Journal of Cellular Biochemistry 18:121-133 (1982)
Mechanisms of Chemical Carcinogenesis 229-241

Differential Reactivation of Zinc-Mediated Metallothionein Induction in Ultraviolet-Irradiated Normal and Repair-Deficient Human Cells

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The ubiquitous, low-molecular-weight, thiol-rich, metal-binding protein, metallothionein (MT), can be induced in cultured normal human fibroblasts (NF) and xeroderma pigmentosum (XP) cells by exposure to $ZnCl_2$. Both NF and XP cells tolerate up to 200 μM $ZnCl_2$ in the growth medium. Upon addition of $ZnCl_2$ (200 μM) to monolayer cultures, both NF and XP cells showed similar kinetics for the induction of MT synthesis: Within 7 hours the MT synthesis rate rose from a low, marginally detectable rate to a maximal rate at least 50-fold greater than the basal rate. The induction of MT synthesis in both cell types was inhibited by actinomycin D (5 μg/ml), indicating that the induction process is controlled at the level of transcription. Exposure of NF or XP cells to far ultraviolet light (UV) followed by induction with $ZnCl_2$ resulted in a UV dose-dependent decrease in the maximal rate of MT synthesis measured 8.5 hours postirradiation. The UV sensitivity of the MT induction was greater in XP cells than in NF cells. However, considerations of the differential repair capacities of NF and XP cells superimposed upon the kinetics of MT induction were invoked to explain the apparent differential UV sensitivity of MT induction. Liquid holding recovery experiments showed that NF cells possess the capacity to reactivate this inducible gene function rapidly while XP cells are deficient in the reactivation capacity. These results are discussed in the context of both UV transcriptional mapping of this inducible gene function and development of techniques for measuring repair of transcription-blocking lesions.

Key words: metallothionein, zinc, xeroderma pigmentosum, human fibroblasts, ultraviolet radiation, gene repair, liquid holding recovery, transcription unit mapping

Previous studies of repair of various types of lesions in cellular DNA have utilized direct measurements of lesion removal, DNA repair synthesis in the damaged region, recovery of cell survival, or host cell reactivation of damaged viral DNA [1,2]. Repair of damage to specific gene functions in mammalian cells has been studied only in the context of repair of lesions which lead to mutation induction at the HPRT locus in human cells [3,4]. In the present paper, evidence is presented to suggest the general utility of an ubiquitous inducible gene function in studies of DNA damage and repair both in cultured cells and in vivo.

Received April 14, 1981; accepted October 9, 1981.

0730-2312/82/1801-0121$04.00

The specific genes of interest in this study encode low-molecular-weight, metal-binding proteins, metallothioneins ([5] and references therein). These proteins have been studied extensively in various eukaryotic systems in relation to the role of the proteins' metal-binding capacity in heavy metal detoxification [5,6] and in essential trace-element metabolism [7–9]. In mammals, metallothioneins (MTs) generally contain 61 amino acids (of which 20 are cysteine), bind group IIB metals (Zn, Cd, Hg) as well as Cu, with high affinity, and are synthesized in response to exposure of cells in vivo or in culture to Zn, Cd, or Cu ([5] and references therein). Recently, several laboratories have reported on studies of the regulation of metal-mediated induction of MT synthesis in cultured mammalian cells [10–12]. In general, those studies indicated that induction of MT synthesis by Cd or Zn is regulated at the level of transcription of thionein (the apoprotein of MT) mRNA [10,12]. Further, the response is not tissue specific and has been observed in human cell lines and explants [13–17].

The properties of the inducibility and transcriptional regulation of this specific gene function suggested that this system might be used 1) to classify DNA damaging agents according to their capacity to block gene transcription, 2) to study various types of repair deficiencies involving removal of transcription-blocking lesions, or 3) to establish relative repair proficiencies among human populations and to identify individuals having specific repair deficiencies.

In the present study we characterize the Zn^{2+}-mediated induction of metallothionein in normal human skin fibroblasts and in xeroderma pigmentosum (XP) cells having a defect in the ability to repair lesions introduced into DNA by ultraviolet (UV) light [18,19], an established transcription-terminating agent [20,21]. The UV sensitivity of the inducible gene function and the capacity to reactivate the inducible gene function are compared in normal (NF) and repair-deficient (XP) cells.

