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Rapid changes of nucleotide excision repair gene expression following UV-irradiation and cisplatin treatment of *Dictyostelium discoideum*

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ABSTRACT

Organisms use different mechanisms to detect and repair different types of DNA damage, and different species vary in their sensitivity to DNA damaging agents. The cellular slime mold *Dictyostelium discoideum* has long been recognized for its unusual resistance to UV and ionizing radiation. We have recently cloned three nucleotide excision repair (NER) genes from *Dictyostelium*, the *rep*B, D and E genes (the homologs of the human xeroderma pigmentosum group B, D and E genes, respectively). Each of these genes has a unique pattern of expression during the multicellular development of this organism. We have now examined the response of these genes to DNA damage. The *rep*B and D DNA helicase genes are rapidly and transiently induced in a dose dependent manner following exposure to both UV-light and the widely used chemotherapeutic agent cisplatin. Interestingly, the *rep*E mRNA level is repressed by UV but not by cisplatin, implying unique signal transduction pathways for recognizing and repairing different types of damage. Cells from all stages of growth and development display the same pattern of NER gene expression following exposure to UV-light. These results suggest that the response to UV is independent of DNA replication, and that all the factors necessary for rapid transcription of these NER genes are either stable throughout development, or are continuously synthesized. It is significant that the up-regulation of the *rep*B and D genes in response to UV and chemical damage has not been observed to occur in cells from other species. We suggest that this rapid expression of NER genes is at least in part responsible for the unusual resistance of *Dictyostelium* to DNA damage.

INTRODUCTION

All organisms are continuously exposed to a variety of DNA damaging agents such as UV-light, ionizing radiation and chemicals. A variety of damage repair mechanisms have evolved to correct each of the specific types of lesions (1–3). The mechanisms used appear to be at least partially organism and/or cell-type specific. A major question is how cells recognize the specific type of DNA damage and mount the correct response.

One mechanism of DNA repair, which repairs mainly damage caused by external assaults such as UV-irradiation or chemicals, is nucleotide excision repair (NER) (4–6). Defects in the individual components of this multistep pathway result in impaired DNA repair and increased sensitivity to UV. In humans, defects in NER result in a disease known as xeroderma pigmentosum (XP), an autosomal recessive inherited disorder that is characterized by increased UV-sensitivity in exposed areas of the skin and eyes, and an increased incidence of skin cancer. There are eight XP complementation groups (XPA–XPG and XPV), and the corresponding genes for most of these groups have been cloned (7–10).

The DNA helicases encoded by the XPB and XPD genes have dual function in transcription as well as in repair. Thus the XPB and XPD are part of a six polypeptide core complex that can be part of the transcription initiation factor, the holo-TFIIH, which functions in transcription, or part of the repairosome, which functions in NER, including transcription coupled repair (TCR) (11–14). This structural relationship between these processes may account for the wide pleotropy observed in many XP patients, and may explain why individuals homozygous for mutations in some NER components often exhibit an array of neurological and developmental abnormalities associated with two other human disorders, Cockayne syndrome (CS) and Trichothiodystrophy (TTD) (8,15).

The cellular slime mold *Dictyostelium discoideum*, which has been widely used in studies of cell and developmental biology (16,17), is unusually resistant to DNA damage (18,19). We have recently identified and characterized the *Dictyostelium rep*B, D and E genes, homologs of the human *XPB*, *XPD* and *UV-DDB/XPE* genes, respectively. The mRNAs of the *Dictyostelium rep*D and E genes rapidly accumulate following the shift from mitotic growth to multicellular development (20,21). This pattern of *rep* gene expression may result from an increased need for transcription coupled repair due to the rapid burst of gene transcription at the onset of development (22–24). Irrespective of the mechanism,

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there is a close coupling of *rep* gene expression with the transition from DNA replication and cell division to development.

We now show that *Dictyostelium* cells at all stages of growth or development can be further induced to express *rep* genes in response to either UV-light or the widely used chemotherapeutic agent cisplatin. This rapid induction of NER genes may be at least partially responsible for the unusual resistance of the cells to DNA damaging agents. The patterns of development, UV and cisplatin induced gene expression are each unique, suggesting different mechanisms of induction.

MATERIALS AND METHODS

Strains and conditions of growth and development

Strain Ax4 was grown axenically in rich HL5 medium (25), or defined minimal FM medium (26). The cell density was never allowed to go beyond $2–3 \times 10^6$ cells/ml, and new cultures were started monthly from stored spores. To initiate development, cells were washed twice by centrifugation in LPS [40 mM Na/KPO_4 pH 6.4, 20 mM KCl, 2.5 mM $MgCl_2 \cdot 6H_2O$, 0.5 mg/ml streptomycin sulfate (27)], resuspended in the same buffer and either shaken in LPS at 2×10^6 or deposited on 40 mm black paper filter discs (Thomas Scientific, Swedesboro, NJ), supported by LPS saturated paper pads, at 7.5×10^7 cells/filter (27,28). The radiation sensitive strains HPS64, HPS512 and HPS517 were a gift from Reg Deering, Penn State University (29). The *ras*G mutant was a gift from Gerry Weeks, University of British Columbia (30,31).

