Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 1087–1092, February 1996
Medical Sciences

DNA damage enhances melanogenesis

(pigmentation/tanning/melanocyte-stimulating hormone/tyrosinase/SOS response)

MARK S. ELLER, KRISTIN OSTROM, AND BARBARA A. GILCHREST*

Department of Dermatology, Boston University School of Medicine, Boston, MA 02118-2394

Communicated by Aaron B. Lerner, Yale University School of Medicine, New Haven, CT, October 23, 1995

ABSTRACT **Although the ability of UV irradiation to induce pigmentation *in vivo* and *in vitro* is well documented, the intracellular signals that trigger this response are poorly understood. We have recently shown that increasing DNA repair after irradiation enhances UV-induced melanization. Moreover, addition of small DNA fragments, particularly thymine dinucleotides (pTpT), selected to mimic sequences excised during the repair of UV-induced DNA photoproducts, to unirradiated pigment cells *in vitro* or to guinea pig skin *in vivo* induces a pigment response indistinguishable from UV-induced tanning. Here we present further evidence that DNA damage and/or the repair of this damage increases melanization. (*i*) Treatment with the restriction enzyme *Pvu* II or the DNA-damaging chemical agents methyl methanesulfonate (MMS) or 4-nitroquinoline 1-oxide (4-NQO) produces a 4- to 10-fold increase in melanin content in Cloudman S91 murine melanoma cells and an up to 70% increase in normal human melanocytes. (*ii*) UV irradiation, MMS, and pTpT all up-regulate the mRNA level for tyrosinase, the rate-limiting enzyme in melanin biosynthesis. (*iii*) Treatment with pTpT or MMS increases the response of S91 cells to melanocyte-stimulating hormone (MSH) and increases the binding of MSH to its cell surface receptor, as has been reported for UV irradiation. Together, these data suggest that UV-induced DNA damage and/or the repair of this damage is an important signal in the pigmentation response to UV irradiation. Because *Pvu* II acts exclusively on DNA and because MMS and 4-NQO, at the concentrations used, primarily interact with DNA, such a stimulus alone appears sufficient to induce melanogenesis. Of possible practical importance, the dinucleotide pTpT mimics most, if not all, of the effects of UV irradiation on pigmentation, tyrosinase mRNA regulation, and response to MSH without the requirement for antecedent DNA damage.**

The prokaryotic response to UV irradiation, the so-called SOS response, is well documented and is now known to include the induction of a set of >20 genes involved in DNA repair and cell survival (reviewed in ref. 1). In this case, the single-stranded DNA generated after UV irradiation interacts with and activates a protease, the Rec A protein (2). Activated Rec A protein then cleaves and inactivates the repressors of specific genes, leading to their induction (2).

In eukaryotic cells, the existence of a UV-induced DNA damage-responsive SOS-like system mediated by one common transcription regulator has been the subject of considerable controversy. Although a variety of genes are known to be induced by DNA damage (3–6), many of these genes are also induced by agents such as phorbol esters (3, 7) and by metabolic or oxidative stress (8–10). Because UV irradiation is reported, like phorbol esters, to activate protein kinase C directly (11, 12) and to produce oxidative damage through generation of free radicals from membrane lipids and other extranuclear cellular constituents, it cannot be determined whether the effects of UV are due directly to DNA damage or instead to other impacts on the cell. Indeed, two of the major UV-induced transcription factors, AP-1 and NF-κB, are now thought to initiate their responses at or near the plasma membrane (13, 14).

Perhaps the best-characterized example of DNA damage-specific gene induction involves a DNA repair enzyme, photolyase, encoded by the *PHR1* gene in *Saccharomyces cerevisiae* (6). This gene is induced by a variety of DNA-damaging agents including UV light, methyl methanesulfonate (MMS) and 4-nitroquinoline 1-oxide (4-NQO). Up-regulation of *PHR1* gene transcription is, at least in part, accomplished by the removal of a damage-responsive repressor which binds to a specific site in the 5′ region of the gene (15).

Another well-studied UV and DNA damage-inducible gene is the mammalian *GADD45* gene. This gene is transcriptionally activated not only by UV irradiation but also by ionizing radiation and chemical agents that specifically cause base damage (10, 16). The induction of *GADD45* by ionizing radiation is mediated by the p53 tumor suppressor protein and the ataxia telangiectasia gene product (17), but the UV- and base-damage responses are less well understood. Recently, GADD45 protein was shown to stimulate DNA excision repair as well as to inhibit DNA replication by blocking the cell cycle at the G_1 checkpoint (18). Thus GADD45 plays a major role in maintaining the integrity of the genome after damage by coordinating repair with replication.

As would be expected teleologically, the majority of the prokaryotic genes responsive to DNA damage are, like *PHR1* and *GADD45*, involved in the repair or prevention of such damage. As well, UV irradiation and DNA-damaging carcinogenic chemical agents appear to induce DNA repair systems in mammalian cells (19), consistent with the existence of an SOS-like response analogous to that in yeast. Because melanin is photoprotective in human skin and is generally acknowledged as the body's major defense against photocarcinogenesis (20), acting to inhibit the formation of UV-induced DNA photoproducts (21, 22) that give rise to "signature mutations" responsible for skin cancer development (23), enhanced melanogenesis might logically be part of the SOS response in skin.

The clinical and histological manifestations of tanning have been extensively studied, but the mechanisms by which UV light stimulates melanogenesis are poorly understood. Several lines of evidence now suggest that DNA damage and/or the repair of this damage is an important signal in the melanogenic response to UV irradiation. (*i*) The action spectrum for the formation of the major UV-induced DNA photoproducts in human skin is essentially the same as that for UV-induced tanning (24, 25). (*ii*) Increasing DNA repair after UV irradiation by addition of T4 endonuclease V (T4N5), the prokaryotic DNA repair enzyme that catalyzes the rate-limiting step in

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: MSH, melanocyte-stimulating hormone; MMS, methyl methanesulfonate; 4-NQO, 4-nitroquinoline 1-oxide.