MATERIALS AND METHODS

Tissue Culture

Cultures of human skin fibroblasts were purchased from the American Type Culture Collection (Rockville, MD). Both normal (NF) cells (CRL 1295) and xeroderma pigmentosum (XP) cells (CRL 1223 or XP12BE, complementation group A) were obtained at passage numbers 12 and 9, respectively, and were routinely cultured in Dulbecco's modified Eagle's medium (DMEM, Grand Island Biological Co., Grand Island, NY) containing 20% fetal calf serum (FCS, Reheis Chemical Co., Scottsdale, AZ) in a humidified, water-jacketed incubator at 37°C maintaining 5% CO_2 tension. Cells were subcultured (1:3 dilution) weekly with a medium renewal on the fourth day after subculturing. Cell cultures were maintained for approximately 3 months at which time they were discarded and new cultures were seeded from frozen stocks maintained in liquid nitrogen. Routine examinations of the cultures for contamination with PPLO were performed using the method of Chanock et al [23]. No PPLO was detected in any culture for the duration of the experiments.

To obtain cells in the subconfluent state (ie, dividing cells), cultures of NF or XP cells less than one week old were trypsinized, diluted about 50-fold in DMEM with 20% FCS, and the cell densities determined by Coulter counting

(Coulter Electronics, Hialeah, FL). These counts were within 5% of that obtained using a hemocytometer. Culture dishes (100 mm) were seeded with 10^5 cells per dish in 10 ml of DMEM with 20% FCS and incubated for 20 hours. Our observations indicate that the NF or XP cells are completely attached to the dish within this 20 hour period although cell division has not occurred. At this time, the medium is replaced with DMEM with 10% FCS and cell doubling occurs within the following 16–20 hours. Thus, at 36–40 hours after subculturing, there would exist approximately 2×10^5 cells per dish. These cultures were exponentially dividing and were subconfluent.

For confluent cells (stationary phase), 100-mm dishes were seeded with 10^5 cells as described above, and the dishes were incubated for one week with a medium change on the fourth day. On the seventh day the medium was replaced with DMEM containing 10% FCS, and incubation was continued for an additional 2–3 days before the cultures were considered confluent. It was observed for both NF and XP cells that cell number per dish became constant on or before the seventh day of culturing yielding about 3.5×10^6 cells per dish (about five population doublings). The medium was saved from the proliferating cultures, diluted twofold with DMEM only, and was used as "conditioned" medium in the liquid holding experiments described below.

$ZnCl_2$ Cytotoxicity in Human Cells

Trypsinized cells were diluted, counted, and plated at 100 per 100 mm dish (for NF) or 200 per dish (for XP) in 10 ml DMEM containing 10% FCS. After 20 hours incubation, the cells were exposed to various concentrations of $ZnCl_2$ (5–1000 μM). Incubation was continued for 24 hours, at which time the treatment medium was removed and replaced with 10 ml DMEM with 20% FCS. The plates were incubated for an additional 8–10 days to allow for colony development with an intermittent feeding on the fourth day. Colonies (clusters of greater than 30 cells each) were fixed in ethanol, stained with crystal violet, and scored. Plating efficiencies for non-treated cultures were 50–75% (NF) and 25–40% (XP). Normal colony formation was seen for both NF and XP cells treated with 200 μM or less $ZnCl_2$. At higher concentrations of the metal, severe cytotoxic effects were observed, and at 400 μM or greater, no colony formation occurred.

Far UV Irradiation

Cells to be irradiated (primarily 254 nm wavelength) were subcultured at least 20 hours prior to exposure to UV light. The culture medium was removed, and the plates were exposed (minus their covers) to one 15-W germicidal lamp (General Electric, #G15T8) which was fitted with a camera shutter device. The incident fluence was determined to be 0.5 J per m^2 per sec using an Epply thermopile (Epply Laboratories, Inc., Newport, RI) connected to a Keithly Model 150-B microvolt ammeter (Keithly Instruments, Cleveland, OH). The thermopile was calibrated against a National Bureau of Standards (NBS) carbon filament secondary standard of total irradiance, in accordance with NBS recommended procedures.

Liquid Holding Recovery

It has been reported that recovery of radiation-induced damage (UV or X-rays) is observed as an increase in the cloning ability of stationary human skin

fibroblasts [4,22]. Cultures of NF or XP cells were grown to the confluent state as described above. After UV irradiation, 10 ml of "conditioned" medium was added to each dish and incubation was continued for noted times. For determining cell survival (colony assay), cells were trypsinized at noted times, counted, and appropriate dilutions plated into 100 mm dishes containing DMEM with 20% FCS. The development and scoring of colonies was as described above.

