Characterization of a DNA-damage-recognition protein from F9 teratocarcinoma cells, which is inducible by retinoic acid and cyclic AMP

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A nuclear protein that recognizes u.v.-damaged DNA was detected in extracts from murine F9 embryonic stem cells using a DNA-binding assay. The nuclear-protein-binding activity was increased in cells after treatment with retinoic acid/dibutyryl cyclic AMP (dbcAMP), with optimum induction at 6 days. *In vitro* treatment of nuclear extracts with agents that affect protein conformation (such as urea, Nonidet P40 and Ca^{2+}) slightly modulated the damage-recognition activity. Furthermore, treatment of nuclear extracts with phosphatase dramatically inhibited the binding activity. In addition, damaged-DNA recognition of

the nuclear extracts was effectively inhibited by damaged double- and single-stranded DNA. The expression of the nuclear protein with similar characteristics was abundant in HeLa cells and was increased in drug- or u.v.-resistant cells. The findings suggest that the recognition of a u.v.-DNA adduct is modulated, at least in part, by an activity that is induced during retinoic acid/dbcAMP-induced differentiation. These results also imply that the identified damage-recognition protein may be important for the sensitivity or resistance of mammalian cells to DNA damage.

INTRODUCTION

U.v. radiation causes the production of cyclobutane-type pyrimidine dimers as well as other DNA base adducts in cells. In response to this naturally occurring DNA-damaging agent, various systems have evolved for the repair of damaged DNA. In human cells, the majority of u.v. damage is repaired by nucleotide excision [1]. We and others have found a rough correlation between the binding activity of u.v.-damaged-DNA-recognition protein (u.v.DRP) and cellular sensitivity or resistance to u.v. damage [2]. This provides a potential indicator of responses of cells to DNA-damaging agents. Since u.v.-type excision repair involves multiple steps that require different enzymes and accessory proteins that presumably must have access to the damaged DNA for effective repair to occur [1,3], it is reasonable that one or more of these factors could be identified through DNA-binding activity. This is supported by evidence that nuclear extracts isolated from mammalian and yeast mutants during DNA repair fail to interact with damaged DNA [4,5]. Inducible u.v.DRP has also been demonstrated in primate cells by pretreatment of cells with u.v. [6]. However, the regulation of u.v.DRP-binding activity is not clear. We have recently reported that some cells resistant to cisplatin, a potent chemotherapeutic agent, expressed enhanced plasmid re-activation and inducible binding activity for cisplatin-DNA adducts [7–9]. Analogously to the repair of u.v.-DNA adducts, cells probably manage cisplatin-DNA adducts in the same way. It has also been shown that the removal of the major cisplatin adducts involves nucleotide excision repair in bacterial [10–12] and mammalian cells [13,14]. These results suggest that the repair process of the cisplatin adduct is probably the same, at least in part, as that of 'u.v.-type' excision repair. However, failures to detect an increase in cisplatin-DNA-recognition activity in cisplatin-resistant cells have been reported by others [e.g. 15], suggesting complexities in the regulation of damage-recognition activity in different cell lines or in different culture conditions of the same cell line. In any

case, substantial lines of evidence have indicated that the inducible level of damage recognition can be elicited by DNA-damaging agents.

In this study, we present evidence showing a differentiation-associated mechanism that can also induce cellular u.v.DRP-binding activity. A low abundance of nuclear protein that specifically interacts with u.v.-damaged DNA was identified from murine F9 stem cells. This protein was induced in cells after the induction of differentiation. Functional analyses suggest that the F9-cell u.v.DRP-binding activity has properties similar to that from HeLa cells. The significance of this regulated expression of u.v.DRP is discussed.

EXPERIMENTAL

Cell lines and induction conditions

Murine F9 embryonic adenocarcinoma stem cells and human HeLa S3 cells were maintained according to the supplier's specifications (American Type Culture Collection). XP fibroblasts (XP12RO, SV40-transformed xeroderma pigmentosum complementation group A) and a cisplatin-resistant HeLa derivative cell line were maintained as previously described [8]. Specifically, F9 cells (a gift from Dr. W.-C. Chang, National Taiwan University) were cultured on gelatin-coated (0.1 %) tissue culture dishes in Dulbecco's modified Eagle's medium (Gibco, Gaithersburg, MD, U.S.A.) containing 10 % (v/v) heat-inactivated fetal calf serum, and incubated at 37 °C in a humidified atmosphere of 5 % (v/v) CO_2 in air. Inhibition of cell growth was assayed by the colorimetric MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] method as previously described [16].

