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KEYWORDS: DNA repair, DNA polymerases, aphidicolin, bleomycin, ultraviolet irradiation

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PARTICIPATION OF DNA POLYMERASES α AND β IN UNSCHEDULED DNA SYNTHESIS IN MAMMALIAN CELLS

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Abstract. Repair polymerases participating in unscheduled DNA synthesis in isolated liver nuclei, bleomycin-treated permeable cells and in ultraviolet-irradiated living cells were studied using two specific inhibitors of DNA polymerases, aphidicolin and 2', 3'-dideoxythymidine-5'-triphosphate. Unscheduled, *i.e.*, repair, DNA synthesis in rat liver nuclei, and in bleomycin-treated permeable SR-C3H/He and XC cells was mostly attributed to DNA polymerase β. Unscheduled DNA synthesis in human liver nuclei, bleomycin-treated permeable HeLa and HEp-2 cells, and in ultraviolet-irradiated HeLa, HEp-2 and XC cells was partially inhibited by the polymerase α-specific inhibitor, aphidicolin. The results suggested that both DNA polymerase α and β participated in unscheduled DNA synthesis, though the respective degrees of participation differed depending on cell type and the nature and degree of DNA damage.

Key words: DNA repair, DNA polymerases, aphidicolin, bleomycin, ultraviolet irradiation.

DNA damage unavoidably occurs in cells due to various causal agents. Repair of DNA damage, therefore, is an essential process in living organisms which assures genetic continuity, and the misrepair of damaged DNA is sometimes a driving force of evolution, and perhaps of aging and carcinogenesis. Knowledge of the molecular mechanism of DNA repair in mammalian cells is still fragmental. Even repair polymerases have not been identified clearly. DNA polymerases in mammalian cells have been classified into DNA polymerases α, β and γ (1-5). Previous studies show that the major polymerase engaging in replicative DNA synthesis is DNA polymerase α, and that DNA polymerase γ located in the mitochondria engages in mitochondrial DNA synthesis (2-5). Conflicting results have been reported on which DNA polymerases are engaged in repair or unscheduled DNA synthesis (USD) (3-5). Most previous reports implicate DNA polymerase β in DNA repair (2-10), but some reports, mainly on *in vitro* studies with human cells, implicate DNA polymerase α (11-18). Recently, involvement of both DNA polymerase α and β has been suggested in *in vitro* repair DNA synthesis induced by various agents in Chinese hamster ovary cells and by bleomycin (BLM) in permeable human cells (19, 20).

In the present communication, it is suggested that both DNA polymerase α and β are involved in repair DNA synthesis, and that their participation differs depending on cell type, and the amount and nature of DNA damage.

MATERIALS AND METHODS

Reagents. Hydroxyurea was obtained from Nutritional Biochemical Corp., Cleveland, Ohio. Aphidicolin was kindly provided by Dr. M. Ohashi of the Tokyo Metropolitan Institute of Gerontology and also by Dr. A. H. Todd of Imperial Chemical Industries, Macclesfield, Cheshire. The other reagents used were obtained as described previously (8).

Cells and culture conditions. HeLa-S3, HEp-2 and XC cells were cultured in plastic dishes in Eagle's minimum essential medium (MEM 1, Nissui Co., Tokyo) supplemented with 10 % bovine serum in a humidified 5 % CO_2-95 % air atmosphere. Mycoplasmal and viral contaminations were checked by autoradiography (21) and electron microscopy. Mouse ascites sarcoma (SR-C3H/He) cells were obtained as described previously (22).

Preparation of permeable cells and liver nuclei. Cells were permeabilized by treatment with buffer B (0.25 M sucrose, 10 mM Tris-HCl, 4 mM $MgCl_2$, 1 mM EDTA and 6 mM 2-mercaptoethanol, pH 8.0) supplemented with 0.0175 % Triton X-100 (Triton-buffer B) (23). Rat livers were obtained from adult Donryu rats. Apparently intact pieces of human livers were obtained from surgically resected hepatoma-bearing livers. Liver nuclei were prepared as described previously (24), and were suspended in buffer B.

