Volume 225, number 1,2, 133–138 FEB 05371 December 1987

Loss of inducible photorepair in a frog cell line hypersensitive to solar UV light

Chuck C.-K. Chao and Sue Lin-Chao+

Department of Biochemistry, University of Texas Health Science Center, Dallas, TX 75235 and + *Program in Molecular Biology, University of Texas at Dallas, Richardson, TX 75083, USA*

Received 2 October 1987

The induction of enzymatic photorepair (EPR) in ICR 2A frog cells and a derived mutant cell line DRP36 hypersensitive to solar UV was studied. Using clonogenic assays, when induced wild-type cells demonstrated an 8-fold increase of EPR the mutant cells displayed a near-background level of inducible EPR. The constitutive EPR in mutant cells, however, was the same as in wild-type cells. A mixed culture of ICR 2A and DRP36 cells showed an intermediate inducible EPR depending upon the cell ratio. Inducible EPR was also detected at the DNA level in wild-type cells, but not in mutant cells.

Repair regulation; Hypersensitivity; Photorepair; Solar UV

1. INTRODUCTION

Ultraviolet (UV) light causes cyclobutane-type pyrimidine dimer damages on cellular DNA, which are normally repaired by cells through excision (i.e. dark) repair or photorepair (PR) (i.e. light-dependent) (review [1]). Substantial evidence has shown that cloned genes involved in eukaryotic excision repair can be dually or multiply regulated by exogenous factors [2–6]. PR regulation, however, has never been documented. Both repair mechanisms, nevertheless, compete for the substrate, i.e. pyrimidine dimers [7]. Therefore, cells with strong excision repair or weak PR function often cause problems for the investigation of PR.

Cultured frog cells provide an ideal system for PR study because of the richness of their PR function and a near deficiency in excision repair [8,9]. It has been shown that the PR activity in this system is partially dependent upon the culture condition [10,11], suggesting a regulated function. Recently, we established an assay system [12,13] sensitive enough to functionally measure a low level of inducible enzymatic PR (EPR). We provided evidence that EPR in frog cells is inducible only by DNA damage of the dimer type from radiation [12]; and unlike other genes of DNA metabolism whose activity is essentially in active genomes, the inducible function of EPR is markedly reduced in growing cells [13]. These observations, therefore, suggest that regulation of EPR in cells is independent of cellular ability of repairing non-dimer damages.

Here, we investigated the inducible EPR in a mutant frog cell line DRP36 hypersensitive to solar UV light but normal to 254 nm UV [12,13]. We found that the inducible function in DRP36 cells is lost as judged by clonogenicity and DNA functional assays, indicating that dimer damage is not the only factor mediating EPR induction in these cells. We also showed that changes of DNA size

Correspondence (present) address: C.C.-K. Chao, Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305, USA

+ Present address: Department of Genetics, Stanford University School of Medicine, Stanford, CA 94305, USA

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

during repair incubation in mutant cells is inefficient. Together with our recent findings [14], the results suggest that the inducible EPR in cells is probably also dependent upon DNA domains or chromosome conformation.

2. MATERIALS AND METHODS

2.1. *Cell lines and culture conditions*

ICR 2A (*Rana pipiens*) frog cells and a derived mutant DRP36 cell line [15,16] were maintained as described [17]. Cells growing in normal medium have a population doubling time of 48 h for ICR 2A and 60 h for DRP36. Arrested (nondividing) cultures were established by serum deprivation and maintained as described in [12,13].

2.2. *Irradiation conditions and clonogenic assays*

Cells were washed twice with phosphate-buffered saline (PBS) and irradiated in PBS as described in [18]. The irradiation source was four GE-G15T8 germicidal lamps at a fluence rate of 0.35 W/m^2. Following irradiation or mock treatments, cells were washed, incubated for six generation doublings (i.e. 12 days), fixed and then stained with Giemsa for clonogenic assays. The number of surviving colonies (groups of 32 or more cells) was determined by inspection with a stereo microscope.

2.3. *Photolyase activity assays*

The strategy of the clonogenic assay of photolyase activity was as described [12,13]. In brief, arrested cells which received a low fluence of pre-irradiation were incubated for a period of time, washed with PBS and either sham irradiated or exposed to a challenge UV fluence immediately followed by a sufficiently photoreactivating light (PRL) [16,19]. Cultures were then washed again with PBS, incubated in normal medium, and the clonogenic ability was determined. The induction index was calculated from the optimal survival fraction of cells pre-irradiated divided by that of those not pre-irradiated for each case [20].