For the induction of F9 differentiation, cells were treated with 0.1 μM all-*trans*-retinoic acid (RA) and 1 mM dibutyryl cyclic AMP (dbcAMP) (Sigma Chemical Co., St. Louis, MO, U.S.A.). The establishment of a differentiated phenotype was evident from morphological changes in response to RA/dbcAMP ex-

posure. Biochemical differentiation was verified by analysing cell cultures for expression of plasminogen activator [17].

Preparation of DNA probes

DNA probes were prepared as described previously [8]. The DNA fragment f130 was originally isolated from plasmid pSVT [18] by restriction with *SphI*–*BglII*. This 130 bp fragment was ligated to the *SphI*–*SmaI*-opened vector pBS(+) (Stratagene) at the *SphI* site. The 5' recessed *BglII* site of f130 was then filled with Klenow DNA polymerase (New England Biolabs) and ligated to the *SmaI* site of the vector. The *HindIII*–*EcoRI* f130 fragment containing a 17 bp dA–dT-rich region is a potential target for u.v. modification. *HindIII*–*EcoRI*-generated f130 fragments were [³²P]dCTP-labelled (3×10^4 c.p.m./ng of DNA) using Klenow DNA polymerase and purified in spin columns by standard methods [19]. The f130 DNA at a concentration of 100 µg/ml was irradiated with u.v. germicidal lamps in semidark as previously described [20]. DNA was irradiated with a fluence rate of 25 J/m² per s from a VL-100C u.v. irradiation unit (Vilbert Lourmat). The fluence rate was measured by a VLX-254 radiometer (Vilbert Lourmat).

DNA transfection and chloramphenicol acetyltransferase (CAT) assays

A modification of the electroporation technique [7] was used for the introduction of pRSVcat DNA into F9 cells. Cells were seeded at 1×10^6 cells per 100 mm plate 1 day before electroporation. Cell suspensions (1 ml in HEPES buffer) were added to sterile cuvettes containing 20 µg of pRSVcat plasmid, gently mixed and subjected to electroporation with a GenePulser (BioRad). Conditions were typically 1000 µF capacity, and 200 V. After resting at 25 °C for 10 min after transfection, cells were returned to the incubator. The following day the cells were fed with fresh media and incubated for another 48 h in the presence or absence of RA/dbcAMP. Cells were then harvested into 1 ml of PBS and centrifuged. The cell pellet was then either stored at –80 °C or processed for CAT assay as previously described [7]. The CAT assay reaction mixture was incubated at 37 °C for 1 h, followed by development on a silica t.l.c. plate (Macherey-Nagel). After autoradiography, density on the X-ray film corresponding to the modified chloramphenicol was quantified by using a scanning densitometer (Hoefer GS300). CAT activity was calculated as percentage of chloramphenicol converted into acetylated derivatives.

Preparation of cell extracts and gel mobility shift assay

Crude nuclear and cytosolic extracts were prepared as described by Dignam et al. [21]. The protein concentration was measured by the Bradford assay using the BioRad dye reagent [22], and visualized by SDS/PAGE [23]. Protein–DNA binding was assayed using 8 µg of nuclear extract, unless otherwise indicated, in 15 µl of buffer containing 12% (v/v) glycerol, 12 mM HEPES (pH 7.9), 100 mM KCl, 5 mM MgCl₂, 4 mM Tris/HCl, 1 mM EDTA, 1 mM dithiothreitol, 300 µg/ml BSA at 25 °C for 30 min as previously described [24]. The reaction mixtures were then subjected to 4% PAGE at low ionic strength (6.7 mM Tris/HCl, pH 7.9, 3.3 mM sodium acetate, 1 mM EDTA) at 25 °C and 15 mA constant current. The resolved gel was dried and exposed to Kodak XAR-5 X-ray film at –70 °C with an intensifying screen. The intensity of the shifted DNA bands was determined by scanning densitometry.

RESULTS

Detection of a u.v.DRP in differentiating F9 cells

Under the standard DNA-binding conditions, a u.v.DRP was detected in RA/dbcAMP-induced differentiating F9 cells. As shown in Figure 1, the probe (6 kJ/m² u.v.-irradiated f130) was bound by a factor or factors in 8 µg of nuclear extracts after 2, 4, 6 or 8 days of induction (indicated with an arrowhead). The binding activities increased with the days of induction (Figure 1, lanes 3–7). Optimal u.v.DRP binding was detected at day 6, followed by a rapid decrease at day 8. Non-specific binding was also seen on this gel (indicated with an asterisk). In contrast, only the non-specific-binding band was detected in mock-treated cells (Figure 1, lane 2). The free probe (indicated by f in Figure 1) was also detected in the extract-free reaction mixture (Figure 1, lane 1).