UV-light treatment

Parallel cultures of 10 ml of cells at 2×10^6 cells/ml were placed in sterile 9 cm diameter Pyrex Petri dishes (0.157 cm fluid depth) which were set on a shaking platform at 180 r.p.m. The cells were irradiated with 254 nm UV-light (germicidal lamp ITT G15T8) and were allowed to recover in the same medium. The incident fluence was measured by using a Black Ray meter (UVP, San Gabriel, CA). Fluence was adjusted for the path length, as well as for the absorbance of the cells and the different media by measuring the transmission of the different cultures and applying the correction factors for shaking cultures described by Morowitz (18,32). Correction values are given in the respective figure legends. At indicated intervals after the UV-irradiation, a culture was removed and an aliquot was serially diluted in SS buffer (0.6 g NaCl, 0.75 g KCl, 0.4 g $CaCl_2–2H_2O$/l) and clonally plated onto SM plates (27) with a lawn of *Klebsiella aerogenes* to determine viability. Surviving colonies were counted starting 3–4 days after plating. The remaining cells from each time point were collected by centrifugation, washed once in 1 ml H_2O and stored as frozen pellets at –80°C, to be used for RNA preparations.

Cisplatin treatment

Cisplatin [*cis*-diamminedichloroplatinum (II); Sigma, St Louis, MO] was dissolved in Pt buffer [3 mM NaCl, 1 mM Na_2HPO_4, pH 7.4 (33)] to a concentration of 3.3 mM. The stock solution was diluted into 10 ml of cells growing in HL5 medium to a final concentration of 330 μM. Samples were removed at indicated time points, and were analyzed for viability and for mRNA levels.

RNA isolation and northern analysis

Total RNA was isolated from the frozen UV-irradiated or cisplatin treated cell pellets, using the TRI reagent (Molecular Research Center, Cincinnati, OH) following the manufacturer's instructions. Ten μg of total RNA were run on 1% agarose formaldehyde gels and blotted to nitrocellulose. The DNA probes for the detection of the *rep*B, D, E and discoidin I mRNAs (20,21,34) were prepared by a two-step method that allows for the sensitive detection of very low abundance mRNAs on blots (35). Hybridization results were quantified by the use of a Fuji PhosphoImager, and were expressed as fold over time zero (21).

RESULTS

Response of DNA repair genes following UV-irradiation of growing and developing wild-type cells

To determine the appropriate range of UV-irradiation for subsequent experiments, we measured the killing of wild-type *Dictyostelium* cells by UV-light in different media and stages of growth or development (data not shown). Cells growing in defined FM medium required an incident fluence of ~300 J/m^2 to reduce viability to 50%. This is the result of the UV-absorbance of this medium. In contrast, UV could not kill cells grown in HL5 medium even at 100-fold greater dose, presumably because of the extremely high level of polynucleotides in this rich medium. Cells harvested from either FM or HL5 medium and then suspended in LPS buffer required considerably lower UV-exposure to achieve the same level of killing. We have measured the absorbance of the media and of cells in the different media and have corrected the fluences accordingly (see legends to Figs 1–4). It is evident from these analyses that the high incident UV-dose required to achieve the same level of killing in FM simply reflects the absorbance of UV by the medium, rather than a unique cellular resistance to UV in this medium. Cells developing on filters at three different stages of development (1, 3 and 10 h) are slightly less susceptible to UV than cells suspended in buffer, especially at 10 h of development when the cells have aggregated and are surrounded by a slime sheath. The UV-sensitivities that we observed were in good agreement with previous reports (reviewed in 18), and are characteristic of systems which are inactivated by single-hit kinetics, but show extensive repair at low UV-doses (36). Based on these initial determinations, we chose a range of UV-doses, giving a range of cell survival, with which to examine the response of the *rep* genes in each of the different conditions.

Figure 1 depicts the expression pattern of the *rep*B, D and E genes in cells which were UV-irradiated and allowed to recover while growing in defined FM medium, and shows a dose dependent induction of the *rep*B and D mRNAs (Fig. 1A and B). The response is quite rapid and transient. At the lowest level of irradiation (>95% survival), the mRNA level of these genes was increased by 20 min following irradiation, accumulated until 60 min and dropped to nearly basal level by 120 min. At increasing doses of UV the peak of induction was higher, and was achieved later. In contrast, the data in Figure 1C show that there is a rapid degradation of the *rep*E mRNA following UV-irradiation. This response is also transient and the *rep*E mRNA level returns to basal level at 120 min post irradiation. The discoidin I gene was used as a control, since its level was not expected to be influenced by UV-treatment and this is confirmed in Figure 1D. Further examination of the levels of additional developmentally regulated

