal killing rate at the wavelength of 313 nm (20). The cell monolayer was washed several times with normal calf serum. The cell monolayer was washed several times with Dulbecco's modified Eagle's medium (Life Technologies, Inc.) devoid of glucose and pressed with 10% calf serum, penicillin (100 units/ml), and streptomycin (Sigma). For UVC treatment, cells were irradiated with UVC for 5 s after removal of medium and one wash with phosphate-buffered saline with a UVC (254 nm) germicidal lamp, 6.3 J/m²/s. For mRNA stability studies transcription was blocked with actinomycin D (Sigma) at a final concentration of 5 µg/ml.

Plasmids—The CHOP promoter-CAT reporter constructs used in this study were generated by cloning polymerase chain reaction amplified mouse CHOP promoter fragments in the pBLCAT3 vector (27). The template for the polymerase chain reaction was a CHOP genomic clone isolated by screening a mouse (129 Sv) genomic library (Stratagene) made of partially Sau 3A digested DNA with a probe encompassing the mouse exon 2, exon 3, and part of exon 4. The 5′ upstream regulatory region of the CHOP gene obtained was 2.5 kilobases. For preparation of constructs, −318/−19 and −318/−9 the forward primers were 5′-CGAGTCGACTGTGTTTCCTCTGATGACCCAGT-3′ and 5′-CGAGTCGACCGGTTGCACTCTTGCCAAATCGCATC-3′, respectively. For construction of the reverse primer was 5′-CGAGGATCCTGTTAGGCTCAAGA-3′ and 5′-CGCCGATCTCCTCTGACGTCCCGGTGTTTT-3′. For the exon 1 containing construct −318/−95 the reverse primer was 5′-CCGGCATCCTCCTCTGACGTCCCGGTGTTTT-3′.

Generation of Stably Transfected Cells—Pools of stably transfected cells were prepared as follows. NIH 3T3 cells were cotransfected with 10 µg of promoter-CAT reporter construct and 2 µg of puromycin-resistant plasmid pPGKpuro (a gift of Peter W. Laird) with the calcium phosphate precipitation technique using a reagent kit (5 Prime – 3 Prime, Inc., Boulder, CO). Selection of puromycin-resistant colonies was performed with 1.5 µg/ml puromycin (Sigma). Pools of at least 40 colonies were used for induction and CAT assay.

Western Blot Analysis—Cells were lysed at 4 °C in RIPA buffer (10 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.1% SDS, 1% deoxycholic acid, 1% Nonidet P-40 containing 5 µg/ml aprotinin, 0.5 µg/ml leupeptin, 5 µg/ml pepstatin, 1 mM dithiothreitol, and 1 mM phenylmethylsulfonyl fluoride). Following SDS-polyacrylamide gel electrophoresis extracts were blotted onto polyvinylidene difluoride membranes (DuPont). Antibody for p53 was from Santa Cruz (Pab 240). For CHOP a polyclonal antibody was used (1). Immunooblots were revealed by ECL detection system (Amer sham Pharmacia Biotech).

Northern Blot Analysis—Total RNA was extracted by using trizol reagent (Life Technologies, Inc.) following the manufacturer’s protocol. RNA was precipitated on a 1% agarose gel containing formaldehyde. After transfer to a Magna Charge nylon membrane (Micron Separation Inc.), UV treatment, and baking, the prehybridization and hybridization were performed at 42 °C in 50% formamide, 5× SSPE, 0.1% SDS, and 5× Denhardt’s solution. Probe was labeled with [α-32P]dATP by random priming reaction. For quantification, blots were scanned with a PhosphorImager (Molecular Dynamics), and data were analyzed with IMAGEQUANT software.