Measurement of Metallothionein Induction

Metallothionein synthesis was measured essentially as described previously [24] for CHO cells cultured in suspension with several modifications for harvesting cells from monolayer cultures. At times prior to or following UV irradiation and/or exposure to $ZnCl_2$, cultures were pulse labeled by addition of ^{35}S-cysteine(Cys) (14 μl of 0.1 mCi/ml ^{35}S-Cys, New England Nuclear Corp.) to each dish containing complete DMEM medium with 10% FCS. After incubation for 1 hour at 37°C in a CO_2 incubator, the medium was removed by aspiration, monolayers were washed gently with 2 ml 0.25% trypsin in phosphate-buffered saline. The trypsin solution was removed by aspiration, and typsinization of cells was accomplished by addition of 2 ml of the trypsin solution followed by incubation for 3–5 minutes at 37°C. Trypsinization was terminated by addition of 10 ml of ice-cold complete growth medium containing soybean trypsin inhibitor (0.2 mg/ml) to each dish. The cell suspension was transferred to a conical 15-ml centrifuge tube, and cells were harvested by centrifugation at 500g for 5 minutes. Cells were washed by resuspension in 5.0 ml 0.15 M saline, 0.01 M Tris-Cl pH 7.4, 0.2 mg/ml soybean trypsin inhibitor, and collected by centrifugation as above. The resulting cell pellet was resuspended in 0.9 ml of 0.01 M Tris-Cl, pH 7.4, 0.01M KCl, 0.0015 M $MgCl_2$, and 0.018 M 2-mercaptoethanol. This cell suspension was frozen quickly in a dry ice-ethanol bath and stored at -20°C for not more than one day. Cell fractionation to obtain cytoplasm and nuclei was performed as described elsewhere [24] with one modification: Immediately following cell lysis by addition of 0.1 ml of 10% Np-40 (Shell Oil Co.), cell lysates were made 0.100 mM $CdCl_2$ by addition of 10 μl of a 10 mM $CdCl_2$ solution (in 1 mM HCl). This step was found to be essential to "stabilize" the metallothionein present by substituting Cd^{2+} for the bound Zn^{2+} [25,26 and unpublished results]. This metal substitution step prevented the oxidative crosslinking and aggregation of metallothionein (unpublished observations).

Metallothionein was quantitated, and relative metallothionein synthesis rates were determined as detailed previously [24,27]. Briefly, this analysis involves separation of metallothionein from other cytoplasmic components by gel filtration chromatography on Sephadex G-75. In all experiments >6000 cpm of incorporated ^{35}S-Cys were analyzed by column chromatography. Most non-metallothionein proteins elute with the excluded volume. Under most conditions including UV irradiations up to ~ 15 J/m^2, incorporation of ^{35}S-cysteine into non-metallothionein proteins during a 1-hour pulse label was approximately constant (ie, ^{35} S-cysteine incorporated/mg protein/60 min) independent of presence or absence of inducing metal and independent of time after irradiation. Exceptions to this observation are noted in Results and Discussion (see legend to Fig. 2). Hence, the relative rate of MT synthesis was determined by measuring the ratio of ^{35}S-incorporation into MT to the ^{35}S-incorporation into non-MT proteins. Further

discussion of this measurement is presented elsewhere [27]. Metallothioneins synthesized by both normal fibroblasts and xeroderma pigmentosum cells were also characterized and quantitated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis after reduction and carboxymethylation of cytoplasmic extracts as described previously [10]. Protein was assayed by the method of Lowry. Metallothionein synthesis rates are presented as relative MT synthesis rates (ie, 100 × ^{35}S-incorporated into MT/^{35}S-incorporated into non-MT cytoplasmic proteins during the 1-hour pulse label). This normalization to non-MT synthesis rates facilitates correction for variable cell recovery from culture dishes and for changing ^{35}S-Cys-specific activity due to decay from experiment to experiment [24,27].

RESULTS

Kinetics of Zn^{2+}-Induction of MT Synthesis in Normal Human Fibroblasts and Xeroderma Pigmentosum Cells

Initial experiments were performed to establish the tolerance of normal and XP cells to continuous $ZnCl_2$ exposure (24 hours). We found that 200 μM $ZnCl_2$ was the maximum subtoxic Zn^{2+} concentration that could be used (data not shown). Previous studies using non human cell lines showed that the maximum subtoxic Zn^{2+} exposure level is also the optimal Zn^{2+} concentration for inducing synthesis of metallothionein (24,28). This observation was also found to be the case for both NF and XP cells (data not shown).

Measurements of the kinetics of induction of MT synthesis in confluent normal and XP cultures are shown in Figure 1. These data were obtained by determining the relative MT synthesis rate during a 1-hour ^{35}S-Cys pulse-labeling period prior to the time at which each data point is plotted. In experiments performed over the course of several months the induction kinetics for MT synthesis in normal fibroblasts were indistinguishable from those observed in XP cells. The response of this inducible gene function increases from a low, basal rate of synthesis, prior to or immediately following exposure to 200 μM Zn^{2+}, to a level at least 50-fold greater than the basal rate by 6-8 hours postinduction. The relative MT synthesis rate then decreases slowly during continued exposure to Zn^{2+}, and appears to reach a steady state level by 24 hours postinduction. These induction kinetics are strikingly similar to those observed in a cultured Cd^{2+}-resistant subline of the CHO cells [28], and in vivo in the livers of animals exposed to Zn^{2+} [29,30]. The induction kinetics in subconfluent monolayers of either XP or NF cells were indistinguishable from those of confluent cultures.