DNA synthesis in permeable cells and liver nuclei. Replicative DNA synthesis in permeable cells was assayed in a mixture with a final volume of 0.6 ml containing 1×10^6-2×10^6 cells, 0.0117 % Triton X-100, 0.167 M sucrose, 0.67 mM EDTA, 4 mM 2-mercaptoethanol, 40 mM Tris-HCl, 5 mM $MgCl_2$, 0.08 M NaCl, 2.5 mM ATP, 50 μM dATP, 10 μM dCTP, 50 μM dGTP and 2.5 μM [^{3}H]dTTP (0.5 Ci/mmol), pH 8.0 (8). Bleomycin-induced USD in permeable cells was measured in the same assay mixture except that ATP was omitted and 0.22 mM bleomycin A_2 was added. CTP, at a final concentration of 1 mM, was added to the assay mixture in some bleomycin-experiments for enhancing bleomycin-induced USD (25 and unpublished results). USD in liver nuclei was measured in the same assay mixture except that 4×10^6 nuclei in place of permeable cells were added to the assay mixture, and that ATP and Triton X-100 were omitted. For inhibition tests, 0.01 ml of either 2', 3'-dideoxythymidine-5'-triphosphate (ddTTP) or aphidicolin dissolved in dimethylsulfoxide (DMSO) was added to the assay mixture (8). An equal volume of DMSO was added to control tubes of aphidicolin reactions. Duplicate samples were incubated at 37℃ for 10 min for replicative DNA synthesis and for 60 min for USD unless otherwise stated. The radioactivity incorporated into acid insoluble materials was measured by a disc methods (22).

DNA synthesis in intact cells. Cells were cultured at 37℃ in 100-mm diameter plastic dishes until they became 60-80 % saturation density. Culture medium was removed. Half of the cultures were irradiated with 50 J/m^2 ultraviolet (UV) at room temperature. The flux of the GL-15 germicidal lamp (peak emission at 254 nm, Mitsubishi Electric Co.) used as the UV source was 0.83 W/m^2 as measured with a UV radiometer (Topcon UVR-254). Irradiated and non-irradiated cultures were harvested by trypsinization. Cells were washed once at 0°-4℃ with fresh culture medium, and suspended in fresh culture medium. Cells were carefully counted and distributed into assay tubes at 1×10^6 cells per tube. The final volume was adjusted to 0.6 ml per tube with fresh culture medium. When added, the concentration of hydroxyurea was 10 mM. Aphidicolin dissolved in DMSO was added at 10 μg/ml in a volume

of 0.01 ml, and an equal volume of DMSO was added to control tubes. The suspension was incubated at 37℃ for 30 min, and then 1 μCi of [^{3}H]deoxythymidine ([^{3}H]dThd, 5 Ci/mmol, Amersham) was added to the suspension. The incubation was continued for 4 h. Following the labeling period, the tubes were rapidly chilled to 0℃, and centrifuged at 1,000 xg for 10 min. The precipitated cells were suspended in 0.6 ml of Triton-buffer B, and 2 ml of ice-cold 7.5 % trichloroacetic acid containing 1 % sodium pyrophosphate was added to the suspension. The radioactivity was measured by a disc method (22). Autoradiography was conducted as described previously (22).

RESULTS

Effect of increasing the concentration of aphidicolin on DNA synthesis. Aphidicolin, a specific inhibitor of DNA polymerase α, inhibited replicative DNA synthesis (Fig. 1). Over 95 % of the replicative DNA synthesis in permeable HeLa cells and XC cells was inhibited by aphidicolin at 5 μg/ml (15 μM) or higher. Bleo-