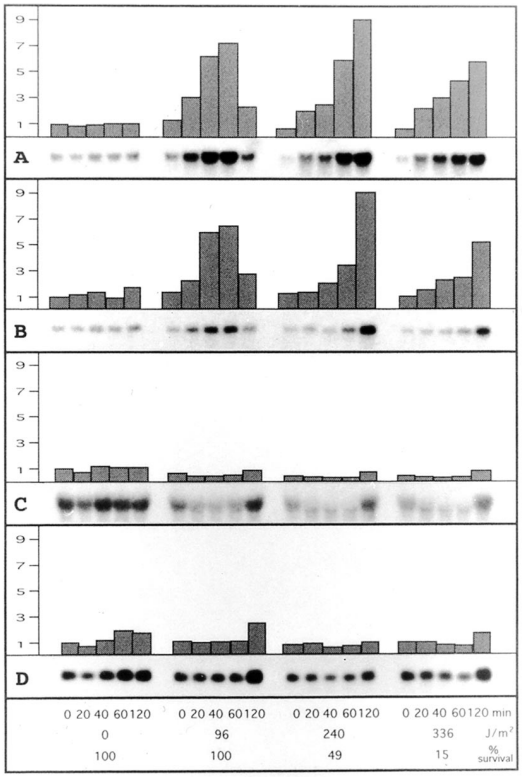

Figure 1. The response of the *rep* genes following UV-irradiation of growing wild-type cells. Parallel dishes of Ax4 cells growing in FM medium at a density of 2×10^6 cells/ml were irradiated at the indicated doses (and corresponding levels of survival). Cells were allowed to recover for 0, 20, 40, 60 and 120 min before harvesting. The UV-light induced mRNA levels were depicted as fold over the level of the non-UV-irradiated cells at 0 min recovery. (**A**) *rep*B; (**B**) *rep*D; (**C**) *rep*E; (**D**) discoidin I. The hybridized bands are shown below the graphs. Incident UV-dose (J/m^2), time after UV-irradiation (min) and the percent survival at each UV-dose are shown at the bottom. The absorbance of the cells in FM medium at 254 nm, corrected for the path length of 0.157 cm, yielded a transmission value of 9.6% and a correction factor of 39% (32). Thus incident doses of 96, 240 and 336 J/m^2 correspond to actual doses of 38, 94 and 130 J/m^2 respectively.

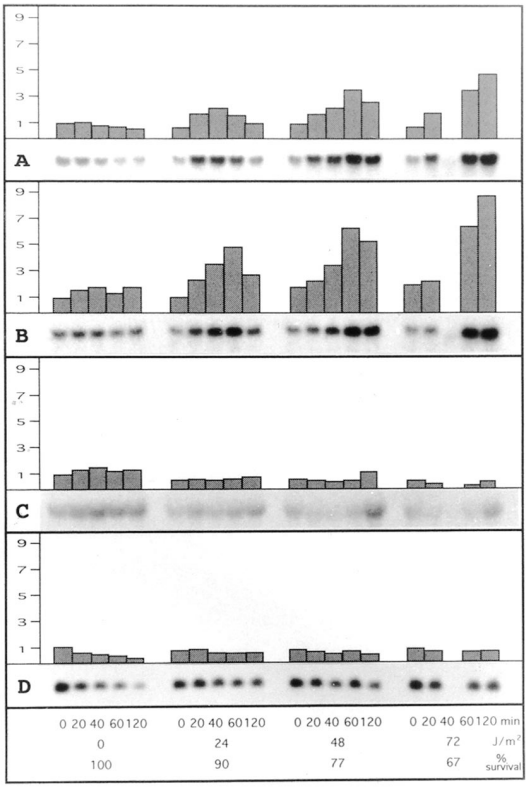

Figure 2. The response of the *rep* genes following UV-irradiation of wild-type cells at the onset of development. Ax4 cells grown to 2×10^6 cells/ml in HL5 medium were harvested, washed and resuspended in LPS buffer to the same density. The cells were then irradiated at the indicated doses (and corresponding levels of survival), and allowed to recover for indicated times. The UV-light induced mRNA levels were depicted as fold over the level of the non-UV-irradiated cells at 0 min recovery. (**A**) *rep*B; (**B**) *rep*D; (**C**) *rep*E; (**D**) discoidin I. The hybridized bands are shown below the graphs. Incident UV-dose (J/m^2), time after UV-irradiation (min) and the percent survival at each UV-dose are shown at the bottom. The correction factors for cells that were irradiated in LPS (75.2% transmission) was 88% (see legend to Fig. 1). Thus incident doses of 24, 48 and 72 J/m^2 correspond to actual doses of 21, 42 and 63 J/m^2 respectively.

mRNAs including cAR1 (cAMP receptor), PDE (cAMP phosphodiesterase) and csA (contact site A) showed no effect of the UV-irradiation (data not shown).

We then examined the pattern of *rep* gene induction when cells grown in HL5 were washed free of medium, resuspended, irradiated and allowed to recover in non-nutrient LPS buffer. The removal of nutrients stops DNA synthesis and cell division and initiates development. Figure 2 shows that the response of all four genes to UV-irradiation of the cells in LPS was virtually identical to that of cells which were growing and irradiated in FM medium (Fig. 1). The same pattern of UV-induction was also observed with cells which were grown in defined FM medium prior to UV-treatment in LPS (data not shown).

The results shown above revealed a dramatic change in *rep* gene expression in response to UV, in both mitotically growing cells and in cells which have just entered the developmental program after the removal of nutrients. To extend these studies to later times of development we irradiated cells that were developing on filter discs (Fig. 3). Once again, the *rep*B and D genes showed a similar response to UV-irradiation at all three of the developmental time points examined (1, 3 and 10 h), although the induction becomes less robust later in development. The decrease in *rep*E mRNA level is not as pronounced, probably reflecting the fact that *rep*E gene expression is normally elevated between 1 and 4 h of development (21). The increase in discoidin I mRNA level during the 2 h recovery following irradiation of cells at 3 h of development is the normal developmentally regulated increase in expression of this gene, and is not related to UV-treatment (34). It is important to re-emphasize that the cells are not dividing during development.

