Involvement of DNA Polymerase δ in DNA Repair Synthesis in Human Fibroblasts at Late Times after Ultraviolet Irradiation†

Steven L. Dresler,*,‡ Bonnie J. Gowans,§ Rona M. Robinson-Hill,‡ and Darel J. Hunting§

Department of Pathology, Washington University School of Medicine, St. Louis, Missouri 63110, and MRC Group in the Radiation Sciences, University Hospital Center, University of Sherbrooke, Sherbrooke, Quebec J1H 5N4, Canada

Received March 10, 1988; Revised Manuscript Received May 6, 1988

ABSTRACT: DNA repair synthesis following UV irradiation of confluent human fibroblasts has a biphasic time course with an early phase of rapid nucleotide incorporation and a late phase of much slower nucleotide incorporation. The biphasic nature of this curve suggests that two distinct DNA repair systems may be operative. Previous studies have specifically implicated DNA polymerase δ as the enzyme involved in DNA repair synthesis occurring immediately after UV damage. In this paper, we describe studies of DNA polymerase involvement in DNA repair synthesis in confluent human fibroblasts at late times after UV irradiation. Late UV-induced DNA repair synthesis in both intact and permeable cells was found to be inhibited by aphidicolin, indicating the involvement of one of the aphidicolin-sensitive DNA polymerases, α or δ. In permeable cells, the process was further analyzed by using the nucleotide analogue (butylphenyl)-2′-deoxyguanosine 5′-triphosphate, which inhibits DNA polymerase α several hundred times more strongly than it inhibits DNA polymerase δ. The (butylphenyl)-2′-deoxyguanosine 5′-triphosphate inhibition curve for late UV-induced repair synthesis was very similar to that for polymerase δ. It appears that repair synthesis at late times after UV irradiation, like repair synthesis at early times, is mediated by DNA polymerase δ.

The biphasic time course of DNA repair synthesis following ultraviolet (UV)[1] irradiation of human fibroblasts [reviewed by Lan and Smerdon (1985)] has led to the suggestion that excision repair of UV damage may have early and late phases mediated by biochemically distinct excision repair systems. Studies of removal of UV damage from the DNA of human cells have also shown biphasic kinetics (Kantor & Setlow, 1981), and the possible existence of separate early and late repair systems receives further support from the finding that some xeroderma pigmentosum cell types are selectively deficient in either the early or the late phase of the UV damage removal curve (Sullivan & Kantor, 1986; Kantor & Player, 1986). Lan and Smerdon (1985) found a nonrandom distribution of repair-incorporated nucleotides in nucleosome core DNA at early times after UV damage and suggested that biphasic repair kinetics may be the net result of rapid repair of damage located near the ends of nucleosome core DNA and slow repair of damage in central regions of the core. As yet, enzymatic differences between the early and late phases of UV-induced excision repair have not been identified.

The existence of specific DNA polymerase inhibitors has facilitated identification of the polymerases involved in DNA repair synthesis after various forms of DNA damage. Studies in intact (Waters, 1981; Snyder & Regan, 1981, 1982; Collins et al., 1982; Dresler & Lieberman, 1983a) and permeable (Berger et al., 1979; Ciarrocchi et al., 1979; Hanaoka et al., 1979; Dresler et al., 1982; Miller & Chinault, 1982a,b; Dresler & Lieberman, 1983a) nongrowing mammalian cells at early times after damage have shown that repair synthesis induced by high doses of UV irradiation is mediated almost entirely by an aphidicolin-sensitive DNA polymerase (i.e., polymerase α or δ; Huberman, 1981; Lee et al., 1981). In intact cells, aphidicolin inhibits removal of UV-induced pyrimidine dimers (Snyder & Regan, 1981, 1982) and potentiates killing by UV irradiation (Tyrrell, 1983), indicating that aphidicolin-sensitive repair synthesis is biologically important. Studies using N^2-(*p*-*n*-butylphenyl)-2′-deoxyguanosine 5′-triphosphate (BuPh-dGTP), a nucleotide analogue which inhibits DNA polymerase α much more strongly than DNA polymerase δ (Byrnes, 1985; Lee et al., 1985; Crute et al., 1986), have specifically implicated polymerase δ as the enzyme mediating DNA repair synthesis at early times after UV damage (Dresler & Frattini, 1986, 1988). Involvement of polymerase δ in repair is supported by biochemical complementation experiments in UV-irradiated permeable human fibroblasts (Nishida et al., 1988). In this paper, we use aphidicolin and BuPh-dGTP to study DNA polymerase involvement in DNA repair synthesis at late times (greater than 12 h) after irradiation of confluent human fibroblasts with high doses of UV light.