RESULTS

We isolated a mouse CHOP genomic clone from which we prepared CHOP promoter-CAT reporter constructs. The reporter constructs were stably introduced into NIH 3T3 fibroblasts by cotransfection with a plasmid encoding puromycin resistance. Pools of puromycin-resistant colonies were challenged with different stresses known to induce CHOP expression. The mouse promoter (−980/+19) behaves very similarly to what is known from studies of the hamster promoter (−778/+21) (21, 22). Strong activation of the promoter in response to MMS, actinomycin D (data not shown), or UVC was observed. Tunicamycin, an inhibitor of protein glycosylation and endoplasmic reticulum function, is also a strong inducer of CHOP expression (2, 14, 16). We show that the induction of CHOP by tunicamycin is mediated by the CHOP promoter (−980/+19 promoter (Fig. 1A). As a control the thymidine kinase promoter activity does not change upon treatment with UVC or tunicamycin (Fig. 1A). Under the same stress conditions that stimulate CHOP promoter activity, we also examined endogenous levels of CHOP mRNA and proteins (Fig. 1, B and C). MMS and tunicamycin show the expected induction of both mRNA and protein levels, in accordance with the promoter data. Following UVC treatment, however, no changes in CHOP mRNA and protein levels are seen at 4 or 24 h. A substantial increase in p53 protein level is seen, demonstrating the effectiveness of the UVC treatment (Fig. 1B).

The absence of CHOP induction by UVC is in contradiction with our promoter data obtained using the same cell line and same treatment conditions. One possible explanation is that UVC induces CHOP gene transcription in a transient manner, such that at the time points we examined (4 and 24 h) the mRNA and protein levels are either not yet induced or have already returned to the non-induced level. The high stability of the CAT protein on the other hand may have allowed its accumulation and detection even long after the promoter is in the inducible state. To explore this hypothesis, we examined CHOP mRNA levels at more frequent time intervals (Fig. 2). A rapid but modest elevation of CHOP mRNA is observed as early as 5 min after UVC exposure (2.5-fold above control). The induction of CHOP mRNA peaks at 1 h (4.5-fold above control) and decreases after 1 h, reaching control levels at 5 h. This modest increase in CHOP mRNA levels, however, does not correspond to the large increase in the activity of the CHOP promoter-CAT reporter activity observed in response to UVC. This discrepancy could be explained by a dual action of UVC on CHOP expression: a positive action on the CHOP promoter activity (as revealed by our construct) and a negative action (acting on transcription initiation, elongation, or RNA stability) that counteracts the promoter activity and consequently attenuates the induction of expression. If so, this hypothesis would predict that UVC may inhibit the induction of CHOP expression by other inducers of cellular stress. This hypothesis was explored. UVC treatment performed 1 h or 30, 15, or only 5 min (lanes 6, 5, 4, and 3, respectively) prior to MMS treatment significantly inhibits the strong induction of CHOP expression observed with MMS treatment alone (lane 2 and con-

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2 M. Schmitt-Ney, unpublished results.
trol level of CHOP mRNA, lane 1) (Fig. 3). This effect of UVC is long lasting, because 16 h after UVC treatment cells that were challenged with MMS show no or very little induction of CHOP mRNA or protein levels (Fig. 3B). This inhibition by UVC of CHOP induction is not restricted to MMS induction, because the induction of CHOP in response to tunicamycin, glucose deprivation, or methionine deprivation is also inhibited by pre-treatment of the cells with UVC (Fig. 4).

Recently, Gorospe et al. (28) have analyzed in detail the p53-dependent elevation of p21 expression in response to UV light. The study showed that in the absence of p53 not only did induction of p21 by UVC not occur, but that the induction of CHOP in response to tunicamycin, glucose deprivation, or methionine deprivation is also inhibited by pre-treatment of the cells with UVC (Fig. 4).

CHOP expression occurs independently of p53 because it is observed in both p53+/- and p53−/− mouse embryonic fibroblasts (Fig. 5). We then analyzed the effect of UVC on the stability of the CHOP mRNA. Cells treated with tunicamycin during 4 h with or without receiving a UVC pretreatment were subjected to a block in transcription by actinomycin D. The fate of the CHOP mRNA was analyzed at different times (Fig. 6). As expected, the level of CHOP mRNA is reduced in cells pre-
UVC.