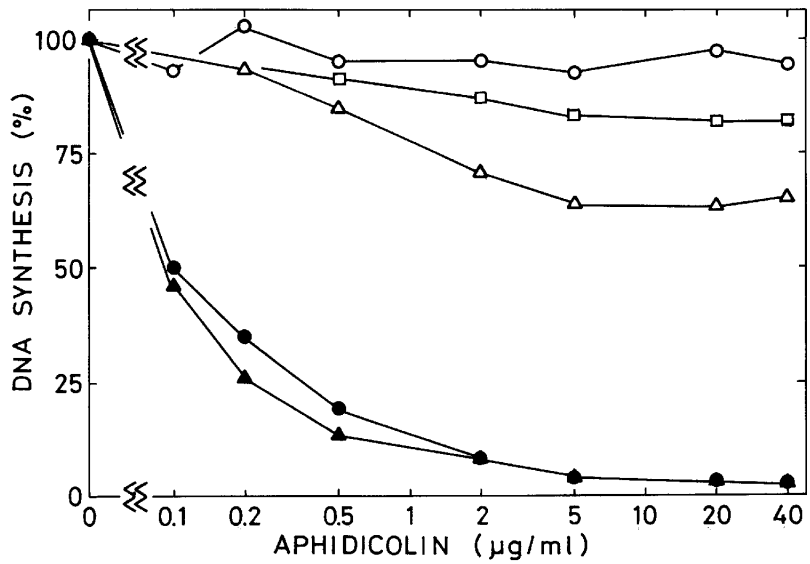

Fig. 1. Effects of increasing aphidicolin concentrations on replicative DNA synthesis and USD. HeLa and XC cells were permeabilized, and replicative DNA synthesis and bleomycin-induced USD were measured as described in Materials and Methods. Human liver nuclei were prepared, and USD in them was measured without bleomycin, as described in Materials and Methods. Aphidicolin dissolved in DMSO was added to the assay mixture at the indicated final concentrations in a final volume of 0.01 ml. An equal volume of DMSO was added to the control tubes. Results are expressed as a percentage of the activity measured in the absence of aphidicolin. Activity measured in the absence of aphidicolin was 13660cpm incorporated per 10^7 cells per 10min for replicative DNA synthesis in permeable HeLa cells (▲), 4180cpm incorporated per 10^7 cells per 60min for bleomycin-induced USD in permeable HeLa cells (△), 16570cpm incorporated per 10^7 cells per 10min for replicative DNA synthesis in permeable XC cells (●), 14300cpm incorporated per 10^7 cells per 60min for bleomycin-induced USD in permeable XC cells (○), and 15465cpm incorporated per 10^7 nuclei per 60min for USD in isolated human liver nuclei (□).

mycin-induced USD in permeable XC cells was not significantly inhibited by aphidicolin, whereas bleomycin-induced USD in permeable HeLa cells and USD in human liver nuclei were partially inhibited by aphidicolin (Fig. 1).

In vitro effects of aphidicolin and ddTTP on USD. That DNA synthesis in permeable cells treated with bleomycin was of the unscheduled type was confirmed autoradiographically (Fig. 2) (25). Few isotope grains were present in cells incubated with the ATP-, CTP- and bleomycin-free assay mixture (Fig. 2A). Some

