Thymidine Dinucleotides Induce S Phase Cell Cycle Arrest in Addition to Increased Melanogenesis in Human Melanocytes

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Although the induction of pigmentation following exposure of melanocytes to ultraviolet light *in vivo* and *in vitro* is well documented, the intracellular mechanisms involved in this response are not yet fully understood. Exposure to UV-B radiation leads to the production of DNA damage, mainly cyclobutane pyrimidine dimers, and it was recently suggested that the thymidine dinucleotide pTpT, mimicking small DNA fragments released in the course of excision repair mechanisms, could trigger melanin synthesis. We now report that the thymidine dinucleotide pTpT induces melanogenesis both in human normal adult melanocytes and in human melanoma cells. Thus, the SOS-like response suggested by Gilchrest's work to be evolutionary conserved, based primarily on work in murine cells and guinea pigs, is also apparently present in the human. Thymidine dinucleotide is non-toxic to melanoma cells and does not induce apoptosis in these cells, but induces S phase cell cycle arrest and a proliferation slow down. Because thymidine excess in culture medium leads to the synchronization of cells in S phase, we investigated whether this phenomenon was involved in the increase in melanin synthesis. We show that melanin synthesis is specifically triggered by the dimeric form of the thymidine and not by the monomeric form pT. Thus, our data strongly support that thymidine dinucleotides pTpT mimic at least part of the effects of ultraviolet irradiation, and may hence represent an invaluable model in the study of the molecular events involved in melanogenesis induction triggered through DNA damage. ***Key words: human adult melanocytes/human melanoma/proliferation/pyrimidine dimers. J Invest Dermatol 111:472–477, 1998***

Ultraviolet exposure of human skin triggers melanin pigmentation and skin darkening, a response familiarly known as tanning. Ultraviolet wavelengths and histologic mechanisms involved in melanogenesis and tanning are well documented (Gilchrest *et al*, 1996). Cellular and molecular mechanisms of melanogenesis, resulting in the activation of tyrosinase (the principal rate-limiting enzyme of melanin synthesis) have been actively investigated (Ferguson and Kidson, 1997). Ultraviolet irradiation can directly affect epidermal melanocytes, but ultraviolet-induced melanogenesis also involves other cell types among which keratinocytes appear to play a major role by secreting paracrine factors such as bFGF and ET-1 (Halaban *et al*, 1987; Imokawa *et al*, 1992). Both direct effects of UV photons on melanocytes and indirect effects of photons interacting with keratinocytes, fibroblasts, and conceivably other cells in the skin contribute to the final tanning response.

Little is known, however, of the molecular mechanisms leading to tyrosinase activation. Data support a role for photochemical reactions involving membrane lipids in modulating melanogenesis. Some studies have implicated the diacylglycerol/protein kinase C pathway (DAG/PKC) (Gordon and Gilchrest, 1989; Friedman *et al*, 1990; Punnonen and Yuspa, 1992) in melanin synthesis: tyrosinase activity is regulated at least in part by PKC-β mediated phosphorylation of its cytoplasmic domain (Park *et al*, 1993a, b). Release of DAG from cell membranes immediately after ultraviolet irradiation is likely to be a mechanism for enhanced melanogenesis following UV exposure (Nishizuka, 1992; Punnonen and Yuspa, 1992).

More recently, UV-induced DNA damage and/or repair have been linked to melanogenesis. The action spectrum for production of DNA photoproducts is essentially identical to that for ultraviolet-induced pigmentation (Parrish *et al*, 1982; Freeman *et al*, 1989). The great majority of UV-B induced DNA photoproducts involve adjacent pyrimidine moieties, ≈80% specifically involve thymidine dinucleotides (Setlow and Carrier, 1966). In the DNA of ultraviolet-irradiated cells, these photoproducts are recognized and repaired by nucleotide excision repair (Tornaletti and Pfeifer, 1996). The repair process leads to the release of short DNA fragments containing the cyclobutane pyrimidine dimer or other dipyrimidine photoproducts (Huang *et al*, 1992; Tateishi *et al*, 1993). A prokaryotic endonuclease, T4 endonuclease V, which enhances rate-limiting incision of thymidine dimers, has been shown to increase melanogenesis in ultraviolet-irradiated melanocytes, suggesting that DNA repair directly stimulates the tanning response (Gilchrest *et al*, 1993). Indeed, treatment of mouse melanoma cells or guinea pig skin with small DNA fragments or pyrimidine dinucleotide pTpT results in increased melanogenesis (Eller *et al*, 1994, 1996). These results strengthen the idea of an evolutionary conserved response to UV irradiation. Indeed, it has long been known that the prokaryote response to UV, the so-called SOS response, induces a set of more than 20 genes involved in DNA repair and cell survival (Walker, 1984). These genes are triggered by a protein (RecA) activated by interaction of the single stranded DNA generated following UV irradiation (Phizicky and Robert, 1981). Because melanin is photoprotective in