NIH 3T3 cells were irradiated (by hybridization with a 32P-labeled probe).

RNA. After electrophoresis and blotting the CHOP mRNA was detected for 3.5 h. Cells were then harvested for the preparation of total cellular methionine for 6 h. Total RNA was prepared after electrophoresis and blotting the CHOP mRNA was detected by hybridization with a 32P-labeled probe.

5 min later cells were treated with tunicamycin (10 μg/ml) for 6 h. Total RNA was prepared after electrophoresis and blotting, the CHOP mRNA was detected by hybridization with a 32P-labeled probe.

FIG. 6.

UVC does not decrease CHOP mRNA stability. NIH 3T3 cells were irradiated with UVC (30 J/m2) or not irradiated as indicated. 5 min later cells were treated with tunicamycin (TUN, 10 μg/ml) for 3.5 h. Transcription was then blocked with actinomycin D (5 μg/ml). Cells were harvested at the time indicated for total RNA preparation. A, after electrophoresis and blotting, the CHOP mRNA was detected by hybridization with a 32P-labeled probe.

p53 +/+ and p53 −/− mouse embryonic fibroblasts were or were not pretreated with UVC light (30 J/m2). 5 min later cells were treated as indicated: untreated control (−), tunicamycin (TUN, 10 μg/ml), or MMS (50 μg/ml) for 3.5 h. Cells were then harvested for the preparation of total cellular RNA. After electrophoresis and blotting the CHOP mRNA was detected by hybridization with a 32P-labeled probe.

FIG. 5.

The inhibitory effect of UVC on CHOP expression is not dependent on the presence or absence of p53, p53 +/+ and p53 −/− mouse embryonic fibroblasts were or were not pretreated with UVC light (30 J/m2). DNA damage has been shown to be one of the initial main focus of studies on UV action. The short wavelength component of UV light, UVC, has been used extensively as a source of genotoxic stress. In fact DNA damage was shown to be one of the initial signals in two important pathways induced by UVC: the p53 and NF-κB (33). Other DNA damage-dependent pathways leading to the production of secreted factors have been described (34, 35). But UVC is not only a genotoxic stress (reviewed in Ref. 31). It is absorbed by other biomolecules in the cell, like proteins and lipids. Suspicions about extra-nuclear targets capable of initiating UVC stress signaling (36) were confirmed by the demonstration that plasma membrane receptors are activated very early after UVC exposure (37–39). Ligand-independent receptor dimerization upon UV irradiation was suggested as a mechanism of activation, but several reports have now shown that it is the inactivation of tyrosine phosphatases by UVC that cause phosphorylation and activation of the receptors (40, 41).

FIG. 4.

The action of diverse CHOP inducers is inhibited by UVC. NIH 3T3 cells were irradiated (lanes 2, 4, 6, 8, and 10) with UVC (30 J/m2) or not irradiated (lanes 1, 3, 5, 7, and 9). 5 min later cells were treated as indicated with MMS (50 μg/ml) for 4 h, tunicamycin (TUN, 10 μg/ml) or MMS (50 μg/ml) for 3.5 h. Transcription was then blocked with actinomycin D (5 μg/ml). Cells were harvested for the preparation of total cellular RNA. After electrophoresis and blotting the CHOP mRNA was detected by hybridization with a 32P-labeled probe.

The UV stress response is an ensemble of changes in gene and protein expression occurring in cells irradiated with UV light (30, 31). DNA damage has been the initial main focus of studies on UV action. The short wavelength component of UV light, UVC, has been used extensively as a source of genotoxic stress. In fact DNA damage was shown to be one of the initial signals in two important pathways induced by UVC: the p53 and NF-κB (33). Other DNA damage-dependent pathways leading to the production of secreted factors have been described (34, 35). But UVC is not only a genotoxic stress (reviewed in Ref. 31). It is absorbed by other biomolecules in the cell, like proteins and lipids. Suspicions about extra-nuclear targets capable of initiating UVC stress signaling (36) were confirmed by the demonstration that plasma membrane receptors are activated very early after UVC exposure (37–39). Ligand-independent receptor dimerization upon UV irradiation was suggested as a mechanism of activation, but several reports have now shown that it is the inactivation of tyrosine phosphatases by UVC that cause phosphorylation and activation of the receptors (40, 41).