Experimental Procedures

Chemicals. Aphidicolin, provided by the Natural Products Branch, Division of Cancer Treatment, National Cancer Institute, was dissolved in DMSO at either 1 or 10 mg/mL and stored at −20 °C. Hydroxyurea (HU) and 5-bromo-2′-

† This work was supported by USPHS Grant CA37261 from the National Cancer Institute, by a grant from the Anna Fuller Fund, by the Medical Research Council of Canada, and by Brown and Williamson Tobacco Corp., Phillip Morris, Inc., R. J. Reynolds Tobacco Co., and the United States Tobacco Co. D.J.H. is a scholar of the FRSQ.

* Address correspondence to this author.
‡ Washington University School of Medicine.
§ University of Sherbrooke.

[1] Abbreviations: UV, ultraviolet; HU, hydroxyurea; BrdUrd, 5-bromo-2′-deoxyuridine; dThd, thymidine; dATP, 2′-deoxyadenosine 5′-triphosphate; dGTP, 2′-deoxyguanosine 5′-triphosphate; BrdUTP, 5-bromo-2′-deoxyuridine 5′-triphosphate; dCTP, 2′-deoxycytidine 5′-triphosphate; EDTA, ethylenediaminetetraacetic acid; Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol; dCMP, 2′-deoxycytidine 5′-monophosphate; PBS, phosphate-buffered saline; dNTPs, the four common 2′-deoxyribonucleoside 5′-triphosphates; BuPh-dGTP, N^2-(*p*-*n*-butylphenyl)-2′-deoxyguanosine 5′-triphosphate.

0006-2960/88/0427-6379$01.50/0

deoxyuridine (BrdUrd) were dissolved in water immediately prior to use. BuPh-dGTP, the generous gift of Dr. George Wright, was dissolved at 10 mM in 30 mM Tris, pH 7.6, and stored at −20 °C.

Cell Culture and UV Irradiation. Human diploid fibroblasts (AG1518; Institute for Medical Research) were passed into plastic culture dishes, prelabeled with [*methyl*-^{14}C]dThd, and grown to confluence as described (Dresler et al., 1982). For autoradiography, cells were grown on ethanol-washed glass microscope slides in plastic culture dishes and were not prelabeled. After culture medium was removed, cell monolayers in culture dishes were exposed to UV radiation (primarily 254 nm) from a germicidal lamp (G15T8) at a flux of either 1 or 2 W/m^2. UV flux was measured by using an International Light IL770A radiometer.

Measurement of DNA Repair Synthesis in Intact Cells Using the BrdUrd Density Shift Technique. Culture dishes of confluent fibroblasts, prelabeled with [^{14}C]dThd and either unirradiated or irradiated with the indicated dose of UV light, were incubated during the indicated time period with [^{3}H]-dThd (20 or 50 μCi/mL; 50–60 Ci/mmol), 50 μM BrdUrd, and either no aphidicolin or 120 μM aphidicolin. DNA was isolated by sodium dodecyl sulfate–proteinase K digestion and subjected to isopycnic centrifugation in alkaline CsCl (Dresler & Lieberman, 1983a) to separate radioactive label incorporated by residual semiconservative DNA replication, which is very dense, from that incorporated by repair synthesis, which has a density very close to that of parental DNA (Hanawalt & Cooper, 1971). Repair synthesis was determined by taking the difference between specific dThd incorporation (^{3}H/^{14}C) in the parental DNA peaks of corresponding irradiated and unirradiated samples (Dresler et al., 1982).

Measurement of DNA Repair Synthesis in Intact Cells in the Presence of HU. Culture dishes of confluent fibroblasts, prelabeled with [^{14}C]dThd and either unirradiated or irradiated with the indicated dose of UV light, were incubated during the indicated time period with 20 μCi/mL [^{3}H]dThd, 10 mM HU, and either no aphidicolin or 120 μM aphidicolin. DNA was isolated by sodium dodecyl sulfate–proteinase K digestion (Dresler & Lieberman, 1983a), precipitated with trichloroacetic acid, and collected on glass fiber filters as described (Dresler et al., 1982). Radioactivity was determined by liquid scintillation counting, and repair synthesis was calculated by taking the difference between specific dThd incorporation (^{3}H/^{14}C) in corresponding irradiated and unirradiated samples (Dresler et al., 1982).