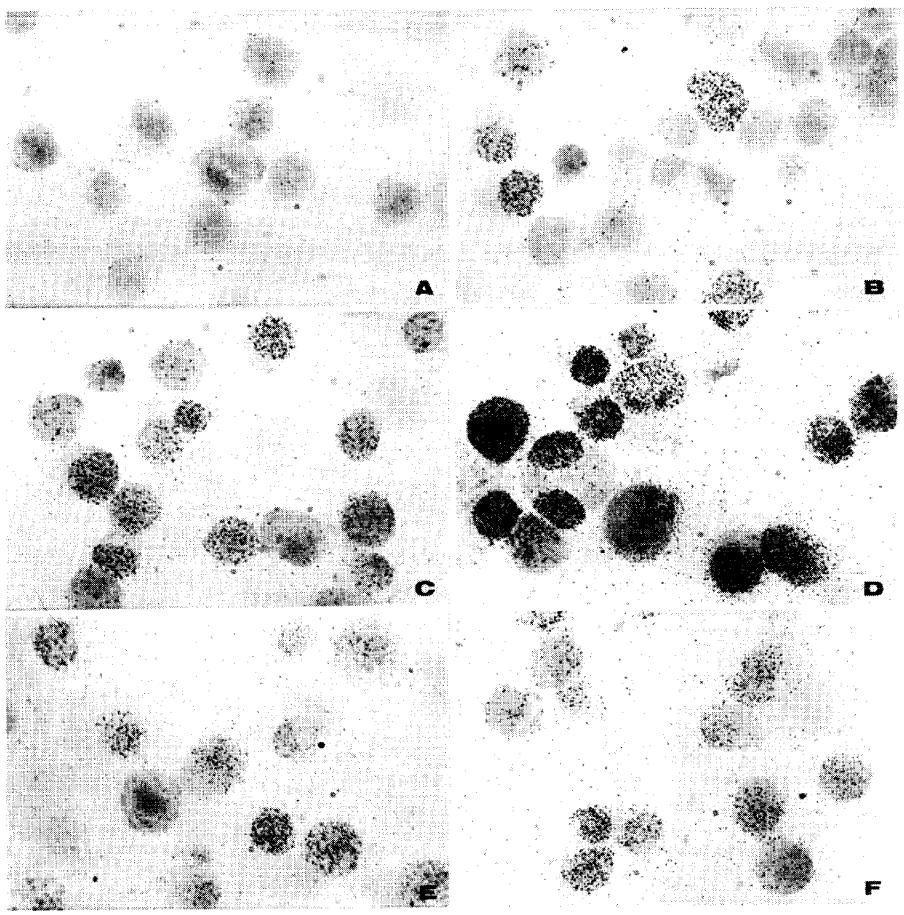

Fig. 2. Autoradiographic demonstration of bleomycin-induced USD in permeable HeLa cells. Permeable HeLa cells were incubated at 37℃ for 60min in the assay mixture for bleomycin-induced USD described in Materials and Methods, (A) except that bleomycin was omitted and specific activity of [^{3}H]dTTP was increased 20 times (10Ci/mmol) or in the assay mixture supplemented with (B) 1mM CTP; (C) 0.22mM bleomycin; (D) 1mM CTP and 0.22mM bleomycin; (E) 1mM CTP, 0.22mM bleomycin and 60μM ddTTP, or (F) 1mM CTP, 0.22mM bleomycin and 120μM aphidicolin. After incubation, cells were gently smeared on microscope slides, air dried and fixed with methanol. The slides were processed for autoradiography.

cells (about 15 % of the total) which were thought to be in the S-phase were significantly labeled with [^{3}H]dTMP when 1 mM CTP was added to the ATP-free assay mixture (Fig. 2B). USD was induced by bleomycin and over 95 % of the cells were labeled with [^{3}H]dTMP upon addition of bleomycin (Fig. 2C). CTP markedly enhanced bleomycin-induced USD (CTP-enhanced, bleomycin-induced USD) (Fig. 2D). Interference by replicative DNA synthesis of the USD determination in bleomycin-treated cells was estimated to be less than 10 % of the total activity, because over 90 % of the DNA synthesis depended on bleomycin which induces USD and concomitantly inhibits replicative DNA synthesis (Fig. 2) (25). Both aphidicolin (a polymerase α-specific inhibitor) and ddTTP (a preferential inhibitor of DNA polymerase β and γ) partially inhibited bleomycin-induced USD in almost all permeable HeLa cells, not partially in the sense of only a part of the cell population (Fig. 2E, F).

USD in rat liver nuclei, in bleomycin-treated permeable SR-C3H/He cells and also in bleomycin-treated permeable XC cells was not significantly inhibited by aphidicolin at 40 μg per ml (120 μM), but was greatly inhibited by 20 μM ddTTP at the ddTTP/dTTP ratio of 8 (Table 1). USD in human liver nuclei, in bleomycin-treated permeable HeLa cells and in bleomycin-treated permeable HEp-2 cells was partially inhibited by aphidicolin and by ddTTP. CTP-enhanced, bleomycin-induced USD in human cells showed higher sensitivity to aphidicolin than bleomycin-induced USD (Table 1). The increase in senitivity to aphidicolin may correspond to the increase in USD. A similar increase in aphidicolin-sen-

TABLE 1. EFFECTS OF APHIDICOLIN AND DDTTP ON USD IN LIVER NUCLEI AND BLEOMYCIN-TREATED PERMEABLE CELLS

Liver nuclei or permeable cells	Activity in control (mean ± S.D.)	% Activity to control (mean ± S.D.)	
		Aphidicolin	ddTTP
Human liver (2)	12770 ± 2695	82.2 ± 1.2	17.9 ± 0.2
Rat liver (3)	3808 ± 990	103.9 ± 3.6	8.2 ± 2.2
HeLa cells (4)	2549 ± 1038	72.9 ± 10.3	32.5 ± 1.9
HeLa cells (5) (CTP)	17168 ± 4156	57.4 ± 6.7	N.D.
HEp-2 cells (1)	2720	80.3	N.D.
HEp-2 cells (4) (CTP)	17315 ± 2329	66.0 ± 3.0	N.D.
SR-C3H/He cells (3)	4902 ± 1635	103.3 ± 3.3	9.4 ± 4.5
XC cells (1)	14300	93.5	7.7

USD in liver nuclei was measured without bleomycin. USD in permeable cells was induced by bleomycin, as described in Materials and Methods. Where added, the concentrations of aphidicolin and ddTTP were 120 μM and 20 μM, respectively. Activity in the inhibitor-free control is expressed as the cpm of [^{3}H] dTMP incorporated/10^7 cells/h. Numbers in parentheses indicate the number of independent experiments each performed in duplicate. CTP in parentheses indicates that 1 mM CTP was added to the assay mixture (20). ND: not determined.