For comparison, 2 µg of nuclear extracts of human cells (Figure 1, lanes 9–11) were run in parallel with uninduced F9-cell extracts (Figure 1, lane 8). U.v.DRP binding is much lower in F9 cells. Resistant HeLa, HeLa and XP group-A cells all exhibited significantly higher u.v.DRP-binding activity than F9 cells. In addition, other human cells (e.g. colon cancer SW620 and VA-13) also expressed higher damage-recognition activity than F9 cells (results not shown). This is not due to differential protein loading because the amount of protein in each extract was the same based on SDS/PAGE analysis as well as the colorimetric (Bradford) assay. In general, human cells exhibited greater u.v.DRP-binding activity than murine cells. Since F9 cells are hypersensitive to u.v. (see below), the results suggest that the u.v.DRP-binding activity identified in F9 cells may be related to the sensitivity or resistance of cells to DNA damage.

To investigate the idea that the induction of F9-cell u.v.DRP is differentiation-associated, the RSV promoter, the transcriptional activity of which is responsive to RA (e.g. [25]), was expressed in F9 cells (Figure 2). Some 20 µg of pRSVcat was introduced into differentiating F9 cells 60 h before assay of CAT (see the Materials and methods section for details). The data indicate a significant increase in CAT activity (indicated by arrowheads) at 6 days and a further increase at 8 days (Figure 2, lanes 6 and 7 respectively). In contrast, only a minor amount, if any, of activity could be measured up to 4 days of induction

Figure 1 Detection of u.v.DRP-binding activity in differentiating F9 cells

(a) (lanes 1–7), 8 µg of nuclear extracts isolated from cells that had been mock treated (lane 2) or induced with RA/dbcAMP for the indicated number of days (lanes 3–7) were incubated with 6 kJ/m² u.v.-irradiated probe f130. Lane 1, extract-free reaction mixture. f, Free probe; arrowhead, u.v.DRP-binding activity; non-specific binding is labelled with an asterisk. (b) (lanes 8–11), Comparison of u.v.DRP-binding activity in murine F9 and human cells: 2 µg of nuclear extract from F9 (lane 8), resistant HeLa (lane 9), HeLa (lane 10) or XP (lane 11) cells were studied.

Figure 2 Transcriptional activity of plasmid pRSVcat in differentiating F9 cells

The F9 cells were induced for differentiation with RA/dbcAMP for the indicated number of days (see the Materials and methods section for details). Some 20 μ g of pRSVcat DNA was electroporated into F9 cells induced for 0, 1, 2, 4, 6 or 8 days (lanes 2–7) and allowed to express for 60 h. Some 20 μ g of cellular extract was used for assay of CAT activity. cm, Chloramphenicol; arrowheads, acetylated chloramphenicol.

Figure 3 Localization of u.v.DRP to cell nucleus

Nuclear and/or cytosolic extracts at the indicated amounts (μ g) from 6-day-induced F9 cells were incubated with the probe (lanes 2–10). Lane 1 is extract-free reaction mixture. This autoradiograph was overexposed on purpose to make the cytosolic binding activity more visible. Symbols are as in Figure 1.

(Figure 2, lanes 2–5). Lane 1 shows the CAT reaction using chloramphenicol (cm) only as substrate. Furthermore, morphological and biochemical markers (see the Materials and methods section for details) indicate that more than 70 % of cells after 6 days of RA/dbcAMP treatment were in the process of differentiation. Therefore the induction conditions should be sufficient to induce differentiation in F9 cells.

Cellular localization of the u.v.DRP to the nucleus

To localize the u.v.DRP in cells, nuclear or cytosolic extracts from 6-day-induced F9 cells were used for the DNA-binding assay. As shown in Figure 3, the binding activity roughly increased with the amount of nuclear extract (lanes 2–4). Only slight binding activity was measured in cytosolic extracts (lanes 8–10). A combination study of the nuclear and cytosolic extracts gives supporting evidence (lanes 5–7). A total of 10 μ g of cellular extracts, for example, showed higher binding in the reaction mixture containing more nuclear extract than in that containing more cytosolic extract (compare lanes 6 and 7 of Figure 3).