Measurement of DNA Repair Synthesis in Intact Cells Using Autoradiography. Confluent fibroblasts, grown on glass microscope slides and either unirradiated or irradiated with the indicated dose of UV light, were incubated with 2.5 μCi/mL [^{3}H]dThd and either no aphidicolin or 60 μM aphidicolin during the time period indicated. The cells were washed, incubated with excess unlabeled dThd, fixed, and processed for autoradiography as described (Dresler & Lieberman, 1983a). Silver grains overlying 150–200 nuclei were counted for each sample. Repair synthesis was calculated by taking the difference between the mean grains per non-S-phase cell in corresponding damaged and undamaged samples.

Measurement of DNA Repair Synthesis in Permeable Cells. The assay used has been extensively characterized (Roberts & Lieberman, 1979; Dresler et al., 1982). Confluent fibroblasts, prelabeled with [^{14}C]dThd and either unirradiated or irradiated with the indicated dose of UV light, were incubated in culture medium for 14 h and then harvested. The harvested cells were made permeable and washed as described (Dresler et al., 1982; Dresler & Lieberman, 1983b). Portions of cell suspension were mixed at 4 °C with 0.5 volume of a reaction stock to give the following final concentrations: 40 mM Tris (pH 7.6 at 37 °C), 8 mM $MgCl_2$, 15 mM KCl, 5 mM ATP, 167 mM sucrose, 2 mM dithiothreitol, 0.67 mM EDTA, the indicated concentrations of dATP, dGTP, BrdUTP, and dCTP (one of which was labeled with ^{32}P), and the indicated concentration of aphidicolin or BuPh-dGTP. Samples were incubated at 37 °C for 15 min, and DNA was isolated by sodium dodecyl sulfate–proteinase K digestion and analyzed by isopycnic centrifugation in alkaline CsCl (Dresler & Lieberman, 1983a). Repair synthesis was determined by taking the difference between specific dCMP incorporation (^{32}P/^{14}C) in the parental DNA peaks of corresponding irradiated and unirradiated samples.

Measurement of DNA Strand Break Accumulation in Intact Cells by Alkaline Elution. Confluent fibroblasts, grown in 100 cm^2 culture dishes, prelabeled with [^{14}C]dThd and irradiated with 20 J/m^2 UV light, were incubated in culture medium without addition for the indicated time and then incubated for 30 min with either no addition, 7.5 μM aphidicolin, or 7.5 μM aphidicolin plus 10 mM HU, as indicated. Cells were then harvested by replacing the culture medium in each dish with 3 mL of 0.5 mM EDTA in PBS (2.7 mM NaH_2PO_4, 13.1 mM Na_2HPO_4, 135 mM NaCl, and 4.9 mM KCl) and placing the dishes on ice for 10 min, followed by gentle scraping and resuspension of the cells by repeated pipetting. A 1-mL portion of each sample was added to 20 mL of ice-cold PBS together with 3.3×10^5 L1210 cells which had been labeled for 16 h with 25 nCi/mL [^{3}H]dThd and irradiated with 1000 rad of γ radiation. (The ^{3}H-labeled, γ-irradiated cells were added to provide an internal strand break standard.) The samples were then subjected to alkaline elution essentially as described (Kohn et al., 1981). Briefly, samples were collected on 2-μm pore size polycarbonate filters and washed, and the cells were lysed with 2% (w/v) sodium dodecyl sulfate and 25 mM EDTA, pH 10.0. DNA was eluted from the filters with 20 mM EDTA adjusted to pH 12.1 with tetrapropylammonium hydroxide at a flow rate of 0.2 mL/min, and 2-mL fractions were collected. The radioactivity in each fraction was determined by liquid scintillation counting, and the fractions of ^{3}H and ^{14}C DNA remaining on each filter at each time point were calculated. In the assay, the rate at which DNA elutes from the filter is related to the DNA strand break frequency; by plotting the elution of ^{14}C(experimental cell DNA) vs ^{3}H(internal strand break standard DNA) for each sample, the relative strand break frequencies of the samples can be compared directly.