*To whom reprint requests should be addressed at: Department of Dermatology, Boston University School of Medicine, 80 East Concord Street, Boston, MA 02118-2394.

excision of cyclobutane pyrimidine dimers, enhances the melanogenic response in Cloudman S91 murine melanoma cells as well as in human melanocytes (26). (*iii*) Addition of thymidine dinucleotides, the principal target for dimerization by UV light and subsequent release during DNA excision repair (27), stimulates melanogenesis in unirradiated S91 cells and in human melanocytes as well as in guinea pig skin (28).

One of the known physiological effectors of melanization is melanocyte-stimulating hormone (MSH) (reviewed in ref. 29). This family of small peptide hormones is derived from a precursor protein, proopiomelanocortin, synthesized by pituitary cells (30) and by epidermal keratinocytes (31, 32). In S91 mouse melanoma cells, MSH increases the mRNA, protein and activity levels of tyrosinase, the rate-limiting enzyme in melanin biosynthesis (33, 34). Cultured human melanocytes also express functional MSH receptors and respond to MSH by increased growth and pigmentation (35–37). UV irradiation has been shown to increase the number of cell surface MSH receptors on S91 cells and to potentiate the melanogenic response of these cells to MSH (38, 39). Furthermore, the intact skin of mice and guinea pigs shows a greater increase in the number of active melanocytes following UV irradiation and MSH treatment than after either treatment alone. Therefore, it has been proposed (38) that a major factor in UV-induced tanning is the up-regulation of the MSH/MSH receptor system in skin. However, the mechanism by which UV light affects this system is unknown. Here we present evidence that UV-induced DNA damage and/or the repair of this damage stimulates melanogenesis.

MATERIALS AND METHODS

Cells and Culture Conditions. Cloudman S91 mouse melanoma cells were purchased from the American Type Culture Collection and maintained in Dulbecco's modified Eagle's medium (DMEM)/10% calf serum (both from GIBCO/BRL). To prevent rapid cell growth during experiments, serum content was reduced to 2%. Normal human melanocytes were established from newborn foreskin and maintained as described (40). Melanocytes were used after the first or second passage.

Restriction with *Pvu* II. S91 cells were treated with streptolysin O (GIBCO/BRL) essentially as described by Lu and Lane (41) with some modifications. Briefly, 1×10^6 cells in 1 ml of DMEM were treated with 50 units of streptolysin O (GIBCO/BRL) for 30 min of 37°C; then 320 units of *Pvu* II (Pharmacia) was added for an additional 30 min at 37°C. As a negative control, a similar amount of *Pvu* II was inactivated by heating in boiling water for 15 min before adding to the cells. After the final incubation, an equal volume of DMEM/2% calf serum was added to each reaction mixture to inactivate the streptolysin O. The cells were then divided between two P60 dishes (Becton Dickinson) and cultured for 6 days.

Treatment with DNA-Damaging Chemical Agents and pTpT. Cells were plated in P60 dishes (S91) or P35 dishes (melanocytes) at a density of $1–2 \times 10^5$ cells per dish and cultured for several days before treatment. Medium was removed and fresh medium containing either 0.2 mM MMS (Aldrich) or 1 μM 4-NQO (Sigma) was added for 4 h and 1 h, respectively; the medium was then replaced with fresh medium and the cells were cultured for the indicated period of time before collecting for analysis. For treatment with pTpT, cells were provided with fresh medium (DMEM/2% calf serum) containing 100 μM pTpT (Midland Certified Reagent, Midland, TX) and were continually exposed to the dinucleotide for the duration of the experiment.

Effect of MSH on Pigmentation of MMS- or pTpT-Treated Cells. Plates of S91 cells were treated with MMS or pTpT as described above and cultured for 3 days. Duplicate plates of cells were then treated with 1×10^{-7} M α-MSH (Sigma) or diluent for an additional 3 days.

Determination of Melanin Content. Cells were analyzed for melanin content as described (28). Briefly, cells were collected by trypsinization and counted by Coulter Counter, and an equal number of cells from duplicate plates of each experimental condition were pelleted. In some experiments, the cell pellets were then photographed. Pellets were then dissolved in 1 ml of 1.0 M NaOH by vortexing for 20 min. The melanin content was calculated based on the absorbance at 475 nm compared to a standard curve of melanin (Sigma) in 1.0 M NaOH.

Northern Analysis. Total RNA was isolated from cells using Tri Reagent (Molecular Research Center, Cincinnati) following the protocol of the manufacturer. Ten micrograms of RNA from each sample was electrophoresed, transferred to filters, and probed as described (42) using a mouse tyrosinase cDNA (ATCC catalog no. 63164).

MSH Binding to Cell Surface Receptors. The binding of α-MSH to its surface receptor on intact S91 cells was performed essentially as described by Orlow *et al.* (43), with minor changes. Cells were resuspended in binding buffer at a concentration of 1×10^6 per ml and incubated at 10°C for 2 h with 1×10^{-9} M ^{125}I-labeled α-MSH (^{125}I-α-MSH), specific activity >2000 Ci/mmol [Peninsula Laboratories (1 Ci = 37 GBq)], in the presence or absence of 5×10^{-6} M unlabeled α-MSH (Sigma). After incubation, the cells were pelleted through 500 μl of 0.3 M sucrose in binding buffer to separate the unbound from bound α-MSH. Counts detected in an excess of unlabeled α-MSH (nonspecific) were subtracted from the samples incubated with ^{125}I-α-MSH alone.

