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DIFFERENTIAL SENSITIVITY TO APHIDICOLIN OF REPLICATIVE DNA SYNTHESIS AND ULTRAVIOLET-INDUCED UNSCHEDULED DNA SYNTHESIS *IN VIVO* IN MAMMALIAN CELLS

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Abstract. *In vivo* in mammalian cells, ultraviolet-induced unscheduled DNA synthesis was less sensitive to aphidicolin than was replicative DNA synthesis. Replicative DNA synthesis in HeLa, HEp-2, WI-38 VA-13 and CV-1 cells was inhibited more than 97 % by aphidicolin at 10 μg/ml, whereas aphidicolin inhibition of DNA synthesis in ultraviolet-irradiated cells varied between 30 % and 90 % depending on cell types and assay conditions. Aphidicolin inhibition of unscheduled DNA synthesis (UDS) in HeLa cells increased gradually with increasing aphidicolin concentration and reached approximately 90 % at 100 μg/ml aphidicolin. A significant fraction of UDS in ultraviolet-irradiated HEp-2 cells was resistant to aphidicolin even at 300 μg/ml. Considered along with related information reported previously, the present results suggest that both aphidicolin-sensitive and insensitive DNA polymerases, DNA polymerase α and a non-α DNA polymerase (possibly DNA polymerase β), are involved in *in situ* UDS in these ultraviolet-irradiated cells. Comparison of staphylococcal nuclease sensitivity between DNAs repaired in the presence and in the absence of aphidicolin in HEp-2 cells suggested that the involvement of DNA polymerase α in UDS favored DNA synthesis in the intranucleosomal region.

Key words : unscheduled DNA synthesis (mammalian cells), DNA polymerase, aphidicolin, ultraviolet irradiation.

Aphidicolin has been shown to be a specific inhibitor of DNA polymerase α without effect on DNA polymerase β or γ (1, 2). Ample evidence shows that DNA polymerase α is the sole target for aphidicolin not only *in vitro* but also *in vivo* (1). Since DNA replication is highly sensitive to aphidicolin, DNA polymerase α is considered to be the major polymerase required for DNA replication. The application of aphidicolin for studying DNA polymerase involved in DNA repair replication or unscheduled DNA synthesis (UDS) has provided conflicting results. Some studies implicated DNA polymerase α and others DNA polymerase β in UDS, as discussed in a previous paper (3). Involvement of both DNA polymerases α and β in UDS was also suggested recently (3-7). Differences in sensitivity to aphidicolin between UDS and replicative DNA synthesis were shown in previous reports (8-10) in which the involvement of DNA polymerase α in UDS was claimed, but the differences have not been studied in detail.

Comparative study of aphidicolin sensitivity between UDS and replicative DNA synthesis, whose principal polymerase is known to be DNA polymerase α, should provide important information on repair polymerase. In the present paper aphidicolin sensitivity of ultraviolet (UV)-induced UDS in mammalian cells was compared with that of replicative DNA synthesis.

MATERIALS AND METHODS

The reagents used were obtained as described previously (3). HeLa-S3, HEp-2, WI-38 VA-13, CV-1 and XC cells were obtained and cultured as described previously (5).

Assay of DNA synthetic activity in intact cells. DNA synthesis in intact cells was measured as described previously (3). In brief, cells were cultured in 100-mm diameter plastic dishes. After removing culture medium, a half of cultures was irradiated with 50 J/m^2 UV at room temperature. Irradiated and non-irradiated control cultures were harvested by trypsinization. Cells were washed once with fresh culture medium and suspended in fresh culture medium, and then distributed into assay tubes at 1×10^6 cells per tube. Aphidicolin dissolved in dimethylsulfoxide (DMSO) was added in a volume of 0.01 ml. An equal volume of DMSO was added to control tubes for aphidicolin reactions. When added, the concentration of hydroxyurea was 10 mM. The final volume was adjusted to 0.6 ml per tube with fresh culture medium. The suspension was incubated at 37 ℃ for 30 min, and then 1 μCi [^{3}H]dThd (5 Ci/mmol, Amersham) was added. The incubation was continued for 4 h. After the labeling period, the tubes were rapidly chilled at 0 ℃ and centrifuged at 1,000 xg for 10 min. The precipitated cells were suspended in 0.6 ml of a hypotonic buffer (10 mM Tris-HCl, 4 mM $MgCl_2$ and 1 mM EDTA, pH 8.0). The radioactivity incorporated into acid insoluble materials was measured by a disc method (11).