Downstream events in the UV stress response have been characterized in more detail and originate at least in part from the activation of membrane receptors. These include the activation of several kinases cascades ultimately leading to trans-activation of several MAP kinases: c-Jun N-terminal kinase MAP kinase (42), p38 MAP kinase (43–45), and extracellular regulated kinase MAP kinase (45). Consequently, multiple transcription factors in the nucleus, e.g. c-Jun, ternary complex factor, activating transcription factor 2, and the CAMP response element-binding protein, become phosphorylated by these kinases and regulate transcriptional programs of the cell. Among the target genes of these transcription factors are those encoding for transcription factors. Rapidly developing transcriptional cascades are thus initiated. The AP-1 complex (Jun/Fos heterodimers) is the prototype of these immediate early transcriptional responses and is induced at the transcriptional level in a few minutes after UV irradiation.

The CHOP/GADD153 gene was initially isolated in a screen for genes whose expression is induced by UVC (10). The strongest data supporting the UVC inducibility of the CHOP/GADD153 gene come from studies performed with the isolated promoter of the gene. Strong inducibility has been described with the hamster gene promoter spanning from position −778 to +21 relative to the start of transcriptional initiation (21–23).
We have cloned 2.5 kilobases of the upstream regulatory region of the mouse CHOP gene. Promoter-CAT reporter constructs were derived from this mouse sequence and stably transfected in NIH cells. Strong UV responsiveness (up to 100-fold) were obtained and are in agreement with the data on the isolated hamster promoter in the context of transfected transcriptional reporter constructs.

Data on the endogenous CHOP mRNA and protein are more conflicting in the literature. An increase of the GADD 153 mRNA of about 5-fold was reported in the initial cloning publication (10). A similar significant increase was observed in HeLa cells following UVC irradiation (46). Other reports demonstrated a small or even no response (16, 24, 28, 47, 48). In all of these studies the UVC dose was quite similar: 15–30 J/m². One study used a more natural range of UV light source and reported a significant increase in CHOP mRNA (24).

Our findings appear to reconcile the conflicting findings in earlier reports. We observe a 4–5-fold increase in CHOP mRNA levels. Levels of CHOP mRNA increase within 5 min after irradiation, peak at about 1 h and return to the uninduced level at about 3 h. This transient increase in CHOP mRNA levels in response to UVC may explain the lack of an increase observed by others at times later than 2 h after irradiation. This relatively modest and transient increase in CHOP mRNA contrasts with the strong effect that UVC exerts on activity of the CHOP promoter under identical conditions of exposure to UVC and in the same cell line. This discrepancy led us to hypothesize that the CHOP gene responds to two opposing signals from UV...
light: an induction of CHOP gene transcription mediated by the promoter of the gene between positions –318 to +19 relative to the start of transcription and an inhibitory action mediated by exon 1 of the gene that prevents the induction of CHOP by the subsequent application of stress-inducing agents for as along as 20 h after UVC irradiation.

The mechanism(s) by which the presence of the first exon confers a UVC-induced inhibition of subsequent induction of the expression of the CHOP gene for as long as 20 h is unknown. The first exon flanks the promoter and is transcribed and present in the mature CHOP mRNA. Therefore, the UVC inhibitory effect observed in the presence of exon 1 could arise at least at three levels of gene expression: (i) transcriptional initiation by influencing the activity of the upstream promoter, (ii) transcriptional elongation by blocking the passage of RNA polymerase, or (iii) RNA stability because exon 1 is present in the mature mRNA.