UV Irradiation. S91 cells were exposed to a single dose of 10 mJ of UV irradiation per cm^2 from a 1-kW xenon arc solar simulator (XMN 1000-21; Optical Radiation, Azuza, CA), metered at 285 ± 5 nm, using a research radiometer (model IL1700 A; International Light, Newburyport, MA), as described (26).

RESULTS

DNA Damage and Pigmentation. UV light is known to stimulate melanogenesis *in vivo* (44) and in cultured melanocytes and melanoma cells (45). However, the intracellular signal(s) generated by UV exposure that initiates this response is unknown. Recent work has implicated DNA damage and/or its repair in this melanogenic response, but UV light is known to affect cytoplasmic signaling pathways as well (13, 14). To examine the effect of DNA damage alone on pigmentation, S91 cells and human melanocytes were treated with various DNA-damaging agents and the effect of melanin content was determined. First, S91 cells were porated with streptolysin O in order to introduce the restriction enzyme *Pvu* II. Heat-inactivated *Pvu* II and treatment with only streptolysin O were used as controls. Cells treated with active *Pvu* II showed a 5-fold increase in melanin per cell compared to cells treated with only streptolysin O (Fig. 1*A*). Heat-inactivated *Pvu* II produced only a slight increase in melanin content, probably reflecting a low residual activity of the heated enzyme.

In a second approach, S91 cells and melanocytes were treated with the chemical agents MMS and 4-NQO, known to alkylate bases in DNA and form adducts that are repaired by the same excision repair pathways as UV-induced photoproducts (46, 47). These agents are commonly used as "UV mimetics" and induce many genes that are responsive to DNA damage (3, 5, 6). Treatment of S91 cells with MMS produced an up to 10-fold increase in melanin per cell within 6 days (Fig. 1*B*); 4-NQO produced a 3- to 4-fold increase (Fig. 1*C*). Human melanocytes also responded to MMS and 4-NQO treatment, increasing melanin content by 40% and 70%, respectively (Fig. 1*D*). These data demonstrate induction of melanogenesis by