A nontoxic concentration of the antibiotic cycloheximide (3 μM), a protein synthesis inhibitor, is included in some culture dishes during and after the pre-irradiation incubation in low-serum medium.

2.4. *Alkaline membrane elution and calculation of single-strand break (SSB)*

The level of repair-associated DNA SSB was assayed using alkaline membrane elution [21]. Arrested cells were cultured as for clonogenic assays. After challenge exposure, cells were harvested immediately or incubated in normal medium for 24 h before being subjected to elution assays. The elution and calculation of DNA SSB in frog cells were carried out as described [22] in reference to a value of 8.1 SSBs induced in 10^{10} Da of mammalian DNA by 300-rad γ-rays [21]. Therefore, the SSBs in 10^{10} Da DNA was calculated from the following equation:

$$8.1 \times [(B_{\text{irrad}} - B_{\text{unirrad}})/(B_{300\,\text{rad}\gamma\text{-rays}} - B_{\text{unirrad}})],$$

where B equals the logarithm of the fraction of DNA retained on the membrane after 0 h elution minus that after 15 h elution.

2.5. *Alkaline sucrose gradient velocity sedimentation*

The size distribution of DNA molecules was investigated by alkaline sucrose gradient sedimentation modified from the 'paper-strip' method [23]. Details adopted for frog cells were as described in [9].

3. RESULTS

3.1. *Constitutive EPR in mutant cells is the same as in wild-type cells*

Typical survival curves of arrested cells following irradiation with 254 nm or solar UV were generated as described [12,13]. The parameters of the curves for ICR 2A and DRP36 cells were summarised in table 1. As shown in the table, both cells demonstrated similar D_q and D_o values for each case. Exposure of cells immediately after irradiation to PRL enhanced the clonogenicity of the cells. PR sectors, an index of the photolyase-dependent functional role of cyclobutane damage, were calculated according to Jagger [23]. Both cells exhibit a value of PR sector around 0.85, indicating a similarity of the constitutive EPR in both cell lines. PR sectors for solar UV-treated ICR 2A and DRP36 cells are 0.30 and 0.15, respectively, indicating that dimer damage only accounts for part of the cytotoxicity of DRP36 cells.

Table 1

Parameters of survival curves for ICR 2A and DRP36 cells following 254 nm UV and solar UV irradiation

	D_q	D_o	PR sector
254 nm-UV (J/m^2)			
ICR 2A	2.4 ± 0.5	1.5 ± 0.2	0.85
DRP36	2.5 ± 0.2	1.4 ± 0.3	0.86
Solar-UV (kJ/m^2)			
ICR 2A	6.5 ± 0.2	6.6 ± 0.3	0.30
DRP36	5.1 ± 0.3	1.5 ± 0.5	0.15

D_q is the fluence along the shoulder of the dose-response curve that cells can tolerate. D_o is the fluence that reduces the percentage of survival by 63% along the exponential portion of the dose-response curve. The average values of triplicate samples ± SE are indicated

3.2. *Inducible EPR in mutant cells is aborted*

It has been shown that an inducible EPR is measurable in arrested, but not in growing frog cells [13]. Arrested wild-type and mutant cells were therefore exposed to an inducing fluence of 254 nm UV and quantitated for the induction of EPR. Plots of typical survival fractions against time after inducing treatment are shown in fig.1. For wild-type cells (fig.1a), the survival fraction reaches a maximum at day 3 after induction in a transient fashion. The calculated induction index (8.1) is also indicated. In contrast, it was impossible to detect an inducible EPR, if any, in mutant cells after exhausted repeats, i.e. only background EPR (as an induction index = 1.2) was measured in DRP36 cells (fig.1b). The inducible function found in wild-type cells was completely blocked by a nontoxic concentration of cycloheximide (3 μM), a protein synthesis inhibitor (* in fig.1). In addition, a mixed cultivation of the two cell lines displayed an intermediate level of inducible EPR in parallel to the cell ratio. Here, only the survival fraction for a mixture of an equal cell ratio is shown (fig.1c).