Figure 4 U.v.DRP-binding activity in F9 and HeLa cells

(a) Nuclear extracts (8 μ g) prepared from F9 (lanes 2–5) or HeLa (lanes 6–9) cells were incubated with the probe f130 without u.v. treatment (lanes 2 and 6), irradiated with 1 kJ/m² (lanes 3 and 7), 3 kJ/m² (lanes 4 and 8) or 6 kJ/m² (lanes 5 and 9). Lane 1 is probe without extract (control). (b) U.v.DRP-binding of indicated amounts (μ g) of nuclear extracts (NE) from F9 and HeLa cells. The extracts were incubated with unirradiated f130 (lanes 1–7) or 6 kJ/m² u.v.-irradiated f130. Symbols are as in Figure 1.

Interestingly, a reaction mixture containing 8 μ g of cytosolic extract plus 2 μ g of nuclear extract showed higher binding than the sum of 10 μ g of cytosolic extract and 2 μ g of nuclear extract (compare lane 6 with lanes 2 and 10 of Figure 3). Lane 1 indicates the reaction without cell extracts as a control. In every case, a majority of the u.v.DRP-binding activity appeared in the nuclear fraction. The minor binding in the cytosolic fraction is probably due to cross-contamination of the extract, or it could be a real presence in the cytosol.

Low abundance of u.v.DRP in F9 cells

Nuclear extracts from F9 or HeLa cells were prepared for DNA-binding assays. Nuclear extracts (8 μ g) from these cells were used to detect the u.v.DRP by using a DNA probe irradiated with 0, 1, 2 or 6 kJ/m² u.v. light (Figure 4a). HeLa-cell u.v.DRP (lanes 6–9) showed at least a 20-fold higher binding activity than that of F9 cells (lanes 2–5). The probe alone was included as a control (lane 1). For both nuclear extracts, the binding activities roughly increased with the level of u.v. damage, indicating a correlation of u.v. damage level with DRP-binding activity.

Figure 4(b) shows u.v.DRP-binding activities in changing amounts of F9- or HeLa-cell nuclear extracts. Unirradiated f130 (lanes 1–7) or u.v. (1 kJ/m²)-irradiated f130 (lanes 8–14) probe was incubated with the indicated amounts of nuclear extracts. Significant binding activities were detected in the u.v.-damaged probe. However, a large amount of nuclear extract also slightly interacted with unirradiated f130 (e.g. compare lanes 7 and 14 of Figure 4b). The significance of the interaction of u.v.DRP with unirradiated DNA probe is unclear. In any case, u.v.DRP binding in F9 cells is much lower than in HeLa cells. Furthermore,

Table 1 Comparison of u.v.DRP-binding activity in F9 and HeLa cells

Some results for HeLa cells were adapted from ref. [27].

	F9	HeLa
RA/dbcAMP induction	+	—
Cellular location	Nucleus	Nucleus
Abundance (molecules per megabase)	0.0035	0.077
Competition for binding:		
U.v.-damaged DNA	Yes	Yes
Undamaged DNA	No	No
Protein-conformation modifier resistance	++	+++
CIP sensitivity	Yes	Yes

unexpected binding activity was seen in F9 [indicated in Figure 4(b) with an asterisk; also seen in Figure 1], but not in HeLa cells. Since this binding activity was also observed to an equal extent in the reaction using unirradiated probe, we consider it to be non-specific. It is interesting to note that a second retarded band, although smaller, was observed when u.v.DRP binding is increased (see lane 14 of Figure 4b: see also Figure 5). The two complexes are shown to involve similar protein species when resolved by SDS/PAGE (results not shown), suggesting that the slower-migrating complex is probably a modified form of the major binding complex. By using scanning densitometry, the intensity of u.v.DRP-binding activity was quantified. The abundance of u.v.DRP in F9 and HeLa cells is approx. 0.0035 and 0.077 molecules per megabase respectively if 1:1 binding stoichiometry is assumed [4] (see below and Table 1). For this estimation, 8 μ g of nuclear extract and 0.3 ng of 130 bp DNA probe (with 6 kJ/m² u.v. irradiation) were used.