Assay of repair synthesis in permeable cells. UV-irradiated cells suspended at 1×10^6 cells per 0.6 ml in fresh culture medium were incubated with 10 mM hydroxyurea at 37 ℃ for 1 h. Then, the cells were permeabilized with Triton-buffer B (0.0175 % Triton X-100, 0.25 M sucrose, 10 mM Tris-HCl, 4 mM $MgCl_2$ and 1 mM EDTA, pH 8.0), as described previously (5). DNA synthesis in permeable cells was assayed in a mixture with a final volume of 0.6 ml containing 1×10^6 cells, 0.0117 % Triton X-100, 0.167 M sucrose, 0.67 mM EDTA, 40 mM Tris-HCl, 5 mM $MgCl_2$, 0.08 M NaCl, 50 μM dATP, 10 μM dCTP, 50 μM dGTP and 2.5 μM [^{3}H]dTTP (5 Ci/mmol), pH 8.0. The mixture was incubated at 37 ℃ for 30 min. Inhibition tests were performed as described previously (5).

Preparation for autoradiographical examination. Cells were cultured on coverslips in 50 mm-diameter plastic dishes for 3 days. Culture medium was removed. A half of the cultures was irradiated with 50 J/m^2 UV. Fresh culture medium was added to the irradiated and non-irradiated control cultures. Aphidicolin and hydroxyurea were added as described above. The final volume was adjusted to 2 ml per dish with fresh culture medium. The dishes were cultured at 37 ℃ for 30 min, and then 10 μCi [^{3}H]dThd (21 Ci/mmol) was added. The cultivation was continued for 4 h. After the labeling period, cells were fixed for 10 min with ethanol : glacial acetic acid (3 : 1). Autoradiography was conducted as described previously (11).

RESULTS

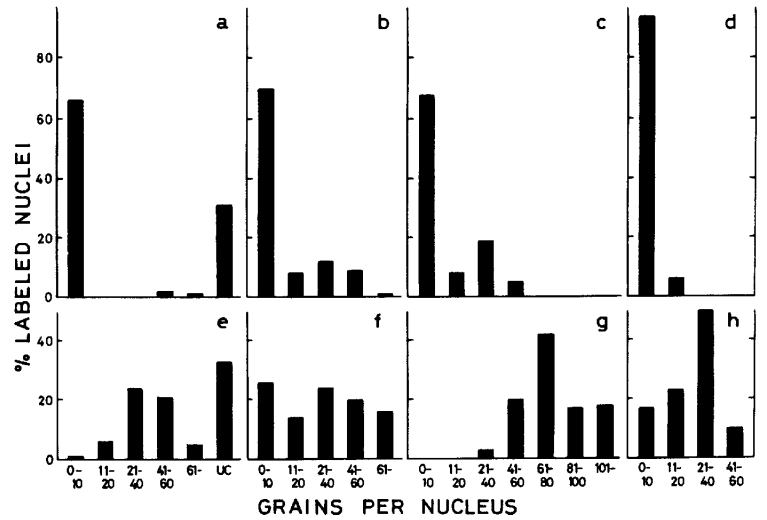
Biochemical and autoradiographical studies showed that UV irradiation markedly suppressed replicative DNA synthesis and induced UDS in almost all cells (Table 1, Figs. 1e and 2e). Cells having uncountable or numerous grains (shown in the columns of "UC" in Figs. 1 and 2) were thought to be in S-phase. Replicative DNA synthesis in non-irradiated control cells (shown in "Control "lines in Table 1, in Figs. 1a-d and in Figs. 2a-d) was mostly inhibited by 10 mM hydroxyurea, whereas DNA synthesis in UV-irradiated cells (shown in "UV" lines in Table 1, in Figs. 1e-h and Figs. 2e-h) was resistant to hydroxyurea, as reported previously (12).