Examination of UV-sensitive mutants

The results presented above suggested that the resistance of *Dictyostelium* to UV-irradiation may be at least partially due to the rapid modulation of components of the NER pathway. A variety of *Dictyostelium* mutants with increased sensitivity to DNA damage from irradiation have been isolated and mapped to eight complementation groups (19,29). Therefore, we wished to test whether the increased UV-sensitivity of some of these mutant

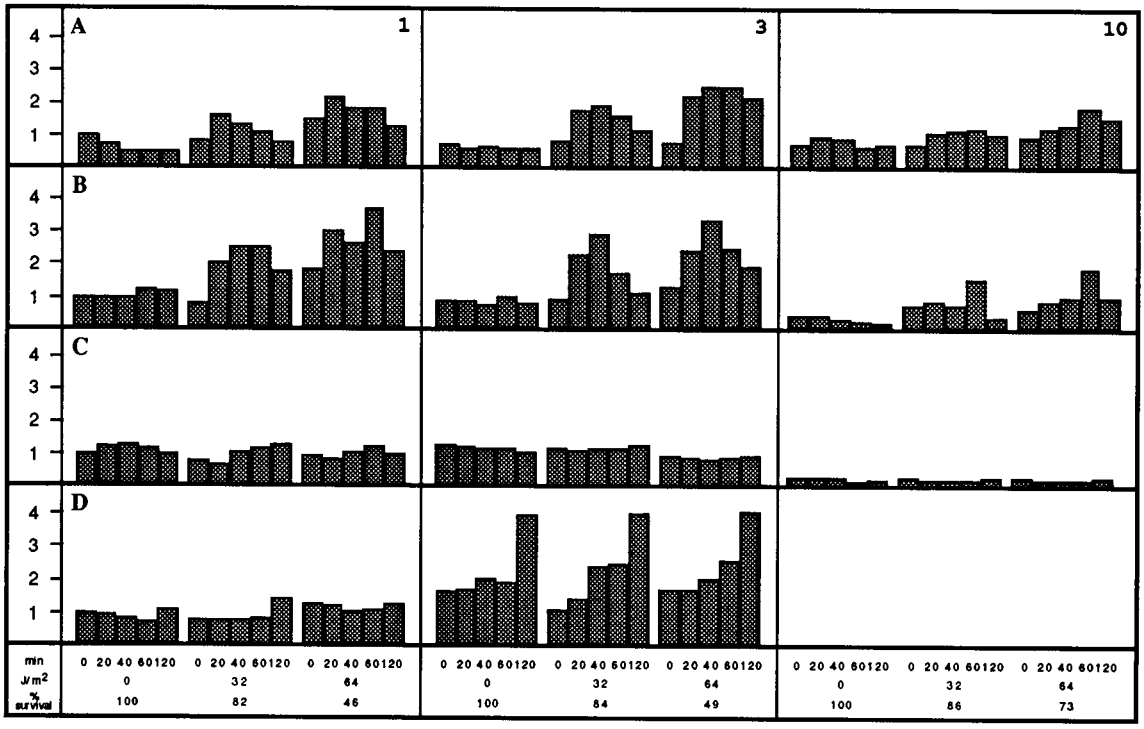

Figure 3. UV-irradiation induced expression of *rep* genes in wild-type cells during development. Ax4 cells were grown in HL5 medium, washed in LPS buffer and plated on filters for development. The cells were then irradiated on the filters at the indicated doses (and corresponding levels of survival), and allowed to recover for indicated times. The UV-light induced mRNA levels were depicted as fold over the level of the non-UV-irradiated cells at 0 min recovery. (**A**) *rep*B; (**B**) *rep*D; (**C**) *rep*E; (**D**) discoidin I. 1, 3 and 10 h refer to the developmental time point at which the cells were irradiated. Incident UV-dose (J/m^2), time after UV-irradiation (min) and the percent survival at each UV-dose are shown at the bottom.

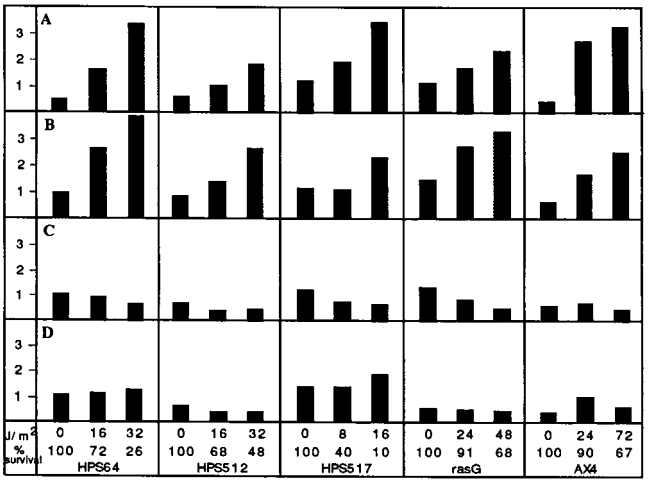

Figure 4. *rep* gene expression in UV-sensitive and *ras*G mutants. All strains were grown in HL5 medium to 2–3 × 10^6 cells/ml, harvested, washed and resuspended in LPS buffer to the same density. The individual cultures were UV-irradiated at the indicated doses (and corresponding level of survival) and harvested at 40 min post-irradiation. The UV-light induced mRNA levels were depicted as fold over the level of the non-UV-irradiated cells at 0 min recovery. (**A**) *rep*B; (**B**) *rep*D; (**C**) *rep*E; (**D**) discoidin I. Incident UV-dose (J/m^2) and the percent survival at each UV-dose are shown at the bottom. The correction factor was the same as in Figure 2 (88%).