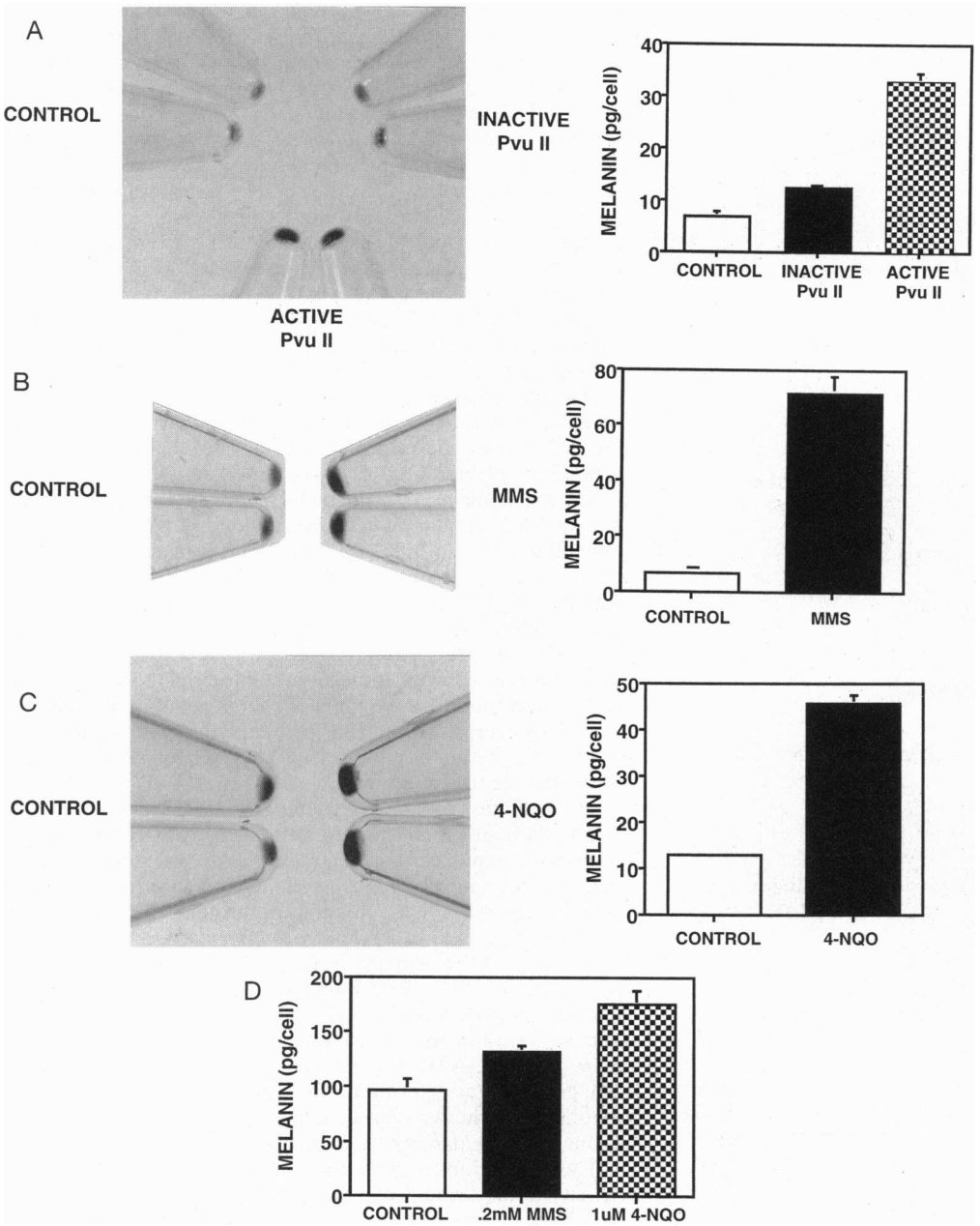

FIG. 1. Effect of DNA-damaging agents on pigmentation in S91 cells and normal human melanocytes. Duplicate cultures of S91 cells were treated with the restriction enzyme *Pvu* II (*A*) or the DNA-damaging chemical agents MMS (*B*) and 4-NQO (*C*) as described. After treatment, cells were cultured for 6 days, collected, and counted and an equal number of cells was pelleted for analysis. Melanin per cell was determined for each pellet. Averaged values of the duplicate cultures of each treatment are represented in the graphs. Normal human melanocytes were similarly treated with MMS and 4-NQO, cultured, and analyzed (*D*). Because of the angle of centrifugation, only the thickest part of lightly pigmented cell pellets is visible, often making these pellets appear smaller. Because of the high melanin content of the melanocytes under basal conditions, the increases produced by MMS and 4-NQO were difficult to appreciate by photographic inspection of the pellets.

agents that selectively damage DNA, suggesting that UV-induced DNA damage is a signal for melanogenesis.

Effect of UV Irradiation and DNA Damage on Tyrosinase mRNA. To better understand the mechanisms by which UV light, DNA-damaging agents, and pTpT increase pigmentation, S91 cells were either UV irradiated or treated with MMS or pTpT and then collected at various times for Northern analysis. The level of tyrosinase mRNA was markedly upregulated in UV-irradiated cells, compared to sham irradiated cells, as early as 24 h after irradiation, and this increase persisted for at least 3 days (Fig. 2*A*). Similarly, MMS treatment increased the tyrosinase message level, compared to that in diluent treated cells, 24–72 h after addition (Fig. 2*B*). The dinucleotide pTpT also increased tyrosinase mRNA on days 3 and 4 (Fig. 2*C*), somewhat later than UV irradiation and MMS. Thus, the UV effect on tyrosinase message appears to be a response to DNA damage, and the dinucleotide pTpT appears to generate the same response.

DNA Damage Potentiates the Melanogenic Response to MSH. The peptide hormone MSH has long been known to induce melanization *in vivo* and *in vitro.* Furthermore, in S91 cells it has been shown that UV light enhances the response to MSH by increasing the number of surface MSH receptors (38, 39), and it has been postulated that this receptor increase mediates the tanning response to UV irradiation (38, 39). We therefore asked whether MMS and pTpT also increase the response of S91 cells to MSH, as reported for UV light. Cells were treated as described in the *Materials and Methods* for 3 days. α-MSH was then added for an additional 3 days before the cells were collected and analyzed for melanin content. The 3-day incubation with α-MSH is shorter than the time required for maximal MSH response in these cells (34, 48) and was selected to emphasize differences that might be masked at maximal response. In the representative experiment shown in Fig. 3*A*, the basal pigmentation level was low and the response to α-MSH alone after 3 days was minimal. However, treatment with MMS did produce a 10-fold increase in melanin content and a synergistic effect was seen with MMS and α-MSH. Therefore, like UV irradiation, pretreatment of S91 cells with MMS enhances the melanogenic response to α-MSH. Treatment with pTpT had the same effect (Fig. 3*B*). These cells had a higher basal pigmentation level and showed a modest

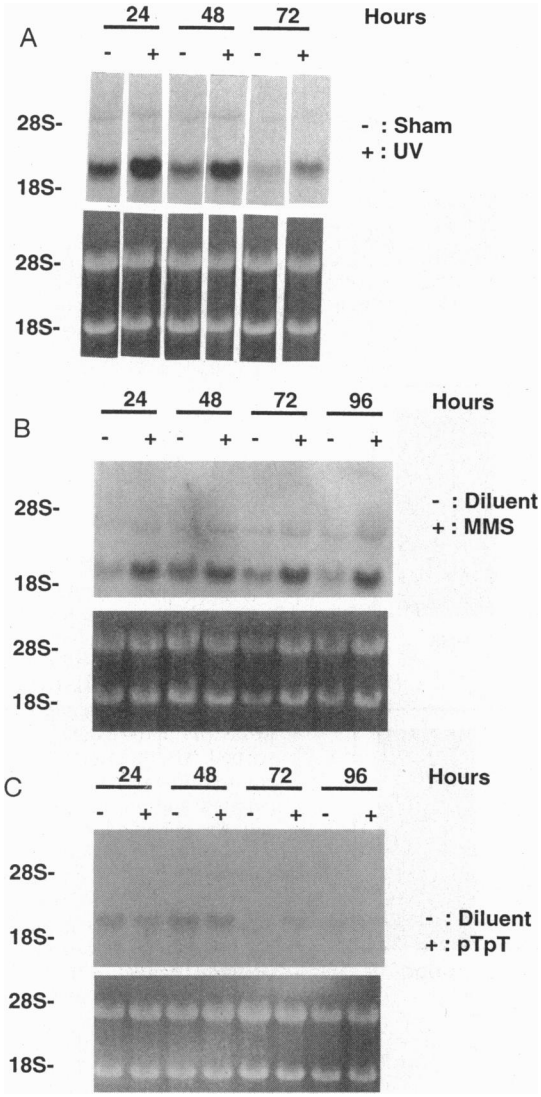

FIG. 2. Regulation of tyrosinase mRNA levels by UV irradiation, MMS, and pTpT. S91 cells were cultured and exposed to UVB irradiation (*A*) or treated with MMS (*B*) or pTpT (*C*) as described. At the indicated times, cells were collected and total RNA was isolated and analyzed for tyrosinase mRNA content by Northern blot analysis. Ethidium bromide-stained gels are included to indicate even loading of the RNA samples.

response to both α-MSH and pTpT within 3 days. However, the response to the two agents combined was more than additive.