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human skin against UV radiation, enhanced melanogenesis might be part of the SOS response in skin.

We now report that the thymidine dinucleotide pTpT induces melanin synthesis in normal human adult melanocytes and human melanoma cells. pTpT induces an S phase cell cycle arrest and a proliferation slow down, but melanin synthesis appears to be independent of effects of pTpT on the cell cycle and proliferation rate.

MATERIAL AND METHODS

Cell culture and treatment Dermatomed skin (0.5 mm thick) was floated on a trypsin solution (0.05%) at 4°C overnight. After separation of the epidermis, epidermal cells were plated at high density in MCDB153 medium (KBM, Clonetics, Rockville, MD) supplemented with 0.5 μg hydrocortisone per ml, 5 μg insulin per ml, and 56 μg bovine pituitary extract per ml. After 24 h, the cells were transferred as previously described (Gilchrest *et al*, 1984) in M199 medium (Gibco BRL, Walkersville, MD), supplemented with 0.18 mM calcium, hydrocortisone (0.5 μg per ml), insulin (10 μg per ml), epidermal growth factor (10 ng per ml), basic fibroblast growth factor (10 ng per ml) (Halaban *et al*, 1987), and phorbol 12,13 dibutyrate (0.5 mM) without fetal calf serum. The following week, the cells were transferred in calcium depleted M199 medium with 5% calcium free fetal calf serum. One week before and during the experiment the phorbol 12,13 dibutyrate was removed from the medium.

A375P human melanoma cells were purchased from the American Type Culture Collection. M4Be human melanoma cells were previously established in the laboratory (Jacubovich *et al*, 1985). Melanoma cells were grown in Dulbecco's modified Eagle's medium or McCoy 5a supplemented with 10% fetal calf serum, 200 units penicillin per ml, and 20 μg streptomycin per ml at 37°C in an humidified 5%CO_2 incubator. 70% confluent cell cultures were used for treatment with nucleotides. Briefly, cells were provided with fresh medium containing 30–50 or 100 μM pTpT (Sigma, St Louis, MO) or 100–500 μM pT (Sigma) or with fresh control medium, and were continually exposed to the nucleotide for the duration of the experiment.

Melanin assay Cells were trypsinized, washed twice with phosphate-buffered saline solution, and solubilized in 1 ml 1N KOH with vigorous vortexing for 10 min after heating for 30 min at 60°C. Melanin concentration was determined by measurement of OD_{405} and comparison with a standard curve obtained using a synthetic melanin (Sigma).

^{14}C-DOPA uptake Cells were incubated with the melanin precursor L-3,4 dihydroxyphenyl [1–^{14}C]alanine (^{14}C-DOPA) for 72 h. Cells were rinsed twice with phosphate-buffered saline, trypsinized, centrifuged, and extracted twice with 2 ml 5% trichloroacetic acid in 95% ethanol at 4°C. The incorporated radioactivity was determined using a scintillation counter.

Cell cycle analysis Cells were trypsinized, washed twice with phosphate-buffered saline, and fixed in 70% ethanol for 30 min at 4°C. The cells were treated with 1 U DNase-free RNase (Sigma) in 1 ml phosphate-buffered saline for 30 min at 37°C. The DNA content was detected by propidium iodide (0.05 mg per ml). The stained cells were analyzed by Facscalibur (Becton Dickinson, San Jose, CA) in the CellQuest program. Fluorescence events due to debris were substracted before analysis.