3.3. *Inducible EPR is also detected at DNA level*

EPR in wild-type and mutant cells was also analyzed at DNA level as described in section 2. Using alkaline membrane elution, labeled DNA from cells with or without inducing treatment was eluted with a differential rate from the membrane

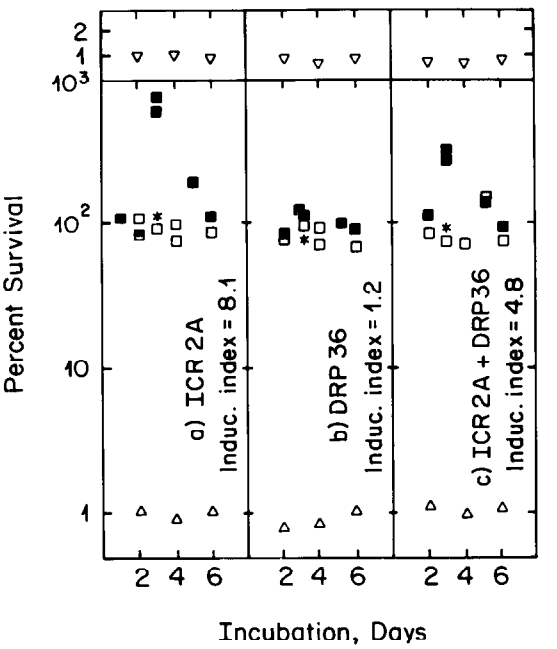

Fig.1. Regulation of EPR in cultured cells following predose of 254 nm UV light. (a) ICR 2A cells; (b) DRP36 cells; (c) Mixed cultures. (■) Cells pretreated and challenged plus PRL; (□) cells not pretreated but challenged plus PRL; (△) cells pretreated and challenged alone; (*) cells pretreated, incubated in 3 μM cycloheximide and challenged plus PRL. Relative cell number to that of day 0 during the course of incubation are shown on the top of the corresponding panel.

depending upon the breaks generated in DNA [21]. A typical result is shown in table 2. Greater than 90% DNA for both unirradiated wild-type and mutant cells stayed on the membrane and there is a similar elution rate between two cell lines (column A). The fraction of eluted DNA was markedly enhanced by a fluence of challenge UV alone: 80% DNA of ICR 2A cells was eluted from the membrane; in contrast, only 32% DNA of DRP36 cells was eluted (column B). Following PR treatment immediately after the challenge, the enhanced effect of elution rate was effectively reduced for both cell lines. However, the EPR effect for DRP36 cells was not as great as for ICR 2A cells, i.e. DNA retention increases from 68% to 80% (column C). This is probably due to a less flexible DNA conformation in the mutant [14]. Nevertheless, the results indicate that the constitutive EPR of both cell lines can be detected

Table 2

Alkaline membrane elution of DNA from 254 nm UV-treated cells expressing the maximum EPR

Cultures	Percent retention of control radioactivity			
	A	B	C	D
ICR 2A	93	20(24)	65(15)	85(5)
DRP36	90	68(13)	80(8)	79(8)
ICR 2A + DRP36	91	40(21)	67(14)	79(8)

Percent radioactivity was collected at 15 h elution from samples incubated for 24 h post-irradiation. An average of duplicate or triplicate samples was shown. Parentheses indicate calculated SSBs per 10^{10} Da DNA (see section 2 for detail). (A) Unirradiated; (B) 15 J/m^2 UV alone; (C) 15 J/m^2 UV + PRL; (D) pre-dose (2 J/m^2) + UV + PRL

from DNA function. This EPR effect on DNA is temperature and cycloheximide-sensitive (not shown). Following a sublethal fluence of inducing UV (2 J/m^2), however, only wild-type cells, but not mutant cells, displayed a further reduced elution rate (column D). Also shown are the calculated SSBs for each case (the numbers in parentheses of table 2). Induced EPR in ICR 2A cells further repaired 10 SSBs in 1010 Da DNA (cf. columns C and D). In contrast, no further SSB repair was measured in DRP36 cells pretreated with inducing UV. In addition, cocultivated cells following the same treatment demonstrated a roughly intermediate effect. The results, therefore, indicate that inducible EPR in ICR 2A cells is also detectable at the DNA level.