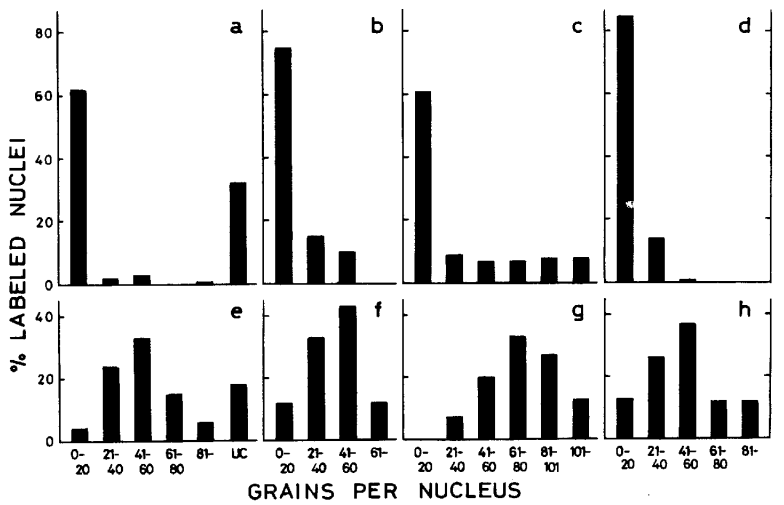
TABLE 1. REPRESENTATIVE DATA OF DNA SYNTHESIS IN UV-IRRADIATED OR NONIRRADIATED CELLS MEASURED WITH OR WITHOUT HYDROXYUREA AND APHIDICOLIN

Cells	Condition	[^{3}H]dThd incorporated (cpm/10^6 cells/4 h)			
		DMSO	Aphidicolin	Hydroxyurea	
				DMSO	Aphidicolin
HeLa	UV	14727	4990	11923	1818
	Control	93503	1909	1877	478
	UV-dependent			10046	1340
HEp-2	UV	6646	3224	7123	4305
	Control	61842	1349	2255	593
	UV-dependent			4868	3712
WI-38 VA-13	UV	2366	1139	2063	366
	Control	18045	383	531	112
	UV-dependent			1532	254
CV-1	UV	3047	976	5809	668
	Control	21728	539	610	154
	UV-dependent			5199	514
XC	UV	16181	3365	2887	1041
	Control	53338	3246	1382	475
	UV-dependent			1505	566

Aphidicolin dissolved in DMSO was added to the assay mixture at 10 μg/ml in a volume of 0.01 ml. An equal volume of DMSO was added to the control tubes. UV-dependent DNA synthesis was calculated by subtracting replicative DNA synthesis (shown in Control lines) measured under the presence of hydroxyurea from DNA synthesis in UV-irradiated cells (shown in UV lines). Each datum shows the mean value of duplicate determinations.

Replicative DNA synthesis in control cells was mostly (up to 98 %) inhibited by aphidicolin at 10 μg/ml (Table 2). Aphidicolin sensitivity of DNA synthesis in UV-irradiated cells varied depending on cell type and on whether hydroxyurea





Figs. 1 and 2. Autoradiographic demonstration of UV-induced UDS in HeLa (Fig. 1) and HEp-2 (Fig. 2) cells. Cells cultured on coverslips were divided into 2 groups, one for nonirradiated control cells (a, b, c, d) and the other for UV-irradiated cells (e, f, g, h). Nonirradiated and UV-irradiated cells were incubated with no inhibitor (a, e), 10 μg/ml aphidicolin (b, f), 10 mM hydroxyurea (c, g), and 10 μg/ml aphidicolin and 10 mM hydroxyurea (d, h). Grains were counted in 100 nuclei per slide. Cells having uncountable number of grains are shown in the columns of "UC". Total grains in 100 nuclei were : (1b) 1180, (1c) 1037, (1d) 208, (1f) 3170, (1g) 7735, (1h) 2203, (2b) 1349, (2c) 2982, (2d) 944, (2f) 4085, (2g) 7639 and (2h) 4818.

TABLE 2. APHIDICOLIN-RESISTANT FRACTIONS OF REPLICATIVE DNA SYNTHESIS AND UDS IN UV-IRRADIATED CELLS

Cells[a]	Aphidicolin-resistant DNA synthesis (%) (Mean ± S.D.)			
	Replicative DNA Synthesis	DNA synthesis in UV-irradiated cells		
		Without hydroxyurea	With hydroxyurea	UV-dependent[b]
HeLa (4)	2.3 ± 0.3	31.8 ± 8.7	17.4 ± 2.9	15.0 ± 3.3
HEp-2 (5)	2.1 ± 0.2	47.8 ± 8.7	54.6 ± 3.8	69.4 ± 7.5
WI-38 VA-13 (2)	2.2 ± 0.1	52.7 ± 4.6	20.2 ± 2.5	20.0 ± 3.4
CV-1 (4)	2.4 ± 0.4	30.8 ± 4.9	12.2 ± 2.1	10.1 ± 2.5
XC (3)	5.9 ± 1.1	19.5 ± 1.2	34.4 ± 3.1	35.7 ± 9.1

a) Numbers in parentheses indicate number of independent experiments each performed in duplicate.
b) UV-dependent DNA synthesis was calculated as described in Table 1.