Cellular proliferation assay Cellular proliferation was measured using the Alamar Blue dye assay (Ahmed *et al*, 1994). Alamar Blue, devoid of any effect on cell viability and cellular proliferation, contains a Redox indicator. Cellular proliferation induces chemical reduction and a change in color from blue to red. The intensity of red color, which can be accurately measured by a fluorimetric assay, reflects the extent of cellular proliferation. Briefly, cells were suspended to a final concentration of 2500 cells per ml and 1 ml cell suspension was dispensed in each well of a 24 well culture plate. To the cultured cells, 100 μl Alamar Blue (10% of incubation volume) was added according to the manufacturer's instructions (Alamar, Sacramento, CA). The cells were incubated for 4 h with the Alamar Blue without washing and the fluorescence was read on a plate reader (Cytofluor, Biosystem, Framingham, MA) without extraction. All assays were performed in triplicate, standard deviations were calculated and never exceeded 5%. Population doubling times were determined graphically from a semilogarithmic linear plot of cell growth.

RESULTS

Pyrimidine dinucleotides induce melanogenesis in human normal adult melanocytes and melanoma cells Pyrimidine dinucleotides were shown to enhance melanogenesis in murine melanoma cells and in human newborn foreskin melanocytes cultured *in vitro* as well as in guinea pig skin (Eller *et al*, 1994). To further examine the effect of pyrimidine dinucleotides on pigmentation of human melanocytes, primary cultures of normal human adult melanocytes and the human melanoma cell line M4Be were continuously exposed to 50 μM pTpT for 7 d. Measurement of the melanin content shows that this treatment resulted in a significant increase in pigmentation of both normal melanocytes and melanoma cells. Comparable results were obtained when pigment formation was measured through incorporation of a melanin precursor, ^{14}C-DOPA, thus showing that pigment formation in dinucleotide-treated human melanocytes results from *de novo* pigment synthesis (**Table I**).

Table I. Melanogenesis induced in human melanocytes by pTpT

	Melanin[a]		^{14}C-DOPA incorporation[b]	
	Control	pTpT[c]	Control	pTpT[c]
Normal adult human melanocytes	14.6 ± 2.6[d]	23.7 ± 4.3	16009 ± 206	30866 ± 4358
M4Be	10.7 ± 1.1	15.2 ± 2.4	945 ± 49	1324 ± 54

[a]μg melanin per 10^6 cells.
[b]cpm per 10^6 cells.
[c]50 μM.
[d]Figures represent means of three independent determinations.

Table II. Melanogenesis induced in M4Be human melanoma cells by pTpT

Liposomes[a]	Control	pTpT[b]
–	11.6 ± 1.0[c]	28.4 ± 1.8
+	47.0 ± 4.2	120.4 ± 3.9

[a]Liposomes were prepared by dispersing 1% (wt/vol) phospholipids with or without 0.21% pTpT in distilled water as described previously (Redziniak and Meybeck, 1986). Soybean phospholipids (Alcolec F100) were obtained from the American Lecithin Company (Bethesda, MD). Liposomes thus prepared have a mean size of 100 nm.
[b]100 μM.
[c]μg melanin per 10^6 cells. Figures represent means of three independent determinations.

Table III. Cell cycle distribution of M4Be melanoma cells following 24 h treatment with pTpT

	Control	pTpT
G1	64.7 ± 1.2 ‡	50.1 ± 0.3
S	26.3 ± 1.0	43.8 ± 0.3
G2	8.9 ± 0.3	6.1 ± 0.3

[a]50 μM.
[b]Percentage of cells.

In an attempt to increase penetration of dinucleotides through cell membranes, which may eventually limit their effect, human melanoma cells were exposed continuously for 7 d to 100 μM pTpT in liposomes incorporated in the culture medium. Surprisingly, control empty liposomes appeared to increase the melanin content of M4Be melanoma cells; however, the results of these experiments consistently show that exposure of human melanoma cells to pTpT results in a 2.5-fold increase in their melanin content, irrespective of the presentation of the dinucleotides, in solution or incorporated into liposomes (**Table II**).

Thus, at concentrations of 50–100 μM, pyrimidine dinucleotide pTpT significantly enhances melanogenesis, by a factor ranging from 1.5 to 2.5 according to experimental conditions and target cells, in normal adult melanocytes as well as in human melanoma cells.

Pyrimidine dinucleotides induce cell cycle arrest and proliferation delay in human melanocytes To further analyze the effect of pTpT on human melanocytes, we studied cell cycle and proliferation of these cells. Studies with human adult melanocytes were rendered uninterpretable by their low proliferation rate and long doubling time under basal conditions, so cell cycle studies were performed with human melanoma cells.