The question of whether the Zn^{2+}-mediated induction of MT synthesis is regulated at the level of transcription of thionein mRNA was, in part, answered by measuring the effect of a transcriptional inhibitor, actinomycin D (AM), on the induction process (Fig. 1). Addition of 5 μg/ml AM (sufficient to block transcription of mRNA precursors [31]) 0.5 hour prior to or 0.5 hour after Zn^{2+} exposure of confluent XP cells resulted in > 95% inhibition of MT synthesis measured at the expected time of maximal MT synthesis. Further evidence of the transcriptional regulation of MT induction is provided below through the analysis of comparative effects of UV irradiation on the induction response in normal and XP cells.

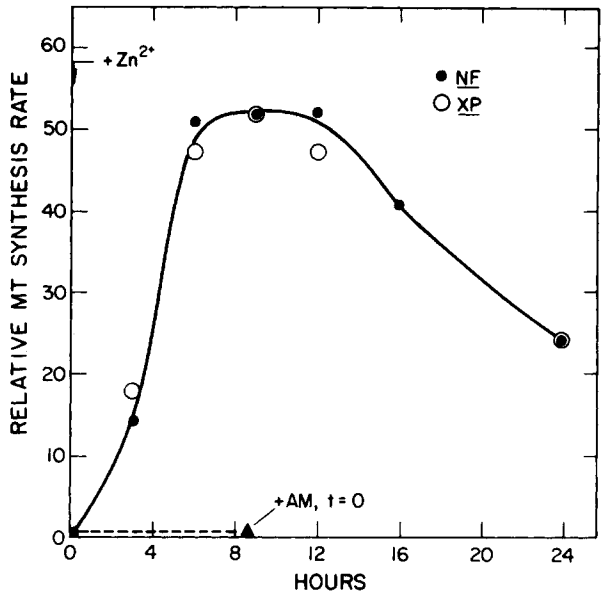

Fig. 1. Metallothionein induction kinetics in NF and XP cells. $ZnCl_2$ was added to 200 μM final concentration at 0 hours to confluent monolayer cultures of NF and XP cells and MT synthesis rates were determined at various times during continuous Zn^{2+} exposure as described in the text. XP cells treated with 5 μg/ml actinomycin D (AM) simultaneously with Zn^{2+} addition at 0 hours showed no detectable MT synthesis at 8.5 hours (—▲—).

Inactivation of Induction of MT Synthesis by UV Irradiation

The UV sensitivity of Zn^{2+}-induced MT synthesis in NF and XP cells was determined initially by measuring the dose-dependence of MT synthesis (6–8 hours) postinduction. In these experiments, confluent monolayers were exposed to various doses of UV light and then immediately (< 5 minutes postirradiation) induced by continuous exposure to 200 μM Zn^{2+}. The rate of MT synthesis was measured from 7.5 to 8.5 hours postinduction (and postirradiation). Results of these measurements are summarized in Figure 2. Several features of these "functional survival curves" are evident. First, MT induction in both NF and XP cells appears to follow exponential inactivation with increasing UV dose. Secondly, there does not appear to be a shoulder on either of these functional survival curves. Finally, UV inactivation of MT induction in NF cells is more resistant than in XP cells although the difference is less pronounced than that for cloning ability (see inset of Fig. 2). This apparent differential sensitivity of MT induction in XP cells compared with that in NF cells can be attributed most likely to the increased repair in NF relative to XP during the MT induction period as will be shown below.

The data presented in Figures 1 and 2 can be used to make several interesting predictions concerning the behavior of this inducible system by taking into account (a) the complex dynamics of the MT induction response together with (b) differential repair proficiencies of the NF and XP cells and (c) the dynamics of the repair process of NF cells. First, the initial phase of the MT induction process occurs over approximately an 8-hour postinduction period. If repair of UV lesions blocking transcription of DNA in normal fibroblasts occurs on a time scale that is faster