was present or absent (Table 2). DNA synthesis measured without hydroxyurea in UV-irradiated cells was a mixture of replicative DNA synthesis and UDS and was less sensitive to aphidicolin than replicative DNA synthesis in the control cells (Tables 1 and 2). Hydroxyurea-resistant DNA synthesis in UV-irradiated cells was largely unscheduled (Figs. 1 and 2). A combination of 10 μg/ml aphidicolin and 10 mM hydroxyurea inhibited most replicative DNA synthesis, whereas reduced but significant isotope labels due to UDS were still observed in almost all cells (Figs. 1d, 1h, 2d and 2h). To calculate aphidicolin sensitivity UDS was roughly estimated in the following two ways. DNA synthesis measured in the presence of 10 mM hydroxyurea in UV-irradiated cells was roughly estimated to be UDS, or UV-dependent UDS was tentatively calculated by subtracting [^{3}H]-dThd incorporated in nonirradiated cells in the presence of hydroxyurea from [^{3}H]dThd incorporated in UV-irradiated cells, as shown in Table 1. Since UV irradiation inhibits replicative DNA synthesis, UDS is underestimated in UV-dependent UDS calculated as described above. The underestimation of UDS is minimized when replicative DNA synthesis is inhibited almost completely by hydroxyurea. Although replicative DNA synthesis was not inhibited completely by 10 mM hydroxyurea, the residual replicative DNA synthesis was much less than DNA synthesis in UV-irradiated cells in the present HeLa and HEp-2 cell experiments. Therefore, the above calculation was thought to provide a rough estimation of UDS. Aphidicolin resistant fractions of UDS estimated by the above two ways showed similar values (Table 2).

Incorporation of [^{3}H]dThd due to UDS in UV-irradiated HeLa or HEp-2 cells was apparently higher in the presence than in the absence of hydroxyurea (Figs. 1e, 1g, 2e and 2g), possibly due to the increase of specific activity of [^{3}H]dThd as a result of the reduction in de novo synthesis of deoxythymidine nucleotide in the presence of hydroxyurea (13).

Patterns of concentration-dependent inhibition by aphidicolin were almost the same in replicative DNA synthesis between HeLa and HEp-2 cells, were different between replicative DNA synthesis and DNA synthesis in UV-irradiated cells, and also were different in DNA synthesis in UV-irradiated HeLa and HEp-2 cells (Figs. 3a and b). Marked differences in the inhibition rates between replicative DNA synthesis and DNA synthesis in UV-irradiated cells were observed at 1 μg/ml aphidicolin. More than 99 % of replicative DNA synthesis in both types of cells was inhibited at 100 μg/ml aphidicolin. About 90 % of DNA synthesis in UV-irradiated HeLa cells was inhibited at the same concentration of aphidicolin (Fig. 3). DNA synthesis in the absence of hydroxyurea in UV-irradiated HEp-2 cells was maximally inhibited by aphidicolin at 5-20 μg/ml, and the inhibition was approximately 50 %. Higher concentrations of aphidicolin did not increase the inhibition. DNA synthesis in the presence of 10 mM hydroxyurea in UV-irradiated HEp-2 cells was maximally inhibited by aphidicolin at 50-300 μg/ml, and the inhibition was approximately 80 %.

Effects of some selective inhibitors for DNA polymerases on UV-induced UDS were studied further by using permeable cells prepared from UV-irradiated HEp-2 cells (Table 3). DNA synthesis measured in permeable cells as described in Materials and Methods was thought to be mostly unscheduled, because more than 80 % of the DNA synthesis depended on UV-irradiation. UDS in UV-pretreated, permeable HEp-2 cells was inhibited about 40 % by 10 μg/ml aphidicolin. The inhibition rate by aphidicolin was almost the same as that of UDS measured *in vivo* in UV-irradiated cells (Tables 2 and 3). UDS was slightly sensitive to 20 μM 2', 3'-dideoxythymidine-5'-triphosphate (ddTTP) and highly sensitive to 1 mM N-ethylmaleimide (Table 3).