strains was due to the inability to induce the *rep* genes. We have tested three of these strains, HPS64 (*rad*C44, linkage group III), HPS512 (uncharacterized complementation group, linkage group I) and HPS517 (*rad*B617, linkage group III). At an incident fluence of 60 J/m^2, HPS64, HPS512 and HPS517 were 4-, 6- and 120-fold more sensitive to UV than the wild-type cells (data not shown). These values agree with those published earlier (29). The results in Figure 4 show that each of these three mutants shows a pattern of *rep* gene expression following UV-treatment which is virtually identical to that seen in wild-type cells.

Examination of a *ras*G mutant

There are reports that the response to UV-irradiation may be transduced through a *ras* mediated pathway (37–39). We examined the effect of UV-irradiation on a *Dictyostelium ras*G gene disruption mutant. *ras*G gene is expressed both in growth and development in *Dictyostelium* (31,40). The *ras*G mutant showed a sensitivity to UV that was identical to the wild type, and a normal pattern of expression of the *rep* and discoidin I genes following UV-irradiation were observed (Fig. 4). It is important to note that there are several closely related *ras* genes in *Dictyostelium* cells, and that they may compensate for each other. This can be analyzed further as additional *ras* mutants become available.

Cisplatin induces *rep* gene expression

Having demonstrated that UV-irradiation dramatically influences the pattern of *rep* gene expression, we were interested in determining whether other DNA damaging agents had a similar effect on the expression of these genes. We chose to examine cisplatin because it is a widely used agent in cancer chemotherapy, because some tumor cells become resistant to the drug and

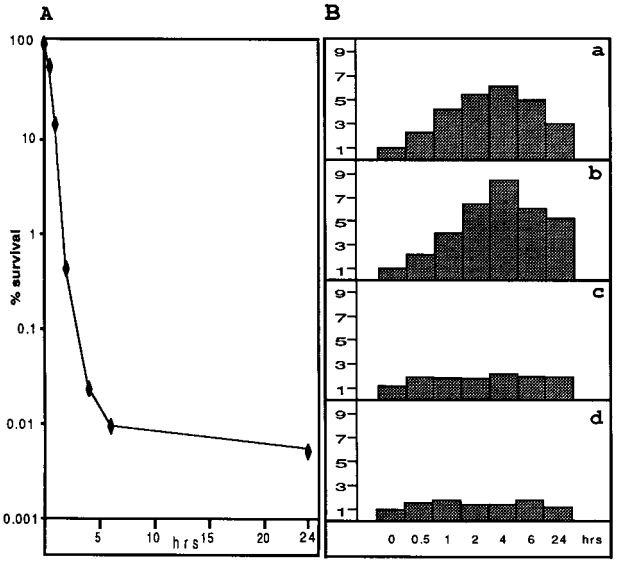

Figure 5. The response of the *rep* genes in cells treated with cisplatin. (**A**) The survival of wild-type Ax4 cells following treatment with 330 μM cisplatin. (**B**) The levels of *rep* gene expression at the corresponding time points following addition of cisplatin to the culture. The cisplatin induced mRNA levels were depicted as fold over the level of mRNA from untreated cells. (a) *rep*B; (b) *rep*D; (c) *rep*E; (d) discoidin I.

because UV-DDB/XPE has been reported to be involved in this resistance (41,42). Figure 5A shows the survival curve for cells treated with 330 μM cisplatin. Preliminary experiments showed that lower doses did not produce substantial killing (data not shown). This is not due to the reaction of cisplatin with the polynucleotides in the medium, because cells growing in defined FM medium had the same sensitivity to the drug (data not shown). Treatment of the cells with 330 μM cisplatin causes the rapid loss of viability of the cells, but the killing plateaus at ~0.01% survival (Fig. 5A). The cisplatin does not appear to be inactivated, because addition of fresh drug did not increase killing (data not shown). RNA was prepared from cells sampled at each of these time points, and analyzed by northern analysis. Similar to what we observed with UV-irradiation, the *rep*B and D mRNAs rapidly accumulate following addition of cisplatin (7- and 9-fold, respectively; Fig. 5B). There is a decrease in mRNA levels at the later time points which we suggest is due to the death of the cells. However, in contrast to the UV-treatment, cisplatin does not cause the rapid degradation of the *rep*E mRNA, but rather a 2-fold increase in the mRNA level. No induction of mRNA from the discoidin I control gene was observed.

DISCUSSION

In order to mount the correct response to repair damaged DNA, a cell must be able to identify the type of damage and then rapidly recruit the appropriate enzymes necessary to execute the repair. Although critical to the survival of all cells, the underlying signal transduction mechanisms responsible for repairing the varied types of DNA damage remain poorly understood.