Similar specificity of u.v.DRP in F9 and HeLa cells

To verify further whether the u.v.DRP identified in F9 and HeLa cells are the same, a competition experiment was carried out. As shown in Figure 5, with increasing amounts of specific competitors (f130-u.v.) the competition became apparent (lanes 2–5 for F9, lanes 9–12 for HeLa cells). Approximately 65% inhibition (or 35% binding) was detected if the amount of competitor was 10-fold (i.e. 3 ng) for F9 (compare lanes 2 and 4) and HeLa cells (compare lanes 9 and 11). A 80–85% inhibition (or 15–20% binding) was seen when competitors were increased by 100-fold (compare lane 5 with 2, and lane 12 with 9) for both cell lines. Binding reaction without nuclear extract is shown in lane 1 as a control. In our hands, there is an approximately 15% deviation in routinely performed DNA-binding assay. It should be noted that a 100-fold molar excess of the specific competitor inhibited less than 80% of u.v.DRP-binding activity, implying that the chemical nature of DNA binding is different from the promoter-binding proteins such as those of human glucose-regulated protein gene promoter to which a near-100% inhibition is observed with the same molarity of sequence-specific competitor (C. C.-K. Chao, unpublished work).

In contrast, non-specific competitor did not effectively inhibit u.v.DRP binding. A 100-fold molar excess of undamaged f103 (Figure 5, lanes 6 and 13) or single-stranded f130 (ssf130) (Figure 5, lanes 7 and 14) did not significantly compete for u.v.DRP. Interestingly, the single-stranded f130 carrying u.v. damage (ssf130-u.v.) effectively competed against u.v.DRP binding (compare lane 8 with 2 and lane 15 with 9 in Figure 5). Nevertheless,

Figure 5 Competition of the u.v.DRP-binding activity of F9 (lanes 2–8) and HeLa (lanes 9–15) cells

Probe (0.3 ng) was incubated with 8 μ g of nuclear extracts in the presence of the indicated molar excesses of specific competitor f130-u.v. (lanes 3–5 and 10–12) or non-specific competitor including unirradiated f130 (lanes 6 and 13), single-stranded f130 (lanes 7 and 14), or irradiated single-stranded f130 (lanes 8 and 15). Control lanes are: probe without extract (lane 1), reaction without competitor (lane 2). Symbols are as in Figure 1. A slow migrating complex is also seen at high u.v.DRP binding (see also legend to Figure 4).

we have not been able to detect similar binding activity in the two cell lines using either unirradiated or irradiated ssf130 probe (results not shown), suggesting that duplex DNA is required for effective u.v.DRP binding.

Modulation of u.v.DRP by agents that modify protein conformation

The u.v.DRP-binding activity detected in F9 cells was assayed *in vitro* in the presence of agents that modify protein conformation. Urea, Nonidet P40 (NP40) and Ca²⁺ all affect the binding activity of a human nuclear factor to the promoter region of a glyceraldehyde-3-phosphate dehydrogenase gene (C. C.-K. Chao, unpublished work). These agents have also been shown to modify the conformation of hsp70, and subsequently modulate its binding to the consensus heat-shock element [26]. Therefore these agents were used to modify F9-cell u.v.DRP binding (Figure 6). Nuclear extracts (8 μ g) were used in the reaction mixture containing the indicated concentrations of Ca²⁺ (lanes 3–5), NP40 (lanes 6–8) or urea (lanes 9–11). The results indicate that CaCl₂ at the concentrations tested slightly modified, if anything, u.v.DRP binding compared with the modifier-free reaction (lane 2). NP40, ranging in concentration from 0.02 to 2%, increased u.v.DRP-binding activity by up to 2-fold. Furthermore, the binding activity was not affected by 0.5 M urea, but was inhibited by urea at concentrations higher than 1 M. These results indicate that the chemical nature of F9-cell u.v.DRP is similar to that identified in HeLa cells [27].

In addition, the effects of phosphate on u.v.DRP-binding activity were studied by treatment of nuclear extracts with calf intestine phosphatase (CIP) or adding ATP to the reaction mixture. Addition of CIP (see the legend to Figure 6) completely inhibited u.v.DRP binding (Figure 6, lane 13) compared with the control reaction (Figure 6, lane 12). Inhibition was also caused by high concentrations of ATP (Figure 6, lanes 14–16). For example, more than 90% binding was inhibited (i.e. less than 10% binding retained) by 10 mM ATP. Since 10 mM ATP will

Figure 6 Modulation of u.v.DRP-binding activity by CaCl_2 , NP40, urea, phosphatase or ATP