No such destabilization of CHOP mRNA was observed. Rather, UVC appeared to increase the stability of the CHOP mRNA. These findings are in agreement with those of Jackman et al. (47), who showed stabilization of the GADD153 mRNA in several situations of cellular stress, one of them being UVC irradiation. Stabilization of mRNA was shown recently to be the mechanism by which UV induces the expression of several important growth regulating genes: the p21 cyclin-dependent kinase inhibitor (28, 49) and the later induction of c-Jun transcripts that follows its early transcriptional induction (50).

Interestingly, the CHOP gene contains a 3′ AU-rich region known to be involved in mRNA stability (28, 51). The AU-rich region sequence is also found in the p21 and the c-Jun transcripts. This sequence is able to bind several proteins; one of these, HuR, is capable of stabilizing the mRNA to which it is bound (52). HuR translocates from nucleus to cytoplasm upon UV irradiation and stabilizes the p21 message (49).

Gorospe et al. (28) describe that in cells lacking the p53 tumor suppressor protein UV was not only not able to induce p21 but also prevented the induction of p21 by mimosine. However, in our studies UVC inhibition is not dependent on the presence or absence of p53, therefore revealing a new mechanism and pathway by which UV is acting.

Inhibition of transcription initiation could be mediated by a cis-acting element located in exon 1 in the DNA. Many examples of regulatory elements downstream of the initiation site have been reported. We have used exon 1 DNA sequences as a probe in a bandshift assay and have not detected binding of proteins in nuclear extracts from cells treated or not treated with UV (data not shown). Transcription initiation could also be modulated by regulatory elements present in the RNA. Such regulation has been demonstrated for example for the HIV trans-activator protein protein that binds the Tat-responsive element sequence in the newly synthesized mRNA and enhances transcription initiation by interacting with the basal transcription machinery.

Finally, transcript elongation could be the target of UVC action. This type of regulation is widely observed in nature and can utilize various mechanism (see Ref. 53 for review). DNA-binding proteins can interfere with the elongation of the nascent RNA. In addition, RNA-binding-proteins like Trp RNA-binding attenuation protein bind to the newly transcribed RNA and arrest progression of RNA polymerase (54).

DNA damage per se is capable of inhibiting transcript elongation (see Ref. 55 for review). Cyclobutane pyrimidine dimers, the most prevalent lesion formed by short wavelength UV radiation, are known to block DNA replication and gene transcription. At the dose used in this study (30 J/m²), the frequency of cyclobutane pyrimidine dimers in the irradiated genome should be of about 1 in 3,000 base pairs (56). Because lesion in the untranscribed strand of DNA is not subject to transcription arrest (57), this calculates a frequency of about 1 in 6,000 base pairs in the transcribed strand. The CHOP gene is a short gene, and cyclobutane pyrimidine dimers are a good substrate for transcription-coupled repair and should therefore be rapidly removed. Furthermore, inhibition of CHOP inducibility could be seen at lower UVC dose of 12 J/m² (data not shown). It is therefore unlikely that DNA lesions in the CHOP gene are responsible for the lack of CHOP induction by UVC. Also the CAT reporter gene under the CHOP promoter is strongly activated by UVC, which could not occur if transcription elongation was blocked by DNA lesions. It is, however, possible that the exon 1 of the CHOP gene represents a hot spot for UVC-induced lesions. These hot spots have been described (58). Also changes in the kinetic of repair of DNA have been described, for example, in the p53 gene leading to mutational hot spots where repair is slow (59).

In summary, we report here a new finding about the regulation of CHOP gene expression by UVC, namely an inhibitory effect on expression. Most studies on UVC have concentrated on the activation of gene and protein expression. The inhibitory effect we describe here is interesting in at least two respects: it reconciles the contradictions observed in the literature and also in our own studies, and it reveals a new mechanism by which UVC acts, namely on an untranslated 5′ exon. This mechanism of inhibition of gene expression by UVC may represent a quick and economical way (in a period of high stress) of turning off genes whose expression is not favorable for the cell at a given moment but might be needed later on.

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