We next asked whether MMS and pTpT increased the binding of MSH to its S91 cell surface receptor, as reported after UV irradiation (38, 39). Cells were treated with either diluent, MMS, or pTpT and harvested after 3 days, and the specific binding of ^{125}I-α-MSH to the cell surface was determined as described in *Materials and Methods.* Both MMS and pTpT treatments approximately doubled α-MSH binding (Fig. 4). Therefore, like UV irradiation, MMS and pTpT enhance the MSH response in S91 cells by increasing the binding of this hormone to its cell surface receptor, again suggesting that this UV response is mediated through DNA damage and/or the repair of this damage. Furthermore, pTpT appears to mimic this effect of DNA damage.

DISCUSSION

This study demonstrates that DNA damage and/or the repair of this damage stimulates melanin synthesis in S91 mouse melanoma cells and in normal human melanocytes. Both enzymatic (*Pvu* II) and chemical (MMS and 4-NQO) damage are effective. Although the repair of *Pvu* II-generated DNA cuts in mammalian cells has not been studied, much more is known about similar lesions induced by ionizing radiation, such as x-rays. These single-strand breaks are rapidly repaired by a "short patch" system in which only a few nucleotides are removed and the gap is closed by DNA polymerase and ligase (46, 49). The repair of MMS-induced DNA lesions proceeds via this "short patch" pathway, while the bulky adducts formed by 4-NQO apparently are repaired by the "long patch" system also commonly used for UV-induced photoproducts in which larger segments of DNA are removed and replaced (46). In our studies, both "short patch" and "long patch" types of DNA damage induced pigmentation. These data agree with studies which show that ionizing radiation and the ionizing radiation mimetic drug bleomycin increase pigment production in skin (50, 51) and in cultured melanocytes (52), as does the more extensively documented UV irradiation.

The mechanism(s) by which DNA damage is recognized and subsequently modulates gene expression is unknown. A DNA-dependent protein kinase (DNA-PK) has been characterized from a variety of cells (53–55) and at least in HeLa cells the activity of this kinase is dependent on DNA ends (56), suggesting it may recognize damaged DNA. Activated DNA-PK has been shown to phosphorylate the transcription factor Sp 1 (57) as well as the large subunit of DNA polymerase II (58) and therefore may play a role in regulating transcription. However, whether this protein kinase is activated by pTpT or whether it is involved in the regulation of the tyrosinase gene is unknown.

The induction of the yeast *PHR1* gene by UV irradiation and DNA damage is better understood. A DNA damage-responsive sequence has been identified in the 5′ region of the *PHR1* gene which binds to a damage-responsive repressor protein, the photolyase regulatory protein, PRP (15). UV irradiation was shown to rapidly remove active PRP from cell extracts. Footprint analysis revealed a 39-base-pair sequence protected by PRP (15). Interestingly, a portion of this sequence, AGGGGTGAAAG, shares homology with a sequence found in the mouse (AGAAGATAAAAG) and human (AGAGGATGAAAG) tyrosinase gene 5′ regions (59, 60). Studies to determine the role of these sequences in regulation of the tyrosinase gene by UV irradiation and other forms of DNA damage, as reported here, are necessary.

The regulation of tyrosinase at the mRNA, protein, and activity levels appears extremely complicated. Aberdam *et al.* (61) have examined the effect of repeated UV irradiation on tyrosinase in human melanocytes and S91 cells, but data from experiments using a single UV exposure sufficient to cause a tanning response are lacking. Multiple UV exposures resulted in transient up-regulation, noted 3 h after the sixth daily irradiation, followed by down-regulation 24 h later, of tyrosinase mRNA, protein, and activity after repeated irradiations in human melanocytes. The response in S91 cells was somewhat different, with elevated levels of tyrosinase message, protein, and activity 3 and 24 h after the last of six daily irradiations. These data agree with our finding of tyrosinase mRNA elevation 24 h after one exposure. However, we could detect no increase at earlier times, possibly reflecting the different experimental designs (single versus multiple exposures), culture conditions, or different UV doses or spectral output of the light sources used. The finding that there is at least a 24-h delay in the induction of tyrosinase mRNA by UV irradiation, MMS, and pTpT is consistent with the delayed onset of the clinical pigmentation response to UV light (62) and pTpT (28) and suggests that this response to DNA damage may require the induction of other genes first. Regulation of tyrosinase protein and activity levels was not addressed in the present study. Tyrosinase is known to be activated by protein