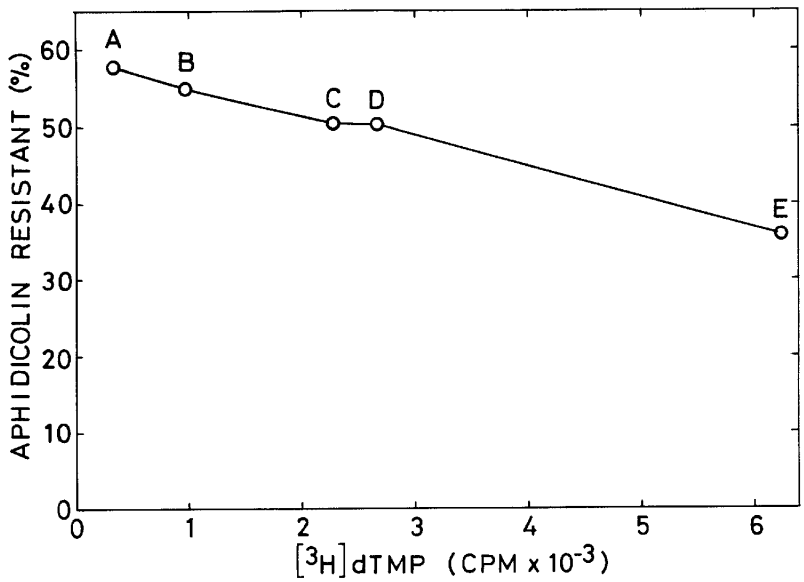

Fig. 3. Correlation between activity of bleomycin-induced USD and aphidicolin-sensitivity of the DNA synthesis. Permeable HeLa cells were pretreated at 0 ℃ for 60min with 1mM ATP and various concentrations of bleomycin. Cells were washed 3 times with Triton-buffer B. USD was measured at 37℃ for 30min with the same assay mixture as bleomycin-induced USD except that bleomycin was omitted. Concentrations of bleomycin were (A), 0; (B), 2.2 μM; (C), 22 μM; (D), 220 μM, and (E), 220 μM. In Experiment (E), 2.5mM ATP was added to the assay mixture.

TABLE 2. EFFECTS OF APHIDICOLIN ON UV-IRRADIATED AND NON-IRRADIATED CONTROL CELLS.

Cells	Condition	[^{3}H] dThd incorporated (cpm/10^6 cells/4 h)			
		DMSO	Aphidicolin	Hydroxyurea	
				DMSO	Aphidicolin
HeLa	UV	14600	3159	10003	1964
	Control	67228	2010	2063	664
	UV-dependent			7940	1300
HEp-2	UV	5332	3247	6423	3444
	Control	62634	1220	1667	407
	UV-dependent			4756	3037
XC	UV	14677	2928	2536	938
	Control	63159	2780	1420	427
	UV-dependent			1116	511

Experimental conditions for cell culture, UV-irradiation and DNA synthesis are described in Materials and Methods. The UV-dose was 50 J/m^2. Where added, concentrations of hydroxyurea and aphidicolin were 10 mM and 30 μM, respectively. DMSO, in which aphidicolin was dissolved, was added to control tubes.

sitivity in accordance with the increase in USD was also observed in bleomycin-preteated permeable HeLa cells (Fig. 3).

Effect of aphidicolin on USD in UV-irradiated living cells. Most of the replicative DNA synthesis in nonirradiated control cells was inhibited by 10 mM hydroxyurea, whereas USD in UV-irradiated cells was resistant or less sensitive to hydroxyurea (Table 2), as was been reported previously (26). Replicative DNA synthesis in control cells was inhibited up to 97 % by 30 μM aphidicolin (Table 2). DNA synthesis in UV-irradiated cells always showed less sensitivity to aphidicolin than that of replicative DNA synthesis, although the sensitivity was variable depending on cell type and the presence or absence of hydroxyurea. Autoradiographical studies showed that replicative DNA synthesis (Fig. 4A) was partially suppressed by UV-irradiation (Fig. 4E), and that USD was induced in almost all cells (Fig. 4E). Replicative DNA synthesis was greatly inhibited by either 30 μM aphidicolin or 10 mM hydroxyurea (Fig. 4B, C, D). Incorporation of [^{3}H]dThd due to USD in UV-irradiated HeLa cells was higher in the presence of hydroxyurea than in its absence (Fig. 4E, G), possibly due to the increase in specific activity of [^{3}H]-dThd as a result of the reduction of de novo synthesis of deoxythymidine nucleotide caused by hydroxyurea (27). Both the autoradiographical study and biochemical study showed that DNA synthesis measured in UV-irradiated cells in the presence of hydroxyurea was largely UV-induced USD. The aphidicolin-resistant fraction of DNA synthesis measured by [^{3}H]dThd uptake in UV-irradiated cells with hydroxyurea was 20 % for HeLa cells, 54 % for HEp-2 cells and 37 % for XC cells (Table 2). UV-dependent USD was tentatively calculated by subtracting [^{3}H]dThd incorporated in nonirradiated cells from [^{3}H]dThd incorporated in UV-irradiated cells (Table 2), but since UV-irradiation causes inhibition of replicative DNA synthesis, the value is somewhat an underestimation. This underestimation of the UV-dependent USD is minimized only when replicative DNA synthesis is inhibited completely by hydroxyurea. Although replicative DNA synthesis was not inhibited completely by hydroxyurea, the residual replicative DNA synthesis was much less than the DNA synthesis in UV-irradiated cells in the present experiment using HeLa and HEp-2 cells. Therefore, a rough estimation of USD was thought to be possible by the above calculation. The aphidicolin-resistant fraction in the calculated UV-dependent USD was 16 % for HeLa cells, 64 % for HEp-2 cells and 46 % for XC cells. The results of the two different methods of calculation of the aphidicolin-resistant fraction have essentially the same tendency, as expected from the result of autoradiographical studies. The much weaker aphidicolin sensitivity of UV-induced USD compared to that of replicative DNA synthesis suggests that both aphidicolin-sensitive and -insensitive DNA polymerases are involved in UV-induced USD in these cells.