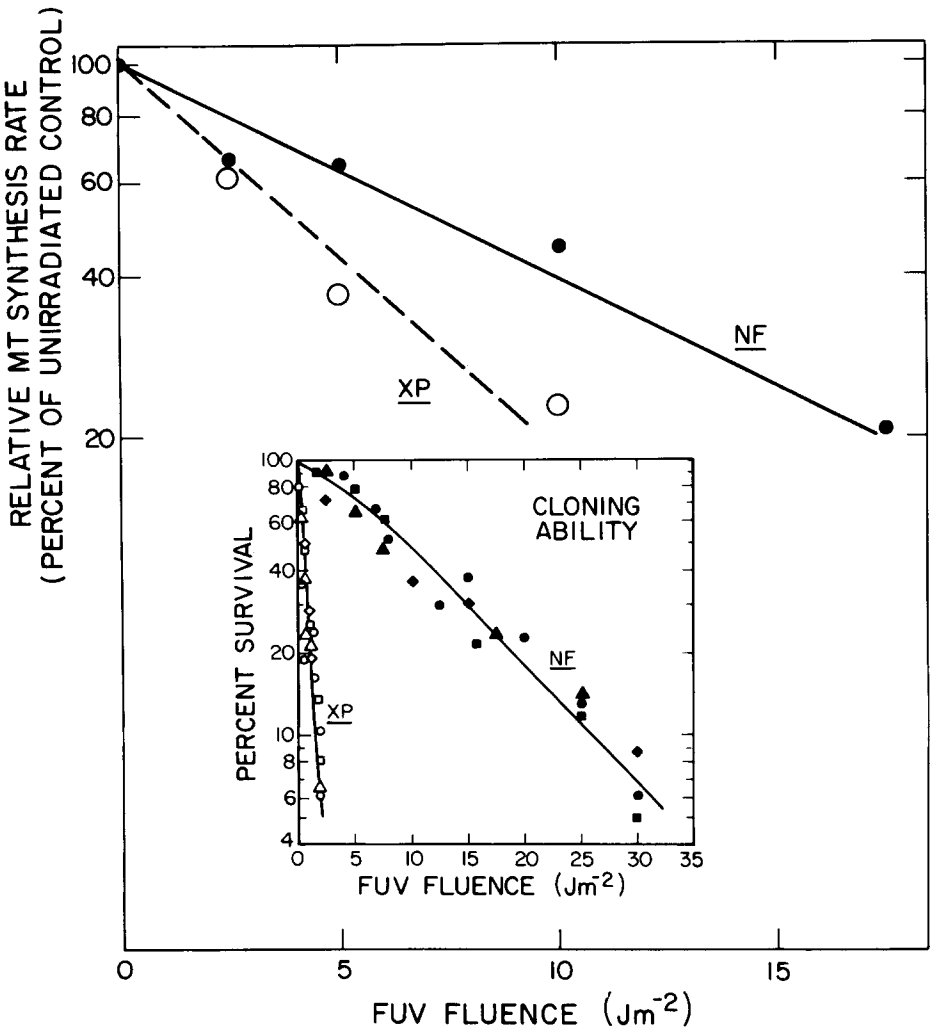

Fig. 2. UV dose-response for inactivation of MT induction. Confluent monolayer cultures were irradiated with the indicated UV dose, and culture medium containing 200 μM $ZnCl_2$ was added to dishes immediately following irradiation. Metallothionein synthesis rates were measured as described in the text by pulse labeling with ^{35}S-Cys for the postirradiation period of 7.5–8.5 hours. The inset shows the UV dose response for cloning ability of NF and XP cells. Different symbols in the survival curves represent different experiments. The relative rate of non-MT protein synthesis in irradiated NF cells did not show significant decrease from unirradiated controls even at 15 Jm^{-2}. However, in XP cells the relative rate of non-MT protein synthesis (measured as ^{35}S-Cys cpm incorporated into non-MT proteins/mg protein/60 min) decreased to ~75% of control by 8.5 hours postirradiation. No correction for this decrease in non-MT protein synthesis is included here. However, such a correction would decrease the calculation of relative MT synthesis rate. Further, during "liquid holding recovery" experiments synthesis of non-MT proteins did not recover.

than, or comparable to, that for maximal induction of MT synthesis, then the repair of the UV lesions in the DNA sequences responsible for MT induction during the postirradiation continuous exposure to Zn^{2+} could produce an apparent shift in the MT induction kinetic response with a maximal MT synthesis rate occurring later in the irradiated cultures than in the unirradiated controls. In this case, the maximal

rate of MT synthesis (ie, representing the maximum number of functioning MT transcription units) would be attained at times later than that observed in unirradiated cells (ie, ~8 hours for NF cells) and would be expected to decrease in a UV dose-dependent manner [3,22]. On the other hand, if the repair processes are slow relative to the MT induction process, then the MT induction response in irradiated cultures should display kinetics similar to the unirradiated control with MT synthesis rate at each time point in the induction kinetics curve for irradiated cultures being a constant fraction of the control. The former situation should exist in normal fibroblasts based upon studies of the rapid (within a few hours postirradiation) recovery of colony forming ability in UV irradiated cells held in "conditioned" growth medium for various intervals prior to plating for survival (termed a "liquid holding recovery" or biological recovery of survival [4,22]). The latter prediction could be anticipated in the case of UV inactivation of the MT induction response in XP cells which are deficient in the capacity to remove UV induced, transcription-blocking lesions [1,3,18,19].