Isolation of DNA Polymerases and Measurement of Polymerase Activity. DNA polymerase α was isolated from HeLa cells as described (Fisher & Korn, 1977). DNA polymerase δ was isolated from calf thymus (Crute et al., 1986). Both polymerases were assayed using poly(dA)–oligo(dT) as template-primer as described (Crute et al., 1986).

Results and Discussion

Effect of Aphidicolin on DNA Repair Synthesis in Intact Human Fibroblasts at Late Times after UV Irradiation. The time course of UV-induced DNA repair synthesis in intact confluent human fibroblasts, as measured by the BrdUrd density shift method, is seen in Figure 1. There is an early phase of rapid [^{3}H]dThd incorporation which lasts 8–10 h, followed by a phase of slower incorporation extending beyond 24 h. To study the effects of aphidicolin on the two phases of repair synthesis, we used the BrdUrd density shift technique to examine repair synthesis in confluent human fibroblasts

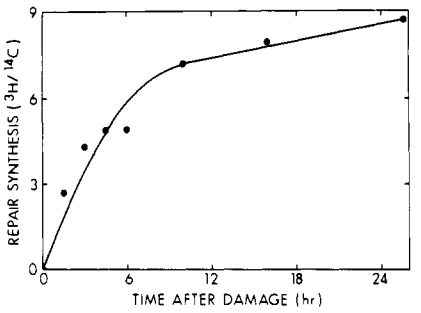

FIGURE 1: Time course of UV-induced DNA repair synthesis in intact human fibroblasts as measured by the BrdUrd density shift technique. Confluent AG1518 cells, prelabeled with [^{14}C]dThd, were irradiated with 11 J/m^2 UV light and incubated with 50 μM BrdUrd and 20 μCi/mL [^{3}H]dThd for the indicated times after damage. Repair synthesis was determined as described under Experimental Procedures.

Table I: Effect of Aphidicolin on UV-Induced DNA Repair Synthesis in Intact Human Fibroblasts at Early and Late Times after UV Irradiation As Measured by the HU and BrdUrd Density Shift Techniques[a]

technique	time of labeling (h after UV irradiation)	repair synthesis ($^3H/^{14}C$) −aphidicolin	repair synthesis ($^3H/^{14}C$) +aphidicolin	effect of aphidicolin
BrdUrd	0–2	4.5	1.5	67% inhibition
BrdUrd	14–33	0.9	1.3	44% stimulation
HU	14–33	27.1	10.3	62% inhibition

[a] Confluent AG1518 fibroblasts, prelabeled with [^{14}C]dThd, were irradiated with 12 J/m^2 UV light and labeled for the indicated time period with 20 μCi/mL [^{3}H]dThd and either 10 mM HU or 50 μM BrdUrd. Repair synthesis was determined as described under Experimental Procedures. Where indicated, 120 μM aphidicolin was present during the labeling period. [This dose of aphidicolin is at least 8 times that which maximally inhibits repair synthesis in intact human fibroblasts at early times after UV irradiation (Dresler & Lieberman, 1983a).]

pulse-labeled with [^{3}H]dThd at early (0–2 h) and late (14–33 h) times after UV irradiation (Table I). As expected, UV-induced [^{3}H]dThd incorporation during the early time period was substantially (67%) inhibited by aphidicolin. Surprisingly, UV-induced [^{3}H]dThd incorporation at late times was stimulated by 44% in the presence of the drug.

We also used autoradiography to evaluate the aphidicolin sensitivity of repair synthesis in intact cells at early and late times after UV irradiation. Consistent with the BrdUrd density shift data, at early times (0–1 h) after damage, UV-induced [^{3}H]dThd incorporation was largely (71%) inhibited by aphidicolin and at late times (19–25 h) after damage, incorporation was stimulated (21%) by the presence of the drug (data not shown).