Nuclease sensitivity of DNA synthesized in UV-irradiated cells. Studies on staphylococcal nuclease sensitivity of DNA synthesized in UV-irradiated HeLa cells in the presence of 10 mM hydroxyurea showed that DNA synthesized in the presence

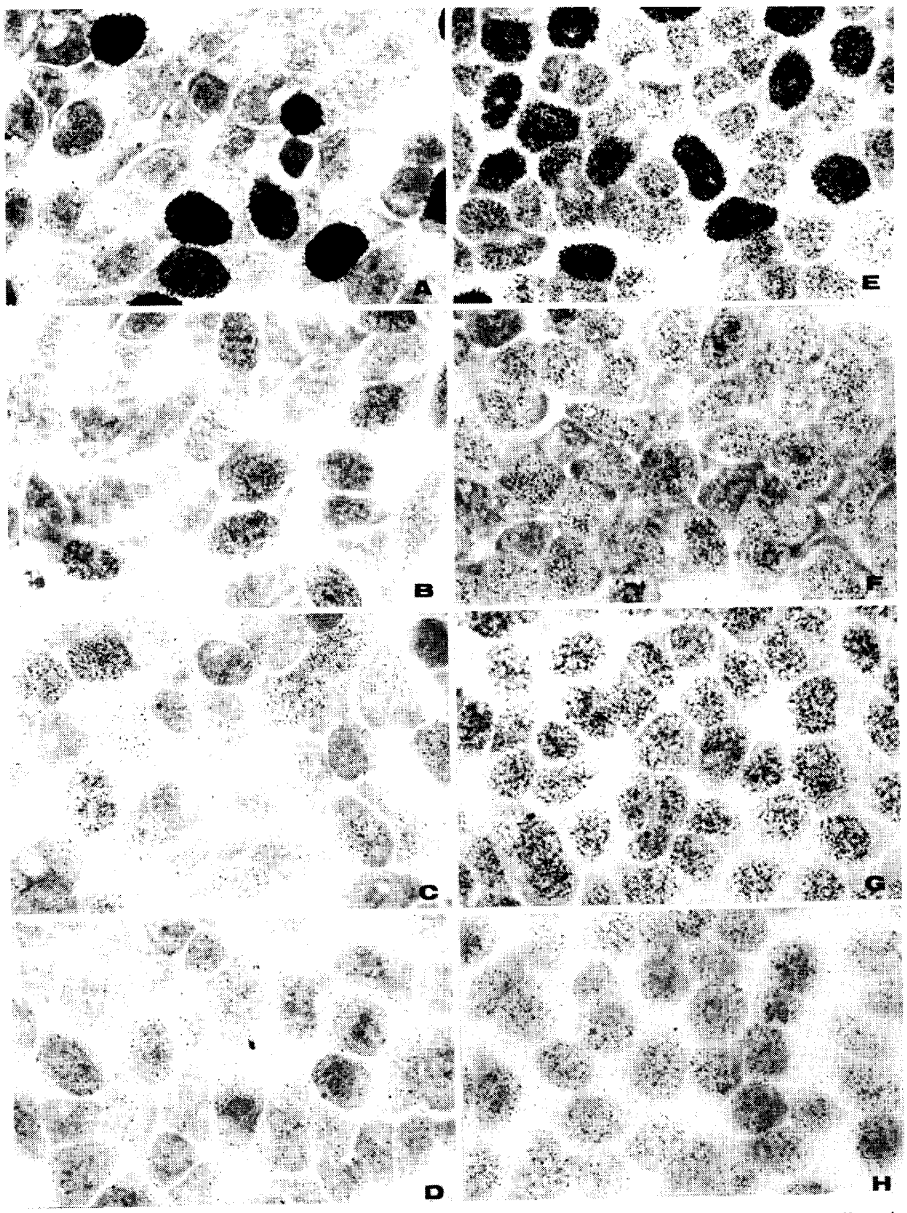

Fig. 4. Autoradiographic demonstration of UV-induced USD in HeLa cells. HeLa cells cultured on coverslips were divided into 2 groups: nonirradiated control (A, B, C, D) and UV-irradiated (50 J/m²) (E, F, G, H). Cells were fed fresh culture medium supplemented with or without 10mM hydroxyurea and 30μM aphidicolin. After 30min incubation at 37℃, [^{3}H]deoxythymidine (21 Ci/mmol) was added at 5μCi per ml, and the incubation was continued for 4h. The specimens were air dried, fixed with ethanol: glacial acetic acid (3:1). Nonirradiated cells were incubated with no inhibitor (A), 30μM aphidicolin (B), 10mM hydroxyurea (C), 30μM aphidicolin and 10mM hydroxyurea (D). UV-irradiated cells were incubated with no inhibitor (E), 30μM aphidicolin (F), 10mM hydroxyurea (G), 30μM aphidicolin and 10mM hydroxyurea (H).

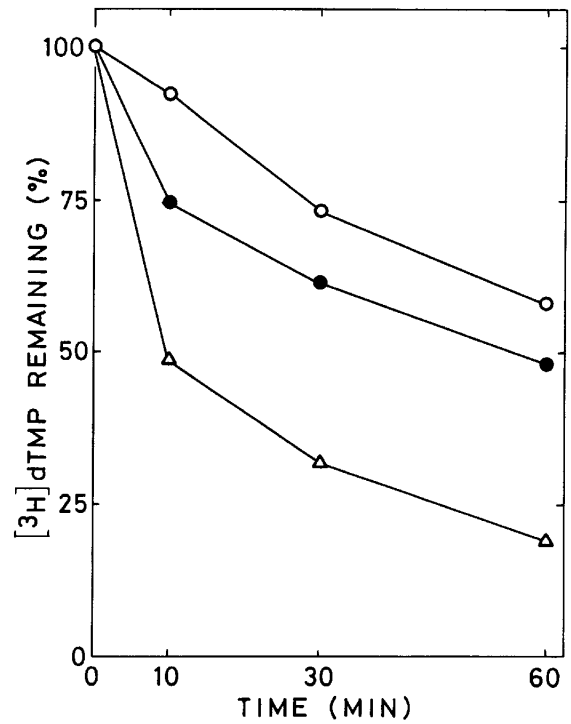

Fig. 5. The percentage of ^{3}H-labeled, newly synthesized DNA undigested by staphylococcal nuclease in HeLa cells after various times of incubation with the enzyme at 37℃. Replicative DNA synthesis with no addition of inhibitors (○), and UV-induced USD in the presence of 10mM hydroxyurea (●) or in the presence of 10mM hydroxyurea and 30 μM aphidicolin (△) were conducted and labeled with [^{3}H]dThd as