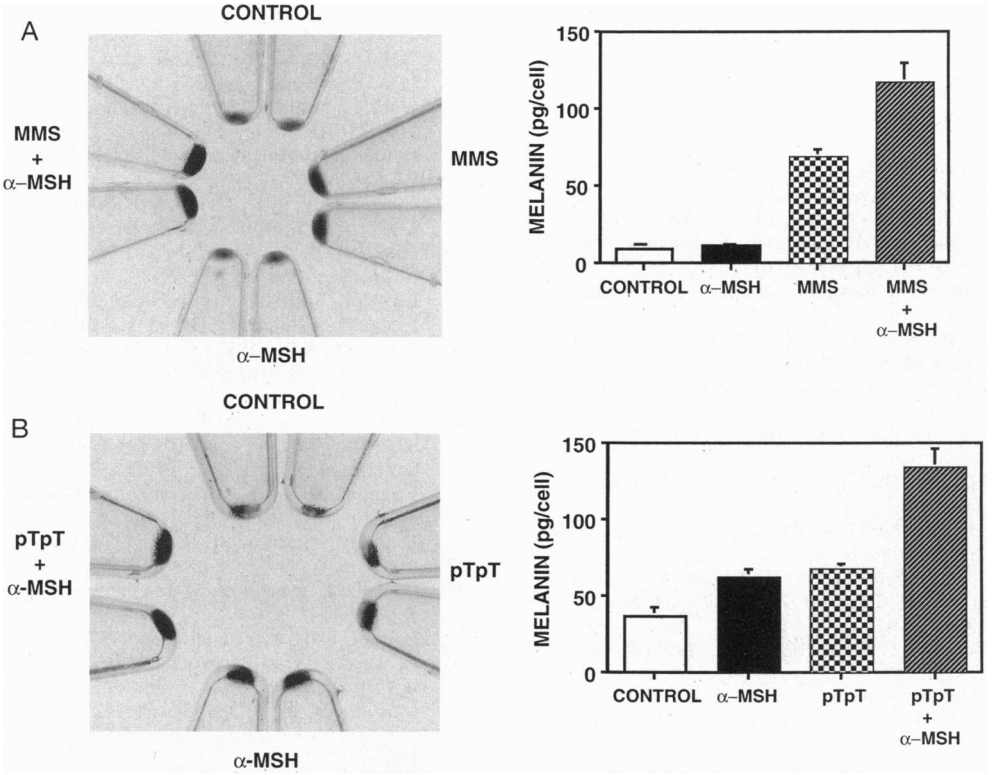

FIG. 3. Effect of MMS and pTpT in the MSH response in S91 cells. Duplicate cultures of cells were treated for 3 days with MMS (*A*) or pTpT (*B*) prior to addition of 1×10^{-7} M αMSH. After 3 additional days of culture in the presence of α-MSH, the cells were collected, counted, pelleted, photographed, and analyzed as described in the legend to Fig. 1.

kinase C (PKC) (63) but the effects of UV irradiation and other forms of DNA damage on the PKC signal transduction pathway are largely unknown. Interestingly, diacylglycerols, released from membrane lipids after UV irradiation, stimulate melanogenesis in S91 cells and in human melanocytes (64, 65), apparently through activation of PKC. Therefore, UV irradiation may have multiple actions, increasing the level of tyrosinase protein as a consequence of the increased mRNA level reported here and concurrently activating this protein by generating the effector molecule for its PKC-mediated phosphorylation.

Our data also suggest that at least part of the melanogenic response to DNA damage is mediated through the MSH/MSH receptor system. UV irradiation is known to up-regulate the number of MSH receptors on the surface of S91 cells and to enhance the MSH response *in vitro* and *in vivo* (38, 39), and we

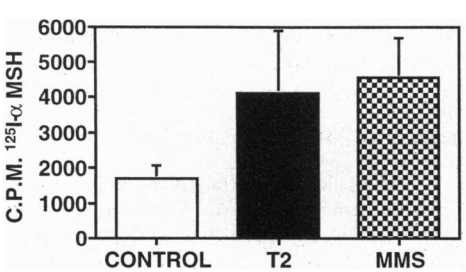

FIG. 4. Up-regulation of MSH binding to S91 cells by MMS and pTpT. Duplicate cultures of S91 cells were treated with either diluent (DMEM), MMS, or pTpT for 3 days. Cells were then collected and 1×10^5 cells were incubated with 1×10^{-9} M ^{125}I-α-MSH alone or in the presence of 5×10^{-6} M unlabeled α-MSH and processed as described. Specific binding was determined by subtracting ^{125}I counts detected in the presence of competing unlabeled MSH from those bound without competition. Data represent averages ± standard deviation of the duplicate cultures.

demonstrate that MMS and pTpT have the same effect. MSH has also been shown to increase the level of tyrosinase mRNA in S91 cells (33, 66), an effect we detect after UV irradiation or treatment with MMS or pTpT. Although no MSH was intentionally added to our S91 cultures, this hormone can be presumed to be present in the calf serum included in the medium (67–69). Furthermore, we find that MMS and pTpT treatments increase the binding of ^{125}I-α-MSH to the cell surface MSH receptor, as has been reported after UV irradiation (38, 70). In the case of UV irradiation, a redistribution of MSH binding sites from an internal to external cellular localization was proposed (43). Although a change in receptor affinity for MSH could not be excluded, up-regulation of MSH binding on S91 cells by other agents, such as retinoic acid, appears to involve a change in the number of binding sites with no change in affinity for ligand (70). Of interest, Varga *et al.* (71) have reported that S91 cells respond to MSH only in the G_2 phase of the cell cycle, possibly through an up-regulation of functional MSH receptors in G_2. UV light and DNA damage are known to stall the cell cycle at G_1 and G_2 checkpoints (72) and this extended period of time in G_2 may, at least in part, explain the enhanced response of UV-irradiated cells to MSH (38, 39). Whether pTpT mimics UV irradiation by arresting cells in G_1 or G_2 requires investigation. It must be noted, however, the proportion of the melanogenic response to DNA damage and pTpT mediated through the MSH/MSH receptor pathway is unknown.

In conclusion, our data suggest that DNA damage and/or the repair of this damage is an important signal in UV-induced melanogenesis. In addition to UV irradiation, that acts on multiple targets including DNA, three agents presumed to act exclusively on DNA increased pigmentation in S91 cells in the same manner. Furthermore, the dinucleotide pTpT, selected as the DNA fragment common to most excised DNA photoproducts, mimics these effects of DNA damage on pigmentation. Determination of the optimal oligonucleotide sequen-

ce(s) for inducing these effects, as well as a better understanding of the cellular systems and proteins recognizing these DNA fragments, should allow modulation of melanogenesis and other UV-induced cellular responses without the requirement for prior DNA damage.