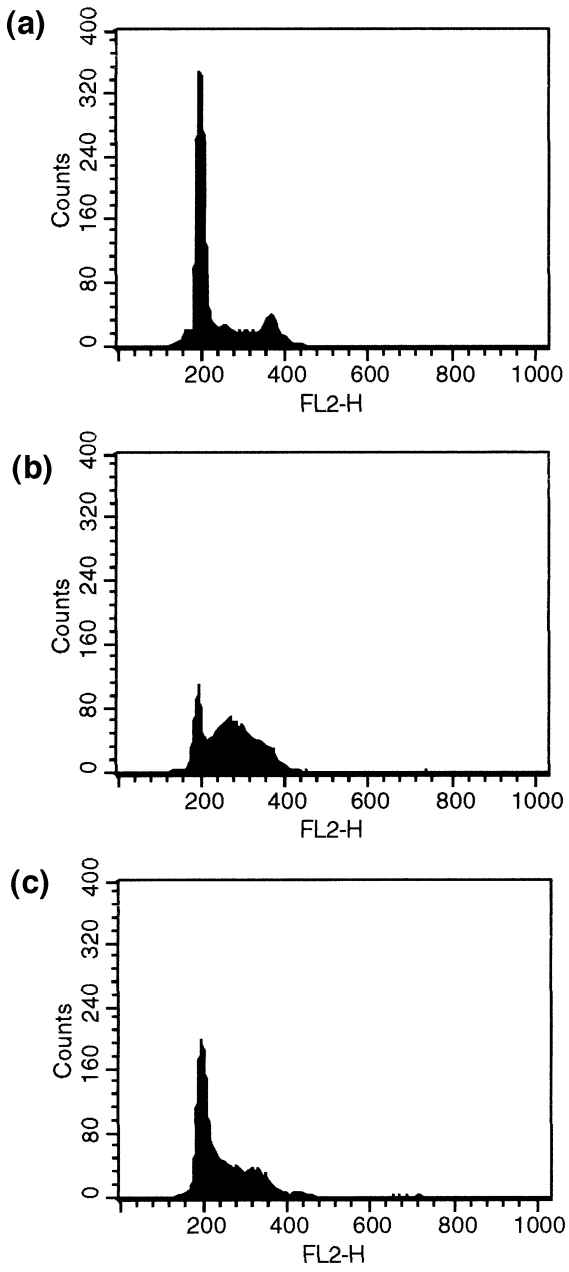

Figure 1. S phase arrest of A375P cells following treatment with pTpT. Cells continuously exposed to pTpT (50 μM) were stained for DNA content with 0.05 mg propidium iodide per ml at selected times. Ordonate, number of cells; FL-2H, fluorescence intensity of propidium iodide. (*a*) Control; (*b*) 24 h; (*c*) 48 h.

M4Be melanoma cells continuously exposed for 24 h to 50 μM pTpT showed an increase in the proportion of cells in S phase: 43.8% ± 0.3 as compared with 26.3% ± 1.0 in control untreated cells (**Table III**). The increase in the proportion of cells in S phase was still demonstrable after 72 h treatment (data not shown); however, because M4Be cells are slow growing with a doubling time exceeding 30 h, similar experiments were performed using the A375P human melanoma cell line. Under the same conditions, A375P melanoma cells continuously exposed to 50 μM pTpT showed a transient accumulation in the S phase after 24 h of treatment. Later, and though pyrimidine dinucleotides were still present in the culture medium, cells appeared to resume cycling at 48 h (**Fig 1**). Attempts to further characterize the cell population gathered in the S phase using bromodeoxyuridine labeling were unsuccessful, probably due to a competition between bromodeoxyuridine and thymidine dinucleotides in the culture medium.

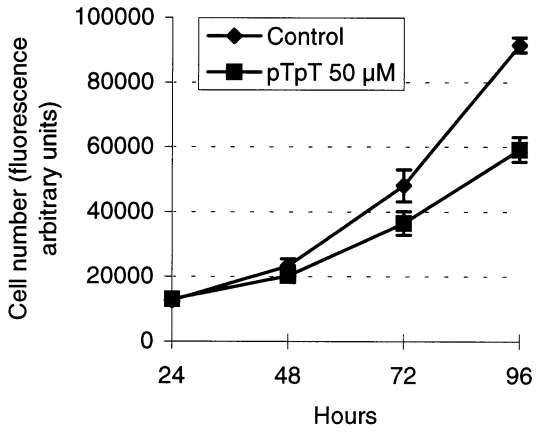

Figure 2. Growth inhibition of A375P cells by pTpT (50 μM). Cells were plated in 24 well plate at a density of 2500 cells per well. Alamar blue was added in medium 24, 48, 72, and 96 h after beginning of treatment and fluorescence was read 4 h later.