described in Materials and Methods. [^{3}H]dThd-incorporated cells were permeabilized and digested at 37℃ for the indicated times with staphylococcal nuclease, and radioactivity in the acid insoluble fraction was measured as described previously (34).

of 30 μM aphidicolin was more sensitive to the nuclease than DNA synthesized in the absence of aphidicolin (Fig. 5). DNA synthesized by replication was more resistant to nuclease digestion than DNA synthesized in UV-irradiated cells in the absence of aphidicolin.

DISCUSSION

DNA polymerases in mammalian cells have been extensively studied (1-5), but their physiological roles are still controversial. The DNA polymerase involved in repair synthesis has not been identified yet, mainly because the machinery of DNA synthesis is very complicated and labile, and suitable mutants for DNA polymerases are not yet available in mammalian cells. Studies of DNA synthesis by using specific inhibitors of DNA polymerases provide indirect but valuable information about this complicated machinery.

Aphidicolin, a tetracyclic diterpene tetraol obtained from *Cephalosporium aphidicola* Petch, has been shown to be a specific inhibitor of DNA polymerase α having no effect on DNA polymerase β or γ (28, 29). There is ample evidence to show that DNA polymerase α is the sole target of aphidicolin *in vitro* and *in vivo* (29). The high sensitivity of DNA replication to aphidicolin provided the most support for the conclusion that DNA polymerase α is the major polymerase required for DNA replication.