1. Peterson, K. R., Ossanna, N., Thliveris, A. T., Ennis, D. G. & Mount, D. W. (1988) *J. Bacteriol* **170,** 1–4.
2. Ogawa, H. & Ogawa, T. (1990) *Adv. Biophys.* **26,** 33–49.
3. Kartasova, T., Cornelissen, B. J. C., Belt, P. & van de Pute, P. (1987) *Nucleic Acids Res.* **15,** 5945–5961.
4. Ronai, Z. A., Lambert, M. E. & Weinstein, I. B. (1990) *Cell Biol. Toxicol.* **6,** 105–126.
5. Fornace, A. J., Jr., Alamo, I., Jr., & Hollander, M. C. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 8800–8804.
6. Sebastian, J., Kraus, B. & Sancar, G. B. (1990) *Mol. Cell. Biol.* **10,** 4630–4637.
7. Rosen, C. F., Gajic, D., Jia, Q. & Drucker, D. J. (1992) *Am. J. Physiol.* **263,** C1103–C1110.
8. Garmyn, M., Yaar, M., Holbrook, N. & Gilchrest, B. A. (1993) *Lab. Invest.* **65,** 471–478.
9. Applegate, L. A., Lauscher, P. & Tyrrell, R. M. (1991) *Cancer Res.* **51,** 974–978.
10. Fornace, A. J., Jr., Nebert, D. W., Hollander, M. C., Luethy, J. D., Papathanasiou, M., Fargnoli, J. & Holbrook, N. J. (1989) *Mol. Cell. Biol.* **9,** 4196–4203.
11. Peak, J. G., Woloschak, G. E. & Peak, M. J. (1991) *Photochem. Photobiol.* **53,** 395–397.
12. Matsui, M. S. & DeLeo, V. A. (1990) *Carcinogenesis* **11,** 229–234.
13. Devary, Y., Gottlieb, R. A., Smeal, T. & Karin, M. (1992) *Cell* **71,** 1081–1091.
14. Devary, Y., Rosette, C., DiDonato, J. A. & Karin, M. (1993) *Science* **261,** 1442–1445.
15. Sebastian, J. & Sancar, G. B. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 11251–11255.
16. Yarosh, D. B., Alas, L., Kibitel, J., O'Connor, A., Carrier, F. & Fornace, A. J., Jr. (1993) *J. Invest. Dermatol.* **100,** 790–794.
17. Kastan, M. B., Zhan, Q., El-Deiry, W. S., Carrier, F., Jacks, T., Walsh, W. V., Plunkett, B. S., Vogelstein, B. & Fornace, A. J., Jr. (1992) *Cell* **71,** 587–597.
18. Smith, M. L., Chen, I.-T., Zhan, Q., Bae, I., Chen, C.-Y., Gilmer, T. M., Kastan, M. B., O'Connor, P. M. & Fornace, A. J., Jr. (1994) *Science* **266,** 1376–1379.
19. Protic, M., Roilides, E., Levine, A. S. & Dixon, K. (1988) *Somatic Cell Mol. Genet.* **14,** 351–357.
20. Okoro, A. N. (1975) *Br. J. Dermatol.* **92,** 485–492.
21. Kobayashi, N., Muramatsu, T., Yamashina, Y., Shirai, T., Ohnishi, T. & Mori, T. (1993) *J. Invest. Dermatol.* **101,** 685–689.
22. Hill, H. Z. & Setlow, R. B. (1982) *Photochem. Photobiol.* **35,** 681–684.
23. Brash, D. E., Rudolph, J. A., Simon, J. A., Lin, A., McKenna, G. J., Baden, H. P., Halperin, A. J. & Pontén, J. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 10124–10128.
24. Parrish, J. A., Jaenicke, K. F. & Anderson, R. R. (1982) *Photochem. Photobiol.* **36,** 187–191.
25. Freeman, S. E., Hacham, H., Gange, R. W., Maytum, D. J. & Sutherland, B. M. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 1119–1126.
26. Gilchrest, B. A., Zhai, S., Eller, M. S., Yarosh, D. B. & Yaar, M. (1993) *J. Invest. Dermatol.* **101,** 666–672.
27. Setlow, R. B. & Carrier, W. L. (1966) *J. Mol. Biol.* **17,** 237–254.
28. Eller, M. S., Yaar, M. & Gilchrest, B. A. (1994) *Nature (London)* **372,** 413–414.
29. Hadley, M. E. & Levine, N. (1993) in *Pigmentation and Pigmentary Disorders*, ed. Levine, N. (CRC, Boca Raton, FL), pp. 95–114.
30. Smith, A. I. & Funder, J. W. (1988) *Endocr. Rev.* **9,** 159–178.
31. Schauer, E. F., Trautinger, F., Köck, A., Schwarz, A., Bhardwaj, R., Simm, M., Ansel, J. C., Schwarz, T. & Luger, T. A. (1994) *J. Clin. Invest.* **93,** 2258–2262.
32. Wintzen, M. & Gilchrest, B. A., *J. Invest. Dermatol.*, in press.
33. Hoganson, G. E., Ledwitz-Rigby, F., Davidson, R. L. & Fuller, B. B. (1989) *Somatic Cell Mol. Genet.* **15,** 255–263.
34. Fuller, B. B., Lunsford, J. B. & Iman, D. S. (1987) *J. Biol. Chem.* **262,** 4024–4033.
35. Donatien, P. D., Hunt, G., Pieron, C., Lunec, J., Taïeb, A. & Thody, A. J. (1992) *Arch. Dermatol. Res.* **284,** 424–426.