The cellular slime mold *D.discoideum* is particularly resistant to DNA damaging agents including UV-light, gamma irradiation and chemicals (19). We have now shown that *Dictyostelium* cells up-regulate the steady state level of the *rep*B and D mRNAs, encoding NER DNA helicase enzymes, following irradiation with UV-light. The response is rapid, dose dependent and transient. The response is also extremely specific in that no effect was observed on the mRNA levels of several other developmentally regulated genes examined. The response is identical in mitotically dividing cells and in developing cells that are not synthesizing DNA or undergoing cell division. These results suggest that the response to UV is independent of DNA replication, and that all the factors necessary for the rapid transcription of these NER genes are either stable throughout development or are continuously synthesized. Moreover, the data point out the importance of DNA repair for this organism at all stages of its life cycle. The ability to repair the DNA of developing, non-dividing cells is necessary to maintain the fidelity of the genetic material that will be packaged into terminally differentiated spores. An apurinic apyrimidinic-specific endonuclease mRNA has also been shown to be induced by UV-light in *Dictyostelium* (43). We suggest that this ability to rapidly modulate the NER and other repair genes may be involved in the resistance of *Dictyostelium* cells to DNA damaging agents. It will be important to extend these results on the modulation of the mRNA levels to the synthesis and stability of the cognate proteins, although it is likely that these increases in mRNA levels will have concomitant increases in proteins levels. Interestingly, the UV-sensitive mutants we examined all exhibited a wild-type response to UV-irradiation. This does not rule out the possibility that the mutations are in one of the induced genes, and additional studies on these strains are necessary to identify the lesions.

It is significant that the up-regulation of the *rep*B and D genes in response to UV and chemical damage has not been observed to occur in cells from other species. In procaryotes, the uvrA, B and D NER genes are induced by UV, as part of the SOS response (44). In contrast, only four out of 18 NER related genes in yeast (*RAD2*/XPG, *RAD7*, *RAD16* and *RAD23* genes) have been demonstrated to be induced after UV-treatment (8,45,46). No UV-induction of NER related genes has been observed in human cell lines, though there is a recent report of a p53 dependent up-regulation of *ERCC3/XPB* in human cells in response to the introduction of thymidine dinucleotides (47). As additional members of the TFIIH complex become available through *Dictyostelium* genome sequencing efforts (48), it will be interesting to study their response to UV as well.

The human homolog of the RepE protein, UV-DDB, is thought to act in a stoichiometric fashion in an early damage recognition step in the repair pathway by binding to UV and chemically damaged DNA (49–53). Thus, its regulation might be expected to be different than that of the *rep*B and D genes which encode products with catalytic activities. In this context, the response of the *rep*E gene to UV is particularly interesting. While it is developmentally up-regulated (21), it shows a dramatic down-regulation in response to UV-damage followed by an increase in transcription back to basal level. It will be very interesting when we are able to measure the levels of RepE protein that accompany the changes we have observed in mRNA levels. However, it is significant that the binding activity of the UV-DDB protein has been shown to decrease transiently following UV-treatment of cells (54). Our data are suggest that this is partially due to the rapid destruction of the mRNA.

Many eucaryotic cells express genes as part of the 'UV-response' which is thought to be analogous to the SOS response in bacteria, though no universal repressor analogous to the procaryotic LexA

has been identified (8). Our data support the idea (55) that the RepE protein may act as a negative regulator of repair of DNA damaged by UV-light, and that its down-regulation may be necessary for the increase in the transcription of the *rep*B and D genes. It should be noted that several studies have suggested a regulatory role for the primate UV-DDB, including the replication of the Hepatitis B virus through interaction with the X-protein (56), the regulated expression of DNA replication genes as a partner of the E2F1 transcrption activator (57), and in the regulation of apolipoprotein B gene expression (58).

Cisplatin is one of the most widely used agents for cancer chemotherapy for treatment of cancers of the testis, ovary, bladder, lung, head and neck (59). The target for the cisplatin mediated cell killing is thought to be DNA (60). The major products of the reaction between cisplatin and DNA are 1,2-intrastrand d(GpG) and d(ApG) crosslinks, which compose ~65 and 25% of cisplatin adducts *in vitro* (42,61). These intrastrand cisplatin cross-links result in the unwinding and kinking of the DNA double helix so that the regions become the target for DNA repair (62). Even though cisplatin is a powerful cancer chemotherapeutic agent, some tumor cells develop cisplatin resistance during chemotherapy. The UV-DDB/XPE protein has been, in part, implicated in this resistance, with up to a 5-fold increase in the level of the UV-DDB/XPE DNA binding activity over that of the parental, sensitive cell line (41), although several other suggestions have been made regarding this resistance (42).

We were interested in examining the effect of this drug on *Dictyostelium* because of the possibility of generating drug resistant mutants that would enable us to do genetic analysis. We have found that the *Dictyostelium* cells appear to be relatively insensitive to cisplatin, as only 40 μM cisplatin caused 50% killing of cultured human fibroblasts (63). At 330 μM cisplatin, the viability of the *Dictyostelium* cells drops dramatically but reaches a plateau of ~0.01% survival by 6 h of treatment. This level of resistance is 10–100-fold greater than the level expected from the natural mutational frequency of 10^{-5}–10^{-6} in *Dictyostelium* and suggests that the resistance is not heritable (64). Indeed, we have clonally isolated cells which survived cisplatin treatment and shown that they have the same sensitivity to cisplatin as did the cells in the original culture (data not shown). The rapid expression of the *rep* genes in response to cisplatin suggests that this may be a mechanism by which cells can acquire at least a transient tolerance to this drug. It will be interesting to determine whether lower, non-lethal doses of cisplatin can induce *rep* gene expression and whether this can protect cells from subsequent higher doses of the drug or UV-irradiation.