Cell proliferation analysis demonstrates that pTpT slows proliferation rate: the doubling time of pTpT-treated A375P cells being 32 h, compared with a doubling time of 25 h in control cells, corresponding to a growth inhibition of 35% in a 96 h experiment (**Fig 2**). Removal of the pyrimidine dinucleotides from the culture medium is accompanied by restoration of their proliferation capacity (data not shown). It should be noted that pTpT concentrations up to 100 μM have no demonstrable cytotoxic effect on human melanoma cells, as evidenced from the raw data scattergrams of pTpT-treated A375P cells shown in **Fig 3**. Similarly, consideration of **Fig 1** shows that there is no sub-G1 peak of apoptotic cells in pTpT-treated A375P cells. Hence, the slow down in proliferation rate is unlikely to result from a cytotoxic effect or from increased apoptosis.

Cell cycle arrest and proliferation slow down are not directly involved in thymidine dinucleotide-induced melanogenesis High concentrations of thymidine prevent cell division, and removal of excess thymidine yields a wave of cell synchrony. Double thymidine block at concentrations of 1–2 mM is widely used to synchronize cells in the S phase (Bootsma *et al*, 1964). Therefore, it can be questioned whether the S phase arrest observed following exposure to 50–100 μM pTpT of melanoma cells results from a potentialization of the thymidine block due to the dimeric form of thymidine, and whether the induction of melanogenesis results from the effects of thymidine dinucleotide on cell cycle and proliferation rate.

Experiments using thymidine monophosphate (pT) at different concentrations showed that at a concentration of 100 μM, corresponding to a thymidine amount comparable with that of 50 μM pTpT, no cell cycle effect was observed (**Fig 4*a***); however, increasing pT concentration up to 300 μM resulted in an S phase arrest similar to that observed after 50 μM pTpT treatment of A375P melanoma cells (**Fig 4*b***). Similarly, thymidine monophosphate displayed only a mild effect onto cell proliferation rate at a concentration of 100 μM (**Fig 5*a***), and an effect comparable with that of 50 μM pTpT, at a concentration of 300 μM, the doubling time of 300 μM pT-treated A375P melanoma cells being 33 h compared with a doubling time of 25 h in untreated control cells (**Fig 5*b***).

Despite the fact that comparable effects on cell cycle and cell proliferation of human melanoma cells are obtained with concentrations of 300 μM pT and 50 μM pTpT, melanogenesis could only be induced in melanoma cells following treatment with the dinucleotide pTpT, and even a nontoxic 500 μM pT concentration was unable to trigger melanogenesis in M4Be cells (**Table IV**), suggesting that the effect on melanin synthesis is specific of the dimeric form of phosphothymidine.

DISCUSSION

The exposure of human melanocytes to thymidine dinucleotide pTpT results in an enhancement of melanogenesis. Our results thus confirm