The finding that aphidicolin, a DNA polymerase inhibitor, stimulates [^{3}H]dThd incorporation at late times after UV irradiation suggests that the drug may produce changes in cellular nucleotide metabolism which confound attempts to study its direct effect on DNA repair synthesis. The plausibility of this hypothesis is supported by the fact that aphidicolin substantially reduces the in situ activities of both ribonucleotide reductase and thymidylate synthetase in mouse 3T6 fibroblasts (Nicander & Reichard, 1985). Reductions in the activities of these enzymes would decrease de novo dTTP synthesis and, in a [^{3}H]dThd labeling experiment, would increase the ^{3}H specific activity of the cellular dTTP pool. This alteration in dTTP specific activity would act counter to any inhibitory effect of aphidicolin on the actual rate of incorporation of dTTP into DNA. To examine this possibility, we studied the effect of aphidicolin on UV-induced [^{3}H]dThd incorporation at late times after damage in intact cells treated with 10 mM HU. The addition of HU, a strong ribonucleotide reductase inhibitor (Timson, 1975; Skoog & Nordenskjold, 1971; Snyder, 1984a,b), should substantially reduce nucleotide flux through that enzyme in both control and aphidicolin-treated cells. This should minimize the impact on [^{3}H]dThd incorporation of aphidicolin-induced changes in the activity either of ribonucleotide reductase or of subsequent enzymes in the de novo dTTP biosynthetic pathway. As seen in Table I, damage-induced [^{3}H]dThd incorporation at late times (14–33 h) after UV irradiation was inhibited by aphidicolin when measured in the presence of HU. These data suggest that DNA repair synthesis in intact human fibroblasts at late times after UV irradiation is largely sensitive to aphidicolin and that the lack of inhibition seen when using BrdUrd density shift and autoradiography is artifactual.

The apparent occurrence of aphidicolin-induced alterations of nucleotide metabolism at late times after UV irradiation is surprising, because such changes have not been noted at early times following damage. Studies of nucleotide metabolism following UV irradiation of mammalian cells have, however, revealed substantial variations in cellular dNTP concentrations during the first 8 h after damage, after which the dNTP concentrations return to control levels (Newman & Miller, 1983; Das et al., 1983). These results suggest that the regulation of dNTP synthesis at early times after UV irradiation may differ from that at late times.

Assessment of Aphidicolin Inhibition of Late UV Repair in Intact Cells by Measuring DNA Strand Break Accumulation. Measurement of DNA strand break accumulation is a sensitive method for detecting inhibition of repair patch synthesis which is largely unaffected by changes in cellular nucleotide metabolism. The basis for this technique is the finding that inhibitors of repair patch synthesis, because they delay the completion and ligation of repair patches, will cause DNA strand breaks to accumulate following DNA damage (Snyder et al., 1981; Bodell et al., 1982; Collins et al., 1982; Cleaver, 1982; Snyder & Regan, 1982; Hunting et al., 1985). Using the alkaline elution technique (Kohn et al., 1981), we studied the effect of aphidicolin on strand break accumulation at early and late times following UV irradiation in intact human fibroblasts. As expected, at early times (3.5–4 h) after UV irradiation (Figure 2A), aphidicolin produced a dramatic increase in the accumulation of DNA strand breaks. At late times (17.5–18 h) after UV irradiation (Figure 2B), aphidicolin also produced a marked increase in strand break accumulation, confirming that aphidicolin strongly inhibits repair synthesis in intact human fibroblasts at both early and late times after UV irradiation.

We have previously shown that addition of HU will potentiate the inhibition of repair patch synthesis produced by a submaximal dose of aphidicolin (Hunting et al., 1985). The fact that, in this experiment, the combination of HU plus aphidicolin produced no more DNA strand break accumulation than aphidicolin alone (Figure 2A,B) indicates that aphidicolin inhibition of both early and late repair was maximal, even though the concentration of aphidicolin used was much lower than the concentrations used in the other experiments described in this paper.

Effect of Aphidicolin on DNA Repair Synthesis at Late Times after UV Irradiation As Measured by the Permeable Cell Technique. Measurement of DNA repair synthesis in permeable cells provides another way of avoiding the confounding effects of changes in cellular nucleotide metabolism. The permeable cell method circumvents this problem by