Results of experiments performed to test the above predictions are shown in Figures 3A,B. Both NF and XP cells were irradiated with identical UV doses, ie, a dose to reduce the "maximal" (8.5 hours) MT synthesis rate to ~50% of the unirradiated control. Following UV irradiation cultures were exposed continuously to 200 μM $ZnCl_2$ and MT synthesis rate was measured at the times indicated. The induction kinetics of the UV-irradiated NF cells display a clear shift of the maximal MT synthesis rate to longer times relative to the unirradiated control. In other experiments, the maximal rate of MT synthesis both decreased in magnitude and occurred at increasingly later times postinduction with increasing UV dose (data not shown). Detailed analyses of the interplay between MT induction kinetics and biological recovery (repair) kinetics following UV are the subject of current studies and will be reported elsewhere. In contrast, the MT induction kinetics in the irradiated XP cultures are similar to the unirradiated control, indicating that the surviving fraction of DNA sequences involved in induction of MT synthesis behaves in a manner similar to the unirradiated control. This type of analysis applied to the shape of curves for MT induction kinetics indicates repair of UV-induced lesions in this inducible gene function. A more direct assessment of cellular capacity to repair UV damage is provided by experiments analogous to those for determining the liquid holding recovery of cloning ability [4,22].

Differential Reactivation of MT Synthesis Induction Capacity in NF and XP Cells During Liquid Holding Recovery

These experiments were designed to determine whether postirradiation incubation at 37°C would enhance the subsequent capacity of the cells to respond to maximally inducing levels of Zn^{2+}. The results of these experiments are shown in Figure 4A. In these experiments cultures of XP and NF cells were irradiated with a UV dose sufficient to inactivate maximal MT induction to ~50% of control values (ie, 10 Jm^{-2} in both XP and NF cells). Cultures were held in "conditioned" medium for various (liquid holding) intervals following UV irradiation. Cultures were then exposed continuously to 200 μM $ZnCl_2$ and MT synthesis rate was measured 7.5 to 8.5 hours subsequent to addition of Zn^{2+}. The results of these measurements show clearly that NF cells reactivate the capacity to induce synthesis of MT while XP cells

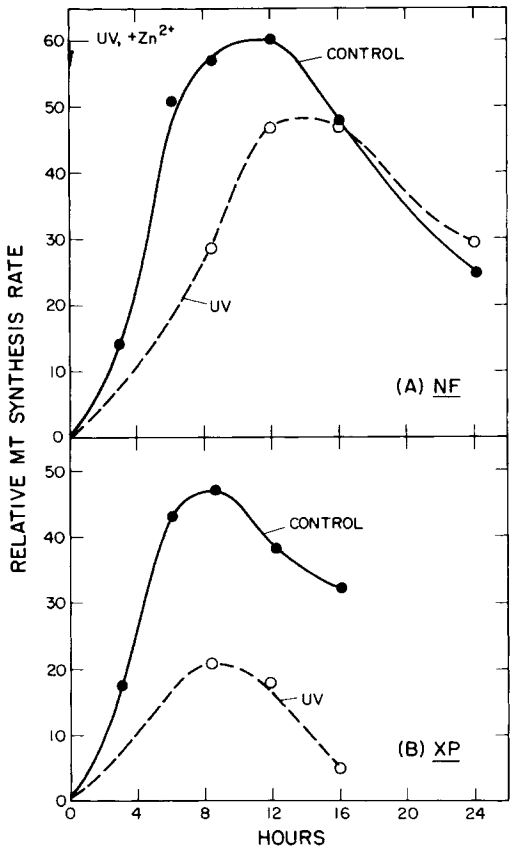

Fig. 3. MT induction kinetics in unirradiated and UV-irradiated NF and XP cells. Confluent monolayer cultures of NF(A) or XP(B) cells were either used as unirradiated controls (—●—) or irradiated (—○—) with 10 Jm^{-2} of UV light. $ZnCl_2$ was added immediately after irradiation (or after the sham-irradiation procedure for controls), and MT synthesis rates were determined at the various times indicated as described in the text.

show no such reactivation capacity even at times long (17 hours) compared with the maximal MT induction phase (see Fig. 1). Measurements of the liquid holding recovery of cloning ability are presented in Fig. 4B. The kinetics of recovery of cloning ability in the NF cells are strikingly similar to the kinetics for reactivation of induction of MT synthesis suggesting that common processes are involved in these presumably independent phenomena. One possible explanation for these results is that a UV dose-dependent delay in cell cycling occurs before normal cycling can begin in the repair-proficient NF cells [3,22]. Recovery of MT induction following UV irradiation may correlate closely with the cell-cycling delay. However, further measurements will be required to test this notion. (Due to the differential sensitivities in the UV inactivation of cloning ability in XP and NF cultures it was necessary to use UV doses of 0.5 and 10 Jm^{-2}, respectively, to achieve ~50% inactivation.)