It is interesting that the patterns of *rep* gene expression are different when the cells are treated with either UV or cisplatin, and that each of these is different than the normal pattern of *rep* genes expression seen in development. Thus, the *rep*B and D genes are turned on following both UV and cisplatin treatment, but only *rep*D is induced as part of the normal developmental program. The *rep*E gene is particularly interesting. It is developmentally up-regulated, shows a dramatic down-regulation in response to UV-damage but shows an increase in transcription following cisplatin treatment. The level of induction (~2-fold) is in agreement with the level of induction seen in animal cells in response to cisplatin (41). It is obvious that the *rep* genes can respond to different transcriptional activators and repressors and that multiple signal transduction mechanisms act on these genes. The goal of future work is to identify the underlying molecular mechanisms that allow cells to respond correctly to the different types of DNA damage.

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REFERENCES

1 Wood,R.D. (1996) *Annu. Rev. Biochem.*, **65**, 135–167.
2 Sancar,A. (1994) *Science*, **266**, 1954–1956.
3 Sancar,A. (1995) *Annu. Rev. Genet.*, **29**, 69–105.
4 Van Houten,B. and McCullough,A. (1994) *Ann. NY Acad. Sci.*, **726**, 236–251.
5 Sweder,K.S. (1994) *Curr. Genet.*, **27**, 1–16.
6 Ma,L.B., Hoeijmakers,J.H.J. and Vandereb,A.J. (1995) *Biochim. Biophys. Acta*, **1242**, 137–163.
7 Kraemer,K.H., Levy,D.D., Parris,C.N., Gozukara,E.M., Moriwaki,S., Adelberg,S. and Seidman,M.M. (1994) *J. Invest. Dermatol.*, **103**, 96S–101S.
8 Friedberg,E.C., Walker,G.C. and Siede,W. (eds) (1995) *DNA Repair and Mutagenesis*. ASM Press, Washington, DC.
9 Bootsma,D. and Hoeijmakers,J. (1994) *Mutat. Res. Fundam. Mol. Mech. Mutagen.*, **307**, 15–23.
10 Tanaka,K. and Wood,R.D. (1994) *Trends Biochem. Sci.*, **19**, 83–86.
11 Drapkin,R., Reardon,J.T., Ansari,A., Huang,J.C., Zawel,L., Ahn,K., Sancar,A. and Reinberg,D. (1994) *Nature*, **368**, 769–772.
12 Sweder,K.S. and Hanawalt,P.C. (1994) *J. Biol. Chem.*, **269**, 1852–1857.
13 Svejstrup,J.Q., Wang,Z., Feaver,W.J., Wu,X., Bushnell,D.A., Donahue,T.F., Friedberg,E.C. and Kornberg,R.D. (1995) *Cell*, **80**, 21–28.
14 Van Vuuren,A.j., Vermeulen,W., Ma,L., Weeda,G., Appeldoorn,E., Jaspers,N., Van der Eb,A.j., Bootsma,D., Hoeijmakers,J., Humbert,S., Schaeffer,L. and Egly,J.M. (1994) *EMBO J.*, **13**, 1645–1653.
15 Chu,G. and Payne,L. (1996) *Trends Genet.*, **12**, 187–192.
16 Loomis,W.F. (ed.) (1982) *The Development of Dictyostelium discoideum*. Academic Press, New York.
17 Alexander,S. and Rossomando,E.F. (1992) In Rossomando,E.F. and Alexander,S. (eds), *Morphogenesis: An Analysis of the Development of Biological Form*. Dekker, New York, pp. 29–61.
18 Deering,R.A. (1988) *Dev. Genet.*, **9**, 483–493.
19 Welker,D. and Deering,R. (1978) *J. Gen. Microbiol.*, **109**, 11–23.
20 Alexander,H., Lee,S.-K., Yu,S.-L. and Alexander,S. (1996) *Nucleic Acids Res.*, **24**, 2295–2301.
21 Lee,S.-K., Yu,S.-L., Garcia,M.X., Alexander,H. and Alexander,S. (1997) *Nucleic Acids Res.*, **25**, 2365–2374.
22 Hanawalt,P. and Mellon,I. (1993) *Curr. Biol.*, **3**, 67–69.
23 Friedberg,E.C. (1996) *Annu. Rev. Biochem.*, **65**, 15–42.
24 Mauldin,S.K., Freeland,T.M. and Deering,R.A. (1994) *Mut. Res. DNA Repair*, **314**, 187–198.
25 Cocucci,S. and Sussman,M. (1970) *J. Cell. Biol.*, **45**, 399–407.
26 Franke,J. and Kessin,R. (1977) *Proc. Natl Acad. Sci. USA*, **74**, 2157–2161.
27 Sussman,M. (1987) *Methods Cell Biol.*, **28**, 9–29.
28 Soll,D. (1987) *Methods Cell Biol.*, **28**, 413.
29 Bronner,C.E., Welker,D.L. and Deering,R.A. (1992) *Mutat. Res. DNA Repair*, **274**, 187–200.
30 Pawson,T., Amiel,T., Hinze,E., Auersperg,N., Neave,N., Sobolewski,A. and Weeks,G. (1985) *Mol. Cell. Biol.*, **5**, 33–39.
31 Khosla,M., Robbins,S.M., Spiegelman,G.B. and Weeks,G. (1990) *Mol. Cell. Biol.*, **10**, 918–922.
32 Morowitz,H.J. (1950) *Science*, **111**, 229–230.
33 Fox,M.E., Feldman,B.J. and Chu,G. (1994) *Mol. Cell. Biol.*, **14**, 8071–8077.
34 Alexander,S., Leone,S. and Ostermeyer,E. (1991) *Mol. Cell. Biol.*, **11**, 3171–3179.
35 Lee,S.-K., Yu,S.-L., Alexander,H. and Alexander,S. (1996) *BioTechniques*, **21**, 630–632.