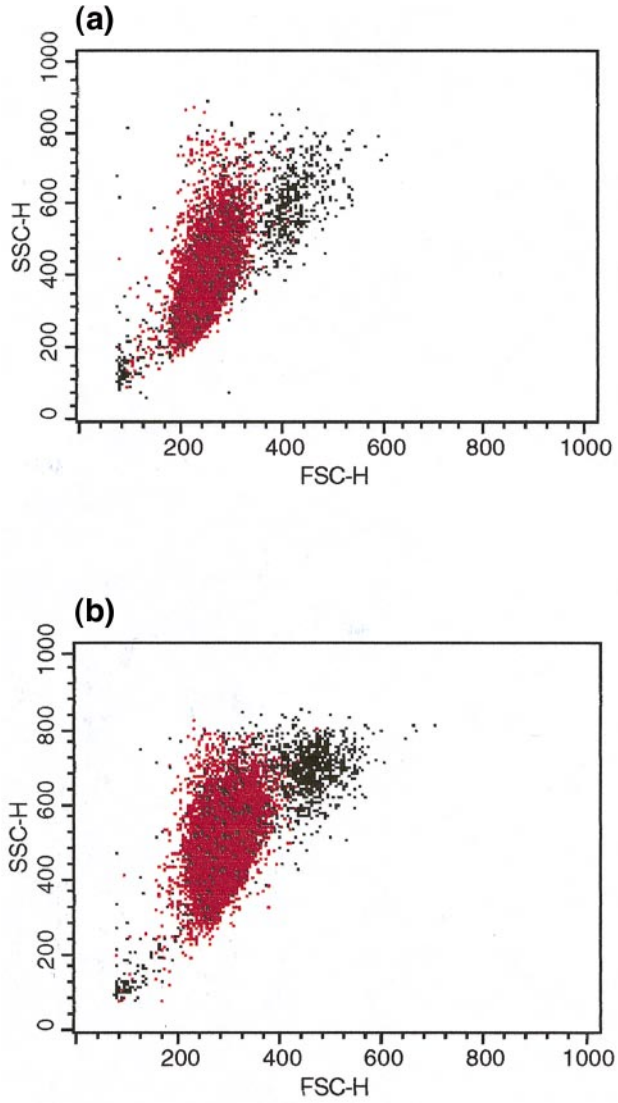

Figure 3. Absence of cytotoxicity of pTpT for A375P cells. Raw data scattergrams of (*a*) control and (*b*) pTpT-treated A375P cells. Cells were exposed to 50 μM pTpT for 24 h and their size and internal structure were analyzed by flow cytometry. FSC-H, forward scatter; SSC-H, side scatter. Red dots represent cell population selected for further analysis, whereas black dots denote either cell doublets (upper right) or cell debris (lower left). pTpT-treated cells show a slight increase in cell volume, compatible with accumulation of cell in S/G2 phases.

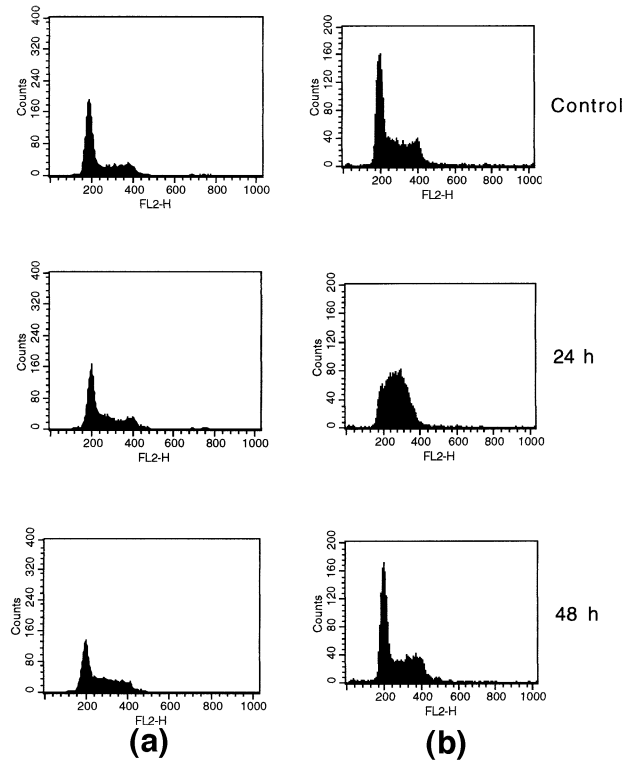

Figure 4. Cell cycle changes in A375P cells following exposure to pT. Cells were exposed to 100 μM (*a*) or 300 μM (*b*) pT and stained for DNA content with 0.05 mg propidium iodide per ml at selected times. Ordinate, number of cells; FL-2H, fluorescence intensity of propidium iodide.