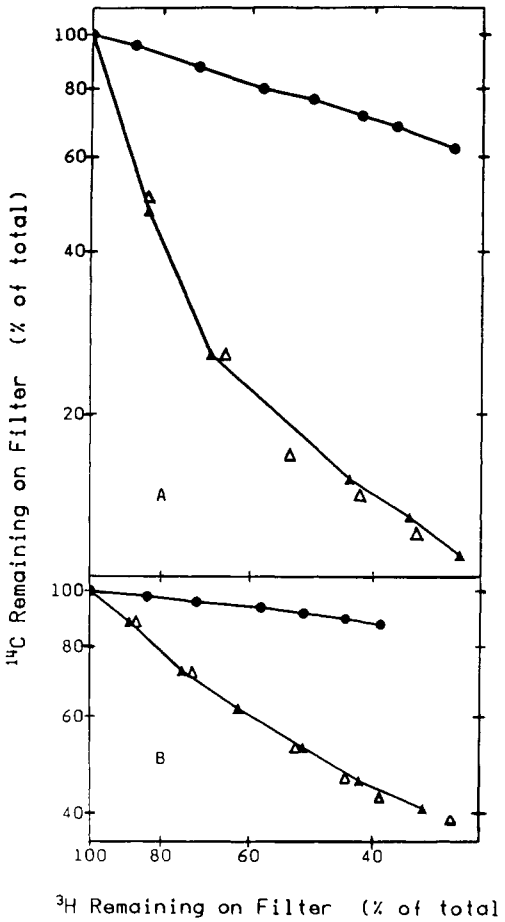

FIGURE 2: Effect of aphidicolin and aphidicolin/HU on DNA strand break accumulation in intact human fibroblasts at early (A) and late (B) times after UV irradiation. Confluent AG1518 cells, prelabeled with [^{14}C]dThd, were irradiated with 20 J/m^2 UV light, incubated for either 3.5 h (A) or 17.5 h (B) in medium without additions, and then incubated for 30 min in medium containing no addition (●), 7.5 μM aphidicolin (▲), or 7.5 μM aphidicolin plus 10 mM HU (△). DNA strand breakage was analyzed by alkaline elution after addition of [^{3}H]dThd-labeled L1210 cells which had been irradiated with 1000 rad of γ radiation as an internal strand break standard.

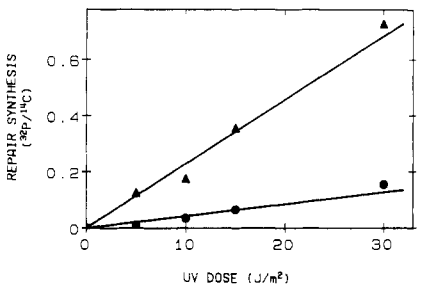

FIGURE 3: Effect of aphidicolin on DNA repair synthesis at late times after UV irradiation as measured by the permeable cell technique. Confluent AG1518 cells, prelabeled with [^{14}C]dThd, were irradiated with the indicated dose of UV light and incubated for 14 h at 37 °C. The cells were then harvested, and repair synthesis was determined by the permeable cell technique with dATP, BrdUTP, dGTP, and [α-^{32}P]dCTP each at 3 μM and with the addition of either no aphidicolin (▲) or 105 μM aphidicolin (●). [The concentration of aphidicolin used is at least 7 times that required to maximally inhibit repair synthesis in permeable human fibroblasts at early times after UV irradiation (Dresler & Lieberman, 1983a).]

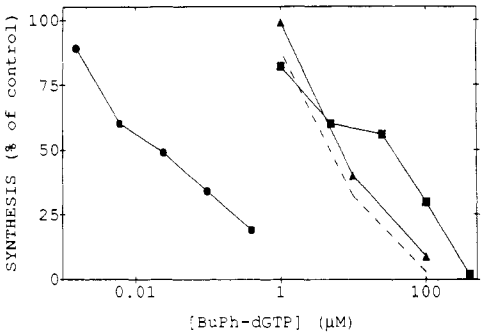

FIGURE 4: Effect of BuPh-dGTP on repair synthesis at late times after UV irradiation and on DNA polymerases α and δ. Confluent AG1518 cells, prelabeled with [^{14}C]dThd, were irradiated with 30 J/m^2 UV light, incubated for 14 h at 37 °C in culture medium, made permeable, and assayed for repair synthesis (▲). Reaction mixtures contained 3 μM dATP, dCTP, and BrdUTP, 0.3 μM [α-^{32}P]dGTP, and the indicated concentrations of BuPh-dGTP. The dashed line represents previously published data for BuPh-dGTP inhibition of early UV repair assayed under identical reaction conditions (Dresler & Frattini, 1986). Polymerases α (●) and δ (■) were assayed in reaction mixtures (see Experimental Procedures) containing the indicated concentrations of BuPh-dGTP. The data are expressed as percentages of the activity seen in samples incubated without BuPh-dGTP.

permitting one to control the concentrations and specific radioactivities of the dNTPs during the assay (Hunting & Dresler, 1985). We irradiated confluent human fibroblasts with various doses of UV light, incubated them in culture medium at 37 °C for 14 h, and then made them permeable and assayed repair synthesis in the presence and absence of aphidicolin. When studied in this way, repair synthesis at late times after UV irradiation was at least 80% aphidicolin sensitive at all UV doses examined (Figure 3). This result, together with the strand break accumulation data presented above, convincingly establishes that DNA repair synthesis at late times after UV irradiation is inhibited by aphidicolin.