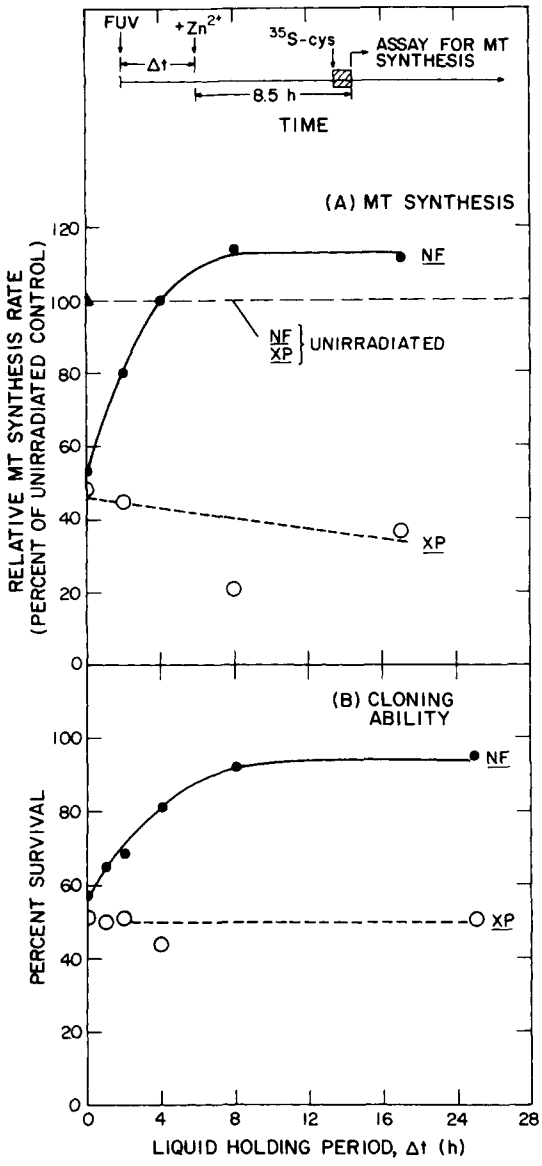

Fig. 4. Liquid holding recovery of (A) MT induction or (B) cloning ability of NF and XP cells. For MT induction experiments, confluent monolayers were held as controls or irradiated with 10 Jm^{-2} UV. Conditioned medium was added to culture dishes immediately after irradiation. At various times post-irradiation, $ZnCl_2$ was added to cultures to a final concentration of 200 μM, and MT synthesis was determined by ^{35}S-Cys pulse labeling from 7.5–8.5 hours following addition of Zn^{+2} as described in the text. The liquid holding period is defined as the time between UV irradiation and addition of $ZnCl_2$ to cultures. For colony survival, confluent monolayers were held as controls or UV irradiated to ~50% surviving fraction (ie, 0.5 and 10 Jm^{-2} for XP and NF, respectively). Assays were performed as described in the text. It should be noted that the inactivation of MT synthesis by 10 Jm^{-2} for XP cells (~48%) appears to be in disagreement with the results of separate experiments shown in Figure 2. In four independent experiments inactivation of MT induction by 10 Jm^{-2} ranged from 10–48% with an average of ~20% in agreement with Figure 2.

DISCUSSION

The major conclusions of these studies can be summarized briefly. 1) An inducible gene function regulated at the level of transcription responds to inducer with similar kinetics in normal (NF) and UV repair-deficient (XP) cell types. 2) The UV inactivation of the inducible response in the NF and XP cells appears to follow first-order exponential inactivation kinetics with the D_0 for the NF cells being slightly greater than that for XP cells. 3) The slight difference in the D_0's of NF and XP cells can be attributed to differences in the capacities of the two cell types to reactivate the induction of MT synthesis presumably by repair of transcription-blocking UV lesions. 4) Differential functional reactivation (repair) capacities of NF and XP cells can be distinguished readily by examination of (a) postirradiation MT induction kinetics or (b) postirradiation liquid holding recovery of the inducible function.