3.4. *The average molecular mass shift of DNA molecules during repair incubation in mutant cells is inefficient*

Since we were unable to detect a significant difference of molecular mass shift of DNA molecules between two cell lines arrested in low serum during repair incubation (not shown), the replicating cells were therefore used for this purpose. Following a typical solar UV fluence (30 kJ/m^2), cells were harvested at 3 h or 24 h during repair incubation and processed for alkaline sucrose gradient sedimentation (see section 2 for details). It should be noted that this treatment caused a maximum inhibition of DNA synthesis at 3 h after irradiation [14,16]. ICR 2A cells normally show a molecular mass shift to a position corresponding to a larger DNA size than the control molecules at this time point, suggesting a replicon inhibition. However, following 24 h incubation DNA molecules were shifted toward a much smaller size (see fig.2a). For DRP36 mutant cells, such a shift pattern was greatly reduced (cf. fig.2A with b for 24 h incubation). This unusual effect is unlikely to have resulted from a defect in incision during DNA repair [14]. Nonetheless, the mutant showed a similar molecular mass shift at 3 h. A mixture of two cell lines was also assayed. As shown in fig.2c, there is an intermediate level of average molecular mass for mixed cultures following extended incubation. Their DNA size at the 3 h point is the same as for independent cultures. The extent of molecular mass shift during incubation is roughly proportional to the cell ratio of ICR 2A to DRP36

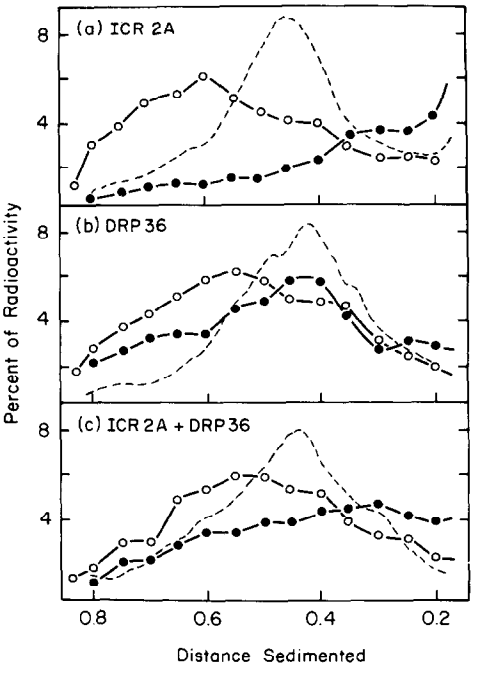

Fig.2. Sedimentation of nascent DNA synthesis in solar UV-irradiated cells. Cells unirradiated (– – –) or irradiated with 30 kJ/m^2 solar UV were incubated for 3 h (○) or 24 h (●) before harvesting for sedimentation. Alkaline sucrose gradient profiles for each cell culture were indicated in separate panels.

(not shown). Taken together, the results indicate that DRP36 cells are inefficient in shifting DNA molecules during repair incubation.

4. DISCUSSION

The results presented here indicate that DRP36 cells are defective or deficient in the induction of EPR. The constitutive EPR in this mutant, however, is the same as in wild-type cells. It has been shown that mammalian cells synthesize a novel class of secretory protein in response to UV damages that effectively transmit UV-specific messages between cells [25,26]. As also seen in frog cells, regulation of EPR is mediated by (a) new protein(s). Assuming there is a commonality between systems, the regulation of EPR activity is probably mediated by (a) secretory factor(s) as well. This aspect is suggestively supported by a homogeneous growth of the cells following UV irradiation (unpublished). A lack of phenotypic complementation of DRP36 cells by cocultivated ICR 2A cells, therefore, is likely to be due to an improperly presented DNA domain or chromosome conformation such that even if the photolyase is induced in DRP36 cells the template affinity is drastically reduced.

Inducible enhancers have been identified for a number of genes including heat shock, metallothionein, B-interferon and c-fos, as well as that responding to steroids (review [27]). The regulation of inducible gene expression in response to environmental changes is probably mediated through a factor modification in general. DNA domains, therefore, are important for presenting a condition with maximum template affinity for regulatory factors. The substantial evidence accumulated in recent years indicates that DNA repair in higher eukaryotes can also be regulated ([12,13] and references therein). In addition, recent works by Hanawalt and colleagues suggest that transcriptionally active DNA domains in mammalian cells are readily accessible to repair enzymes (review [28]). These studies imply that accessibility of higher eukaryote templates to regulatory (ribo-)proteins is dependent upon a flexibility of chromosome conformation or DNA domains. It is consistent with the suggestion from a recent study with a yeast RAD6 mutant that 'chromatin remodelling' plays a central role in DNA metabolism, including DNA repair. The multiple functions of the RAD6 protein are mediated by its ubiquitin-conjugating activity which is conserved between yeast and mammals [29]. In addition, DRP36 cells have a slow growth rate and an unusual overall DNA metabolism [14]. Therefore, the conclusion of this study with frog cells that regulation of EPR is associated with a flexible DNA structure may represent a general mechanism of gene regulation.