Effect of BuPh-dGTP on DNA Repair Synthesis at Late Times after UV Irradiation. The aphidicolin sensitivity of late UV-induced DNA repair synthesis indicates that the process is mediated by one or both of the aphidicolin-sensitive DNA polymerases, α and/or δ. These two enzymes can be differentiated by using the nucleotide analogue BuPh-dGTP which inhibits polymerase α several hundred times more strongly than it inhibits polymerase δ (Byrnes, 1985; Lee et al., 1985; Crute et al., 1986). Using the permeable cell system, we compared the sensitivity to BuPh-dGTP of repair synthesis at late times after UV irradiation with the BuPh-dGTP sensitivities of DNA polymerases α and δ (Figure 4). The concentrations of BuPh-dGTP required to inhibit late UV repair synthesis were several hundredfold greater than those required to inhibit DNA polymerase α but were very similar to those which inhibited DNA polymerase δ. In addition, the BuPh-dGTP inhibition curve for repair synthesis at late times after UV irradiation was almost identical with that for early repair. It appears that repair synthesis at late times after UV irradiation, like repair synthesis at early times (Dresler & Frattini, 1986, 1988; Dresler & Kimbro, 1987; Nishida et al., 1988), is mediated by DNA polymerase δ.

Conclusion. Several characteristics of excision repair at late times after UV irradiation suggest that the process may differ biochemically from repair at early times after UV irradiation (see the introduction). Our data indicate that these differences are not due to the involvement of different DNA polymerases in early and late repair. This conclusion does not rule out the existence of other differences between repair synthesis mechanisms at early and late times after damage. Celis and Madsen (1986) have shown that UV irradiation of cultured human cells induces a dramatic change in the nuclear distribution of PCNA/cyclin, a protein which acts as a specific accessory factor for DNA polymerase δ (Bravo et al., 1987; Prelich et al., 1987). DNA damage-induced synthesis of a number of nuclear proteins has also been described (Lambert

et al., 1986). Changes in the expression and/or distribution of DNA polymerase accessory proteins could dramatically alter the characteristics of repair patch synthesis at late times after damage, even if the DNA polymerase involved remained the same.

ACKNOWLEDGMENTS

We thank Dr. George Wright for providing BuPh-dGTP, Mark G. Frattini for preparing DNA polymerases α and δ, and Joseph A. DiGiuseppe and Brenda Jo Mengeling for helpful comments on the manuscript.

Registry No. DNA polymerase, 9012-90-2; aphidicolin, 38966-21-1.

REFERENCES

Berger, N. A., Kurohara, K. K., Petzold, S. J., & Sikorski, G. (1979) *Biochem. Biophys. Res. Commun. 89*, 218–225.

Bodell, W. J., Kaufmann, W. K., & Cleaver, J. E. (1982) *Biochemistry 21*, 6767–6772.

Bravo, R., Frank, R., Blundell, P. A., & MacDonald-Bravo, H. (1987) *Nature (London) 326*, 515–517.

Byrnes, J. J. (1985) *Biochem. Biophys. Res. Commun. 132*, 628–634.

Celis, J. E., & Madsen, P. (1986) *FEBS Lett. 209*, 277–283.

Ciarrocchi, G., Jose, J. G., & Linn, S. (1979) *Nucleic Acids Res. 7*, 1205–1219.

Cleaver, J. E. (1982) *Carcinogenesis (London) 3*, 1171–1174.

Collins, A. R. S., Squires, S., & Johnson, R. T. (1982) *Nucleic Acids Res. 10*, 1203–1213.

Crute, J. J., Wahl, A. F., & Bambara, R. A. (1986) *Biochemistry 25*, 26–36.

Das, S. K., Benditt, E. P., & Loeb, L. A. (1983) *Biochem. Biophys. Res. Commun. 114*, 458–464.

Dresler, S. L., & Lieberman, M. W. (1983a) *J. Biol. Chem. 258*, 9990–9994.

Dresler, S. L., & Lieberman, M. W. (1983b) *J. Biol. Chem. 258*, 12269–12273.