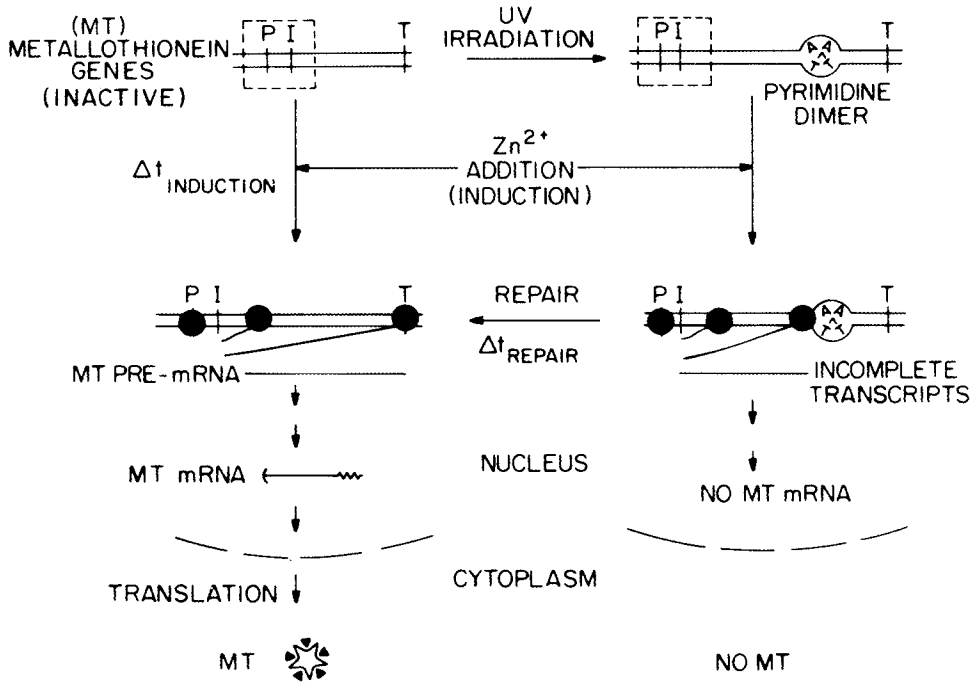

Fig. 5. Proposed model for MT induction, UV inactivation of MT induction, and reactivation of MT induction. In the preinduction state MT genes are transcriptionally inactive as indicated by the dashed line surrounding putative promoter (P) and initiator (I) regions. (The termination signal is indicated by T.) Addition of Zn^{2+} causes activation or derepression of the MT structural genes and RNA polymerase (solid circles) produces primary transcripts (pre-MT mRNA) which are processed to form MT mRNA. Translation of MT mRNA is tightly coupled to the appearance of cytoplasmic MT mRNA [12]. MT is indicated by the star shaped symbol with associated Zn^{2+} (solid triangles). If the MT gene sustains a pyrimidine dimer within the transcription unit prior to Zn^{+2} addition, incomplete transcripts are formed [20,21] following initiation of transcription by Zn^{2+} addition. No MT mRNA is produced and consequently no MT is synthesized. However, in cells proficient in excision repair, the transcription-terminating pyrimidine dimers can be removed and transcription can proceed normally. If the repair processes occur on a time scale of the order or shorter than the time required for induction of MT gene expression, the amount of UV inactivation would be underestimated.

These findings have several implications. The apparent ubiquity of the metal-mediated MT induction response in higher eukaryotes [5] and the ability to induce the response in skin explants or in peripheral lymphocytes [13–17] from humans suggests a general applicability of this inducible system as a diagnostic indicator for differential repair capacities among individuals, and for identification of agents which introduce transcription-blocking lesions into the genome.

One further implication of these studies relates to the use of UV inactivation of gene functions in transcription unit mapping [20,21]. Although it is tempting to use the expression of a protein product in a transcriptionally controlled system, such as MT induction, as a direct indicator of damage to the structural gene (transcription unit) encoding the protein product, caution must be used in interpreting the results of such measurements. As shown in this study (and illustrated in Fig. 5) repair processes operating on a time scale comparable to the MT induction process could cause an underestimate of the size of the UV-sensitive target due to reactivation of damaged targets during the induction process. However, in the case of MT induction, the present study provides two procedures which are diagnostic for the role of repair processes in modulation of the post-UV irradiation MT induction response (Figs. 3,4). Another concern in relating UV inactivation of MT induction to transcription unit size involves the complexity of the regulation of MT induction. For example, could UV damage in a portion of the genome not containing the structural MT transcription unit inactivate MT induction? This question is being addressed in current studies. In any case, it is interesting that the induction of MT synthesis in NF and XP cells is unexpectedly sensitive to UV irradiation especially considering the size of the protein product and its mRNA [32]. Estimates of the size of the transcription unit for thionein induction from the present study indicate a large UV sensitive target (possibly > 20 kilobases based upon a D_0 of 7.5 Jm^{-2} (Fig. 2) and conversions of UV dose to frequency of transcription-terminating lesions summarized by Sauerbier and Hercules [21]). This estimate is by far greater than the size of thionein mRNA ($\sim$400–450 bases) [32,33]. This interesting comparison will require further, more definitive analyses using internal calibrations to accurately measure the size(s) of the UV-sensitive target(s).

ACKNOWLEDGMENTS

We thank John L. Hanners and Brenda Vigil for excellent technical assistance. Also, we are grateful to M.D. Enger, J.K. Griffith, A.G. Saponara, and R.A. Walters for critical examination of the manuscript. This work was supported by the Department of Energy.

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