ACKNOWLEDGEMENTS

The authors are grateful to the generosity of Dr M.E. Gaulden, Department of Radiology, UTHSCD, for supporting us in the completion of a preliminary account of this work.

REFERENCES

[1] Friedberg, E.C. (1985) DNA Repair, Freeman, New York.
[2] Robinson, G.W., Nicolet, C.M., Kalainov, D. and Friedberg, E.C. (1986) Proc. Natl. Acad. Sci. USA 83, 1842–1846.
[3] Maga, J.A., McClanahan, T.A. and McEntee, K. (1986) Mol. Gen. Genet. 205, 276–284.
[4] Cole, G.M., Schild, D., Lovell, S.T. and Mortimer, R.K. (1987) Mol. Cell. Biol. 7, 1078–1084.
[5] McClanahan, T. and McEntee, K. (1986) Mol. Cell. Biol. 6, 90–96.
[6] Vivino, A.A., Smith, M.D. and Minton, K.W. (1986) Mol. Cell. Biol. 6, 4767–4769.
[7] Hoy, C.A. and Rupert, C.S. (1984) Mutat. Res. 140, 199–203.
[8] Freed, J.J., Hoess, R.H., Angelosanto, F.A. and Massey, H.C., jr (1979) Mutat. Res. 62, 325–339.
[9] Rosenstein, B.S. and Setlow, R.B. (1980) Biophys. J. 31, 195–205.
[10] Sutherland, B.M. and Oliver, R. (1976) Biochim. Biophys. Acta 442, 358–367.
[11] Mortelmans, K., Cleaver, J.E., Friedberg, E.C., Paterson, M.C., Smith, B.P. and Thomas, G.H. (1977) Mutat. Res. 44, 433–446.
[12] Chao, C.C.-K. and Lin-Chao, S. (1987) Biochem. Biophys. Res. Commun. 145, 604–611.
[13] Chao, C.C.-K. and Lin-Chao, S. (1987) Mutat. Res., in press.
[14] Chao, C.C.-K. and Lin-Chao, S. (1987) Nucleic Acids Res., submitted.
[15] Rosenstein, B.S. and Chao, C.C.-K. (1985) Somat. Cell Mol. Genet. 11, 339–344.

[16] Rosenstein, B.S. and Chao, C.C.-K. (1985) Mutat. Res. 146, 191–196.
[17] Freed, J.J. and Mezger-Freed, L. (1970) Proc. Natl. Acad. Sci. USA 65, 337–344.
[18] Chao, C.C.-K. and Rosenstein, B.S. (1984) Mutat. Res. 139, 35–39.
[19] Chao, C.C.-K., Rosenstein, R.B. and Rosenstein, B.S. (1985) Mutat. Res. 149, 443–450.
[20] Tyrrell, R.M. (1984) Proc. Natl. Acad. Sci. USA 81, 781–784.
[21] Kohn, K.W., Reickson, L.C., Ewig, R.A.G. and Friedman, C.A. (1976) Biochemistry 15, 4629–4637.
[22] Rosenstein, B.S., Ducore, J.M. and Cummings, S.W. (1983) Mutat. Res. 112, 397–406.
[23] Carrier, W.L. and Setlow, R.B. (1971) Anal. Biochem. 43, 427–432.
[24] Jagger, J. (1967) Introduction to Research in Ultraviolet, Prentice Hall, New Jersey.
[25] Schorpp, M., Mallick, U., Rahnsdirf, H.J. and Herlich, P. (1984) Cell 37, 861–868.
[26] Rotem, N., Axelrod, J.H. and Miskin, R. (1987) Mol. Cell. Biol. 7, 622–631.
[27] Maniatis, T., Goodbourn, S. and Fischer, J.A. (1987) Science 236, 1237–1245.
[28] Hanawalt, P. (1986) in: Mechanisms of DNA Damage and Repair (Grossman, L. and Upton, A.C. eds) pp.489–498, Plenum, New York.
[29] Jentsch, S., McGrath, J.P. and Varshavsky, A. (1987) Nature 329, 131–134.