36 Jagger,J. (1976) In Wang,S.Y. (ed.), *Photochemistry and Photobiology of Nucleic Acids*. Academic Press, New York, pp. 147–186.
37 Engelberg,D., Klein,C., Martinetto,H., Struhl,K. and Karin,M. (1994) *Cell*, **77**, 381–390.
38 Devary,Y., Rosette,C., DiDonato,J.A. and Karin,M. (1993) *Science*, **261**, 1442–1445.
39 Mount,D.W. (1996) *Nature*, **383**, 763–764.
40 Thiery,R., Robbins,S., Khosla,M., Spiegelman,G.B. and Weeks,G. (1992) *Biochem. Cell Biol*., **70**, 1193–1199.
41 Chu,G. and Chang,E. (1990) *Proc. Natl Acad. Sci. USA*, **87**, 3324–3327.
42 Chu,G. (1994) *J. Biol. Chem*., **269**, 787–790.
43 Freeland,T.M., Guyer,R.B., Ling,A.Z. and Deering,R.A. (1996) *Nucleic Acids Res*., **24**, 1950–1953.
44 Walker,G.C. (1985) *Annu. Rev. Biochem*., **54**, 425–457.
45 Madura,K. and Prakash,S. (1986) *J. Bact*., **166**, 914–923.
46 Madura,K. and Prakash,S. (1990) *Mol. Cell. Res*., **10**, 3256–3257.
47 Eller,M.S., Maeda,T., Magnoni,C., Atwal,D. and Gilchrest,B.A. (1997) *Proc. Natl Acad. Sci. USA*, **94**, 12627–12632.
48 Loomis,W.F. and Kuspa,A. (1997) In Maeda,Y., Inouye,K. and Takeuchi,I. (eds), *Dictyostelium: A Model System for Cell and Developmental Biology*. Universal Academy Press, Inc., Tokyo, pp. 15–28.
49 Feldberg,R.S. and Grossman,L. (1976) *Biochemistry*, **15**, 2402–2408.
50 Chu,G. and Chang,E. (1988) *Science*, **242**, 564–567.
51 Takao,M., Abramic,M., Moos,M.J., Otrin,V.R., Wootton,J.C., Mclenigan,M., Levine,A.S. and Protic,M. (1993) *Nucleic Acids Res*., **21**, 4111–4118.
52 Keeney,S., Eker,A., Brody,T., Vermeulen,W., Bootsma,D., Hoeijmakers,J. and Linn,S. (1994) *Proc. Natl Acad. Sci. USA*, **91**, 4053–4056.
53 Payne,A. and Chu,G. (1994) *Mut. Res. Fundam. Mol. Mech. Mutagen*., **310**, 89–102.
54 Hirschfeld,S., Levine,A.S., Ozato,K. and Protic,M. (1990) *Mol. Cell. Biol*., **10**, 2041–2048.
55 Protic,M. (1994) *Ann. NY Acad. Sci*., **726**, 333–335.
56 Lee,T.H., Elledge,S.J. and Butel,J.S. (1995) *J. Virol*., **69**, 1107–1114.
57 Hayes,S., Shiyanov,P., Chen,X.Q. and Raychaudhuri,P. (1998) *Mol. Cell. Biol*., **18**, 240–249.
58 Krishnamoorthy,R.R., Lee,T.H., Butel,J.S. and Das,H.K. (1997) *Biochemistry*, **36**, 960–969.
59 Loehrer,P.J. and Einhorn,L.H. (1984) *Ann. Int. Med*., **100**, 704–713.
60 Hughes,E.N., Engelsberg,B.N. and Billings,P.C. (1992) *J. Biol. Chem*., **276**, 13520–13527.
61 Fichtinger-Schepman,A.M.J., van der Veer,J.L., den Hartog,J.H., Lohman,P.H.M. and Reedijk,J. (1985) *Biochemistry*, **24**, 707–713.
62 Bellon,S.F., Coleman,J.H. and Lippard,S.J. (1991) *Biochemistry*, **30**, 8026–8035.
63 Hansson,J., Edgren,M.R., Egyhazi,S., Hao,X.Y., Mannervik,B. and Ringborg,U. (1996) *Acta Oncol*., **35**, 683–690.
64 Loomis,W.F. (1987) *Methods Cell Biol*., **28**, 31–65.