36. DeLuca, M., Siegrist, W., Bondanza, S., Mathor, M., Cancedda, R. & Eberle, A. N. (1993) *J. Cell Sci.* **105,** 1079–1084.
37. Hunt, G., Todd, C., Kyne, S. & Thody, A. J. (1994) *J. Endocrinol.* **140,** R1–R3.
38. Bolognia, J., Murray, M. & Pawelek, J. (1989) *J. Invest. Dermatol.* **92,** 651–656.
39. Chakraborty, A. K., Orlow, S. J., Bolognia, J. L. & Pawelek, J. M. (1991) *J. Cell. Physiol.* **147,** 1–6.
40. Gilchrest, B. A., Vrabel, M. A., Flynn, E. & Szabo, G. (1989) *J. Invest. Dermatol.* **83,** 700–702.
41. Lu, X. & Lane, D. P. (1993) *Cell* **75,** 765–778.
42. Eller, M. S., Oleksiak, M. F., McQuaid, T. J., McAfee, S. G. & Gilchrest, B. A. (1992) *Exp. Cell. Res.* **199,** 328–336.
43. Orlow, S. J., Hotchkiss, S. & Pawelek, J. M. (1990) *J. Cell. Physiol.* **142,** 129–136.
44. Jimbow, K., Kitzpatrick, T. B. & Quevedo, W. C., Jr. (1985) in *Structure and Function of Melanin*, ed. Jimbow, K. (Fuji-Shoin, Sapporo, Japan), Vol. 2, pp. 71–82.
45. Friedman, P. S. & Gilchrest, B. A. (1987) *J. Cell. Physiol.* **133,** 88–94.
46. Regan, J. D. & Setlow, R. B. (1974) *Cancer Res.* **34,** 3118–3325.
47. Fujiwara, Y. (1975) *Cancer Res.* **35,** 2780–2789.
48. Gordon, L., Peacocke, M. & Gilchrest, B. A. (1992) *J. Dermatol. Sci.* **3,** 35–41.
49. Painter, R. B. & Young, B. R. (1972) *Mutat. Res.* **14,** 225–235.
50. Becker, S. W., Fitzpatrick, T. B. & Montgomery, H. (1952) *A.M.A. Arch. Dermatol. Syphiol.* **65,** 511–523.
51. Mosher, D. B., Fitzpatrick, T. B., Hori, Y. & Ortonne, J.-P. (1993) in *Dermatology in General Medicine*, eds. Fitzpatrick, T. B., Eisen, A. Z., Wolff, K., Freedberg, I. M. & Austen, K. F. (McGraw-Hill, New York), pp. 976–977.
52. Rubeiz, N. G., Park, H.-Y., Rogers, G. S. & Gilchrest, B. A. (1993) *J. Invest. Dermatol.* **100,** 590.
53. Walker, A. I., Hunt, T., Jackson, R. J. & Anderson, C. W. (1985) *EMBO J.* **4,** 139–145.
54. Lees-Miller, S. P., Chen, Y.-R. & Anderson, C. W. (1990) *Mol. Cell. Biol.* **10,** 6472–6481.
55. Carter, T., Vancurová, I., Sun, I., Lou, W. & DeLeon, S. (1990) *Mol. Cell. Biol.* **10,** 6460–6471.
56. Gottlieb, T. M. & Jackson, S. P. (1993) *Cell* **72,** 131–142.
57. Jackson, S. P., MacDonald, J. J., Lees-Miller, S. & Tjian, R. (1990) *Cell* **63,** 155–165.
58. Peterson, S. R., Dvir, A., Anderson, C. W. & Dynan, W. S. (1992) *Genes Dev.* **6,** 426–438.
59. Kwon, B. S., Haq, A. K., Wakulchik, M., Kestler, D., Barton, D. E., Francke, U., Lamoreux, M. L., Whitney, J. B., III, & Halaban, R. (1989) *J. Invest. Dermatol.* **93,** 589–594.
60. Ponnazhagan, S., Hou, L. & Kwon, B. S. (1994) *J. Invest. Dermatol.* **102,** 744–748.
61. Aberdam, E., Roméro, C. & Ortonne, J.-P. (1993) *J. Cell Sci.* **106,** 1015–1022.
62. Norris, P. G., Gange, R. W. & Hawk, J. L. M. (1993) in *Dermatology in General Medicine*, eds. Fitzpatrick, T. B., Eisen, A. Z., Wolff, K., Freedberg, I. M. & Austen, K. F. (McGraw-Hill, New York), pp. 1651–1658.
63. Park, H.-Y., Russakovsky, V., Ohno, S. & Gilchrest, B. A. (1993) *J. Biol. Chem.* **268,** 11742–11749.
64. Gordon, P. R. & Gilchrest, B. A. (1989) *J. Invest. Dermatol.* **93,** 700–702.
65. Friedman, P. S., Wren, F. E. & Matthews, J. N. S. (1990) *J. Cell. Physiol.* **142,** 334–341.
66. Kwon, B. S., Wakulchik, M., Haq, A. K., Halaban, R. & Kestler, D. (1988) *Biochem. Biophys. Res. Commun.* **153,** 1301–1309.
67. Altmeyer, P., Stöhr, L. & Holzmann, (1986) *J. Invest. Dermatol.* **86,** 454–456.
68. Lerner, A. & McGuire, J. S. (1964) *N. Engl. J. Med.* **270,** 539–546.
69. Schally, A. V., Akira, A. & Kastin, A. J. (1973) *Science* **179,** 341–350.
70. Chakraborty, A. K., Orlow, S. J. & Pawelek, J. M. (1990) *FEBS Lett.* **276,** 205–208.
71. Varga, J. M., Dipasquale, A., Pawelek, J., McGuire, J. S. & Lerner, A. B. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 1590–1593.
72. Murray, A. W. (1992) *Nature (London)* **359,** 599–604.