Nuclear extracts (8 μg) were mixed with the indicated concentration of CaCl_2 (lanes 3–5), NP40 (lanes 6–8), urea (lanes 9–11) or ATP (lanes 14–16), and incubated in the binding-reaction mixture. The effect of calf intestine phosphatase (CIP, 1 unit, 37 $^\circ\text{C}$ for 30 min) was also tested (lane 13). Control lanes are: probe without extract (lane 1), without the addition of modulating agents (lanes 2 and 12). Symbols are as in Figure 1.

chelate all of the Mg^{2+} in the reaction mixture, the significance of the inhibition by ATP is not certain. However, the data suggest that u.v.DRP binding is highly sensitive to changes in phosphorylation of the molecule.

DISCUSSION

In this study, we have identified a cellular protein from murine F9 stem cells that recognizes u.v.-damaged DNA. This u.v.DRP-binding activity is present in relatively low abundance in F9 cells compared with HeLa cells. The binding activity in HeLa cells is a minimum of 0.077 molecules per megabase of genomic DNA, if 1:1 binding stoichiometry is assumed [4]. Nuclear extracts further purified by passage through a heparin-agarose column exhibited at least a 20-fold higher binding activity (C. C.-K. Chao, unpublished work). This is comparable with the 1.7 molecules per megabase estimated by others [4]. An analogous calculation yields an estimate of about 0.0035 molecules per megabase of F9-cell genomic DNA (see below). Thus the abundance of murine u.v.DRP is less than one-twentieth of that of human u.v.DRP with respect to genome size. XP cells displayed only trace amounts of u.v.DRP binding activity. Other workers have also found only a trace or none of this type of DNA binding in the XP group-E cells [4,28]. These data imply a potential correlation between u.v.DRP and DNA excision repair. This is consistent with a recent finding by Wood's group that a purified XPA factor exhibited the property of complementing repair synthesis *in vitro* and binding to damaged DNA [29].

The u.v.DRP-binding activity detected in murine F9 cells appears, on the basis of the following, to have properties similar to those in HeLa cells [27] (Table 1). First, most of the F9-cell u.v.DRP is localized in the nucleus. Secondly, the DRP-binding activity is specific for DNA-u.v. adducts. The two binding activities had the same relative affinities for different forms of DNA. Thirdly, the u.v.DRP is resistant to protein-conformation modifiers. Furthermore, both F9-cell and human u.v.DRP exhibit similar mobility on polyacrylamide gel. Therefore u.v.DRP may be conserved in mammalian cells. Nevertheless, the functional similarity between F9- and HeLa-cell u.v.DRP does not guarantee their similarity in structure.

Unexpectedly, we also found that u.v.DRP-binding activity is elicited in differentiating F9 cells. The level of u.v.DRP increased

with cellular differentiation with a maximum of a more than 10-fold increase after 6 days of induction. This regulation of F9-cell u.v.DRP was not detected in HeLa cells after the same treatment (results not shown). The estimated maximum u.v.DRP-binding activity in F9 cells is about 0.04 molecule per megabase, that is about 50% of that in HeLa cells, compared with 5% of HeLa-cell u.v.DRP in uninduced F9 cells. The results imply that u.v.DRP is physiologically regulated. We have previously demonstrated that human DRP specific for cisplatin damage is inducible by treatment of cells with cisplatin [9]. It has also been reported by others that a constitutive u.v.DRP can be induced by pretreatment of monkey cells with u.v. [6]. Clearly, there is an inducible mechanism for DRP regulation in mammalian cells. Altogether, the level of constitutive DRPs is important but it may not necessarily correlate with the sensitivity or resistance of cells to DNA-damaging agents. One should also consider the inducible level of DRPs when pondering the potential importance of cellular proteins to cytotoxicity. Our findings may add one line of support to the notion that, in addition to exogenously caused DNA damage, differentiation-associated pathways can positively regulate u.v.DRP in cells. In this study, we have also shown that phosphorylation may be an additional mechanism in controlling u.v.DRP-binding activity. However, our data are insufficient to permit understanding of the mechanisms that cause the induction of u.v.DRP-binding activity.

We thank Ms. Shang-Lang Huang for technical assistance. This research was supported by grants from Chang Gung Medical College (CMRP318, NMRP166) and the National Science Council, R.O.C. (NSC81-0412-B182-07 to C.C.-K.C. and NSC80-0203-B001-14 to S.L.-C.).

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Received 27 July 1992/27 August 1992; accepted 2 September 1992