and extend the original observation of Eller *et al* (1994), who reported that exposure of mouse melanoma cells S91 and guinea pig skin to pTpT resulted in an increase in melanin synthesis, and that this effect was specific of the thymidine dinucleotide because no such effect was observed following treatment of the melanocytes with similar concentrations of the purine dinucleotide pdApdA. Our experiments, using primary cultures or early passages of normal human adult melanocytes, slowly growing but fully able to trigger a melanogenic response to ultraviolet B irradiation, show that a 100 μM concentration of the dinucleotide pTpT enhances melanogenesis by a factor of 2.5 and that a lower concentration (30–50 μM) still yields a significant response. Our experiments further suggest that the induction of a melanogenic response is dependent on the dimeric form of thymidine, as no significant melanin synthesis was obtained following treatment of melanoma cells with the monomeric form of phosphothymidine (pT) at concentrations as high as 500 μM. The increase in melanin content of M4Be human melanoma cells following treatment with empty liposomes is likely to result from surface membrane events. Further studies will be needed to elucidate whether there is activation of specific transmembrane receptors in the diacylglycerol/protein kinase C pathway, the PKA pathways, or other signaling molecules involved in melanogenesis. This could be an nice model for study of melanogenesis; however, it should be stressed that these events are certainly different from those triggered by the thymidine dinucleotide pTpT, because there is an additive interaction between the effects of thymidine dinucleotide and of liposomes in melanogenesis enhancement: as a matter of fact, treatment of M4Be cells with pTpT enhances melanin content by a factor of 2.5, whereas treatment with empty liposomes enhances melanin content by a factor of 4 and treatment of the same cells by pTpT in liposomes enhances melanin content by a factor of 10.

The most striking observation of the experiments reported here is that following exposure to thymidine dinucleotide, human melanocytes accumulate in the S phase of the cell cycle. It is highly unlikely that the increase in S phase cells might be interpreted as stemming from a larger number of actively cycling cells, because human melanoma cell lines are usually characterized by a large growth fraction, and because an increase in the proportion of cycling cells should have resulted in an increase in proliferation rate or apoptosis. A double block by a thymidine excess has long been used to synchronize cells, and it has long been known that such a synchronization results from a diminution of the progression of cells through the S phase of the cell cycle (Galvazi and Bootsma, 1966; Galvazi *et al*, 1966). The concentrations of thymidine required to achieve cell synchronization, however, were usually 20–50 times higher (Rosenfeld *et al*, 1973) than the concentrations of thymidine dinucleotide used in the experiments reported here that resulted in S phase accumulation of treated melanoma cells. Indeed, exposure of the melanoma cells to comparable amounts of thymidine mononucleotide (100 μM) failed to result in significant cell cycle changes, although further increasing thymidine mononucleotide concentration up to 300 μM resulted in S phase arrest. Thymidine dinucleotide might interfere with DNA synthesis, but it is unlikely that the significant accumulation of melanoma cells in the S phase of the cell cycle that we evidenced following treatment with thymidine dinucleotide would simply result from exposure to thymidine, and

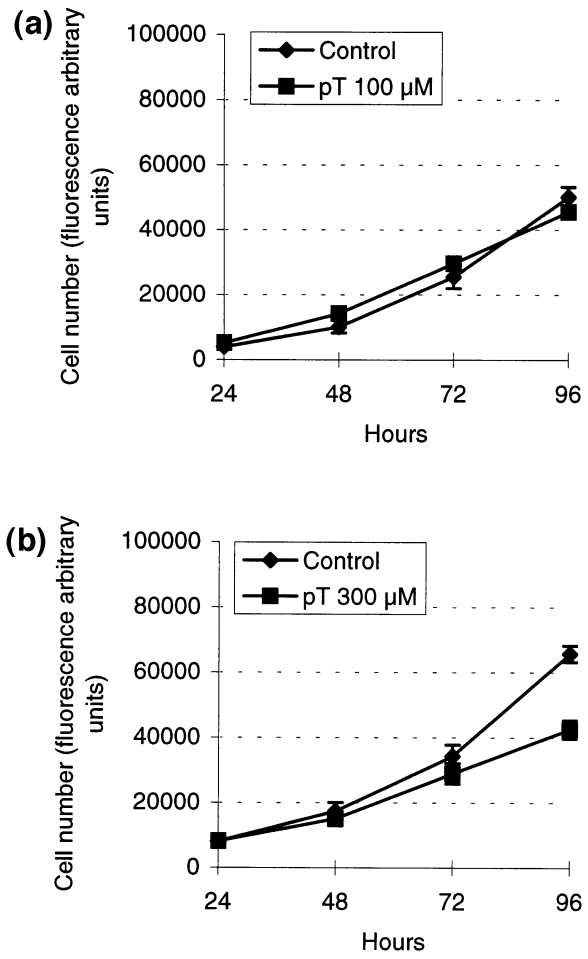

Figure 5. Effect of pT on the growth of A375P cells. Cells were exposed to 100 μM (*a*) or 300 μM (*b*) pT and cell growth was monitored using the Alamar Blue method (see legend to **Fig 2**).