Most previous reports suggested that the major polymerase involved in DNA repair or USD was DNA polymerase β. The involvement of DNA polymerase α in USD was demonstrated recently by using aphidicolin in permeable human cells and human cell nuclei (11-13), though the involvement of DNA polymerase β in USD was also shown by using the same drug (7-10). Involvement of both DNA polymerase α and β in USD was suggested by Miller and Chinault (19) and by us (20). They used Chinese hamster ovary cells permeabilized with lysolecithin, and suggested that the relative extent of involvement of DNA polymerase α and β in USD was related to the amount or type of DNA damage (19). We reported the possible involvement of DNA polymerase α and β in bleomycin-induced USD in permeable human cells and the possible difference in repair polymerases in bleomycin-induced USD between permeable human cells and rodent cells (20). Mattern *et al.* (30) suggested that the DNA polymerization step of repair might involve both the α- and β-type DNA polymerases, and that one type of DNA polymerase might be able to substitute for another.

In the present study, an extention of previous study (20), DNA polymerases involved in USD were examined in various cell preparations using aphidicolin and ddTTP. DNA polymerase β was thought to be the major polymerase to participate in USD in isolated rat liver nuclei and in bleomycin-treated permeable rodent cells, as suggested previously (8, 31). Participation of both aphidicolin-sensitive and -insensitive DNA polymerases (possibly DNA polymerase α and β) was shown in USD in bleomycin-treated permeable human cells, in human liver nuclei, and in UV-irradiated human and rodent cells. Precise estimation of the participation of aphidicolin-sensitive and -insensitive DNA polymerases in USD is difficult in living cells, because concentrations of deoxynucleoside triphosphates (dNTPs) in cells are variable and the sensitivity of DNA polymerase α to aphidicolin is altered by the concentration of dNTPs (16, 32). However, this difficulty was largely overcome in the present experiment by comparing aphidicolin-sensitivity of USD to that of replicative DNA synthesis in which the major polymerase involved is DNA polymerase α, and by using hydroxyurea which is known to inhibit replicative DNA synthesis by reducing dNTP-levels. As a result, the involvement of the two types of DNA polymerases, α and β, in USD was suggested. The participation of both types of DNA polymerases in USD was thought to be variable depending on cell type and the type and extent of DNA damage.

The conclusion of the present study on aphidicolin-sensitivity of USD in UV-

irradiated HeLa cells are incompatible with that reported by Hardt *et al.* (9). Hardt *et al.* measured autoradiographically the aphidicolin-effect on USD in UV-irradiated HeLa cells without using hydroxyurea, and claimed that aphidicolin did not inhibit the repair synthesis. The experimental results shown in Photos 1b and 1d in their paper (9) were obtained under similar conditions as ours shown in Fig. 4E and F. An important difference between the two experiments may be the concentrations of aphidicolin used, 15 μM in their experiment and 30 μM in our experiment. Comparison of the average number of grains per cell between USD measured without aphidicolin in UV-irradiated HeLa cells (Fig. 4E) and that with aphidicolin (Fig. 4F) showed that USD was inhibited about 20 % by 30 μM aphidicolin. The difference between their and our data is not so great and might be due mainly to the difference in the concentrations of aphidicolin used. As reported by others and in the present paper, the inhibition by aphidicolin of DNA synthesis in which DNA polymerase α is involved increased with increasing aphidicolin concentrations, and was higher when resting cells were used rather than rapidly growing cells (16) and hydroxyurea was used to reduce dNTP levels. Namely, the inhibition by aphidicolin fluctuates depending on the concentration ratio of aphidicolin to DNA polymerase α and to dNTPs. The aphidicolin concentration in the experiment reported by Hardt *et al.* (9) might not have been high enough to inhibit USD. Aphidicolin-sensitivity of USD in UV-irradiated cells was clearly demonstrated in the presence of 10 mM hydroxyurea (Table 2, Fig. 4G and H), possibly because hydroxyurea reduced intracellular concentrations of dNTPs which compete with aphidicolin (16, 32).

The finding that DNA synthesized in UV-irradiated cells in the absence of aphidicolin was more resistant to staphylococcal nuclease than that synthesized in the presence of aphidicolin suggested that the involvement of DNA polymerase α in USD was favorable to DNA synthesis in the intranucleosomal region (33). To clarify further the involvement of the two types of DNA polymerases in USD, it will be necessary to study their functional differences and any cooperation that might exist between them.

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