Dresler, S. L., & Frattini, M. G. (1986) *Nucleic Acids Res. 14*, 7093–7102.

Dresler, S. L., & Kimbro, K. S. (1987) *Biochemistry 26*, 2664–2668.

Dresler, S. L., & Frattini, M. G. (1988) *Biochem. Pharmacol. 37*, 1033–1037.

Dresler, S. L., Roberts, J. D., & Lieberman, M. W. (1982) *Biochemistry 21*, 2557–2564.

Fisher, P. A., & Korn, D. (1977) *J. Biol. Chem. 252*, 6528–6535.

Hanaoka, F., Kato, H., Ikegami, S., Ohashi, M., & Yamada, M. (1979) *Biochem. Biophys. Res. Commun. 87*, 575–580.

Hanawalt, P. C., & Cooper, P. K. (1971) *Methods Enzymol. 21D*, 221–230.

Huberman, J. A. (1981) *Cell (Cambridge, Mass.) 23*, 647–648.

Hunting, D. J., & Dresler, S. L. (1985) *Carcinogenesis (London) 6*, 1525–1528.

Hunting, D. J., Dresler, S. L., & Lieberman, M. W. (1985) *Biochemistry 24*, 3219–3226.

Kantor, G. J., & Setlow, R. B. (1981) *Cancer Res. 41*, 819–825.

Kantor, G. J., & Player, A. N. (1986) *Mutat. Res. 166*, 79–88.

Kohn, K. W., Ewig, R. A. G., Erickson, L. A., & Zwelling, L. A. (1981) in *DNA Repair: A Laboratory Manual of Research Procedures* (Friedberg, E. C., & Hanawalt, P. C., Eds.) Vol. 1, Part B, pp 379–401, Marcel Dekker, New York.

Lambert, M. E., Garrels, J. I., McDonald, J., & Weinstein, I. B. (1986) in *Antimutagenesis and Anticarcinogenesis Mechanisms* (Shankel, D. M., Hartman, P. E., Kada, T., & Hollaender, A., Eds.) pp 291–311, Plenum, New York.

Lan, S. Y., & Smerdon, M. J. (1985) *Biochemistry 24*, 7771–7783.

Lee, M. Y. W. T., Tan, C.-K., Downey, K. M., & So, A. G. (1981) *Prog. Nucleic Acid Res. Mol. Biol. 26*, 83–96.

Lee, M. Y. W. T., Toomey, N. L., & Wright, G. E. (1985) *Nucleic Acids Res. 13*, 8623–8630.

Miller, M. R., & Chinault, D. N. (1982a) *J. Biol. Chem. 257*, 46–49.

Miller, M. R., & Chinault, D. N. (1982b) *J. Biol. Chem. 257*, 10204–10209.

Newman, C. N., & Miller, J. H. (1983) *Biochem. Biophys. Res. Commun. 116*, 1064–1069.

Nicander, B., & Reichard, P. (1985) *J. Biol. Chem. 260*, 9216–9222.

Nishida, C., Reinhard, P., & Linn, S. (1988) *J. Biol. Chem. 263*, 501–510.

Prelich, G., Tan, C.-K., Kostura, M., Mathews, M. B., So, A. G., Downey, K. M., & Stillman, B. (1987) *Nature (London) 326*, 517–520.

Roberts, J. D., & Lieberman, M. W. (1979) *Biochemistry 18*, 4499–4505.

Skoog, L., & Nordenskjold, B. (1971) *Eur. J. Biochem. 19*, 81–89.

Snyder, R. D. (1984a) *Biochem. Pharmacol. 33*, 1515–1518.

Snyder, R. D. (1984b) *Mutat. Res. 131*, 163–172.

Snyder, R. D., & Regan, J. D. (1981) *Biochem. Biophys. Res. Commun. 99*, 1088–1094.

Snyder, R. D., & Regan, J. D. (1982) *Biochim. Biophys. Acta 697*, 229–234.

Snyder, R. D., Carrier, W. L., & Regan, J. D. (1981) *Biophys. J. 35*, 339–350.

Sullivan, J. K., & Kantor, G. J. (1986) *Photochem. Photobiol. 43*, 429–436.

Timson, J. (1975) *Mutat. Res. 32*, 115–132.

Tyrrell, R. M. (1983) *Carcinogenesis (London) 4*, 327–329.

Waters, R. (1981) *Carcinogenesis (London) 2*, 795–797.