Table IV. Effect of pTpT and pT on pigmentation of human melanoma cells

	Melanin[a]
Control	19.6 ± 1.48
pTpT[b]	42.2 ± 0.56
pT[c]	26.7 ± 1.76
pT[d]	24.5 ± 1.6

[a]μg melanin per 10^6 cells.
[b]50 μM.
[c]300 μM.
[d]500 μM.

further investigations are needed to eventually document the mechanism by which the dimeric form of thymidine causes cell cycle arrest. It is worthy to note that another pyrimidine analog, bromodeoxyuridine, which induces growth arrest, melanogenesis inhibition (Kreider *et al*, 1974; Silagi, 1976), and a decrease in tumorigenicity of human melanoma cells (Rieber *et al*, 1989; Valyi-Nagy *et al*, 1993), at a concentration of 2.5 μg per ml (8 μM), is able to control human melanoma cell growth, probably by acting at a point in G1 beyond cyclin D synthesis in which it prevents S phase transition of human melanoma cells by influencing the levels of expression of the p21 inhibitor and decreasing the cyclin A activator of cdk2 (Rieber *et al*, 1996). Most recently, Eller *et al* (1997) have described growth retardation and p21 induction by pTpT. The elucidation of the point in the cell cycle where thymidine dinucleotide pTpT acts will require further investigation of the expression of cyclins, more especially cyclin A and cyclin D, and cdk2 in treated human melanoma cells. Friedmann and Gilchrest (1987) have shown years ago that human melanocytes exposed daily to physiologic ranges of ultraviolet radiation show a dose-related increase in melanin content per cell and uptake of melanin precursors, accompanied by growth inhibition. From our experiments, however, it is clear that cell cycle arrest and growth inhibition do not by themselves result in melanogenesis enhancement.

Tanning is a complex protective response of skin triggered by ultraviolet radiation to prevent further ultraviolet radiation-induced damage. A central element of such a protective response is the ultraviolet-triggered induction of melanin synthesis. It is becoming increasingly evident that at least two different signaling pathways could actually be involved in the induction of melanogenesis. One major and well-investigated pathway involves biochemical mediators such as α-melanotropin and ACTH synthesized in skin by epidermal melanocytes and keratinocytes. Thus, Im *et al* (1998) have recently published some evidence of an α-melanotropin mediated protective effect in human melanocytes following ultraviolet B irradiation. In addition to this well-established pathway, it is becoming more and more evident that DNA damage, resulting from ultraviolet exposure but also from exposure to other DNA damaging agents, enhances melanogenesis (Eller *et al*, 1996). Recent work by Gilchrest's group suggests the existence of an SOS-like response in eukaryotic cells similar to the one occurring in prokaryotic cells in response to ultraviolet-induced DNA damage. Indeed, it is well established that in prokaryotic cells, in response to DNA damage or when DNA replication is inhibited, an intracellular signal for SOS induction is generated. Evidence suggests that this signal consists of regions of single-stranded DNA that are generated when the cell attempts to replicate a damaged template or when its normal process of DNA replication is interrupted (Sassanfar *et al*. 1990). The S phase arrest evidenced in our experiments may be explained by this SOS induction. It is likely that pTpT, and perhaps other small nucleic acids, mimics the products of DNA damage or processed DNA-damage intermediates, which may explain why pTpT, which is not the cyclobutane pyrimidine dimer formed following ultraviolet irradiation, is actually inducing melanogenesis. Thus, the SOS-like response suggested by Gilchrest's work to be evolutionary conserved, based primarily on work in murine cells and guinea pigs, is also apparently present in the human.

Several studies have previously shown that melanogenesis is related to DNA repair (Gilchrest *et al*, 1993), and, most recently, it was demonstrated that pTpT enhances DNA repair in human skin cells (Eller *et al*, 1997). Xeroderma pigmentosum (XP) patients, however, who are deficient in DNA repair mechanisms, are able to tan after exposure to ultraviolet, most likely through activation of the major melanogenesis pathway involving biochemical mediators. Hence, it would be of utmost interest to explore the ability of melanocytes from XP patients to trigger melanogenesis when exposed to pTpT, this would help to elucidate whether there is an association between DNA repair and increased melanogenesis, both phenomena triggered by thymidine dinucleotides.

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