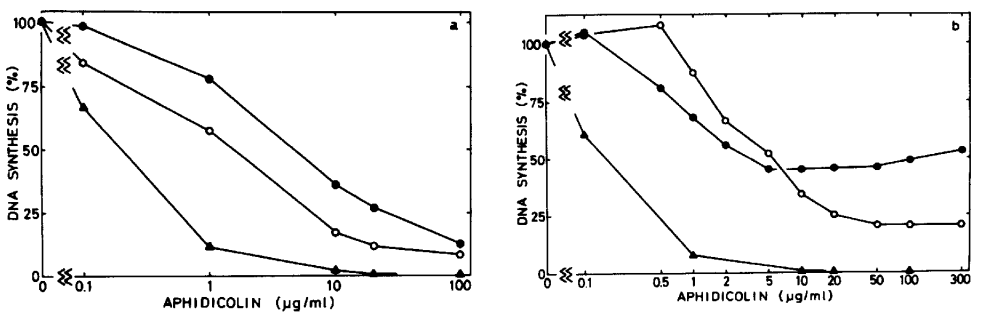


Fig. 3. Effects of increasing aphidicolin concentrations on replicative DNA synthesis and UV-induced UDS. DNA synthesis in HeLa cells (a) and in HEp-2 cells (b) was measured as described in Materials and Methods. Results are expressed as a percentage of the activity measured in the absence of aphidicolin. Assays were performed in duplicate. Variation in the results for duplicate tests was mostly less than 10 %. ▲, replicative DNA synthesis ; ●, DNA synthesis measured without hydroxyurea in UV-irradiated cells ; ○, DNA synthesis measured with 10 mM hydroxyurea in UV-irradiated cells.

TABLE 3. EFFECT OF COMBINATION OF INHIBITORS ON UDS INDUCED BY UV-IRRADIATION IN HEp-2 CELLS AND MEASURED AFTER PERMEABILIZATION

Inhibitor			UDS (c.p.m. per 10^6 cells	% activity to inhibitor-free control
ddTTP	Aphidicolin	N-ethylmaleimide		
−	−	−	1910	100
+	−	−	1745	91
−	+	−	1135	59
−	−	+	179	9
+	+	−	1097	57
+	−	+	84	4
−	+	+	93	5

HEp-2 cells were irradiated with UV at 50 J/m² and incubated with 10 mM hydroxyurea in *in vivo* at 37 ℃ for 1 h, and then permeabilized. DNA synthesis in permeable cells was measured as described in Materials and Methods. 2-Mercaptoethanol was omitted. Where added (+) the concentrations of ddTTP, aphidicolin and N-ethylmaleimide were 20 μM, 10 μg/ml and 1 mM, respectively. Assay was performed in duplicate.

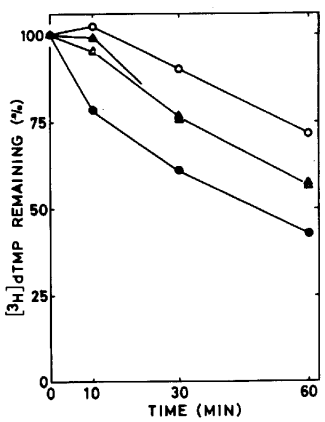


Fig. 4. Percentage of ³H-labeled, newly synthesized DNA in HEp-2 cells undigested by staphylococcal nuclease after various times of incubation with the enzyme at 37 ℃. DNA synthesis was conducted and cells were labeled with [³H]dThd as described in Materials and Methods, except that the incubation time of some samples was reduced to 2 h. Radioactivities incorporated were 66141 cpm/10^6 cells for replicative DNA synthesis measured for 4 h with no addition of inhibitors (○), 8692 cpm for UV-induced UDS measured for 4 h in the presence of 10 mM hydroxyurea (▲), 5041 cpm for UV-induced UDS measured for 2 h in the presence of 10 mM hydroxyurea (△), and 4476 cpm for UV-induced UDS measured for 4 h in the presence of 10 mM hydroxyurea and 10 μg/ml aphidicolin (●). [³H]dThd-incorporated cells were permeabilized and digested at 37 ℃ for the indicated times with 2.5 units staphylococcal nuclease, and radioactivity in acid insoluble fraction was measured as described previously (14). Assays were performed in duplicate. Variation in the results for duplicate tests was less than 10 %.

In the presence of 10 mM hydroxyurea in UV-irradiated HEp-2 cells, DNA synthesized in the presence of 10 μg/ml aphidicolin was more sensitive to staphylococcal nuclease than was DNA synthesized in the absence of aphidicolin (Fig. 4). The similar sensitivity difference was also observed in UDSs measured in the presence and absence of aphidicolin in HeLa cells as reported previously (3). The sensitivity difference was not due to the difference in amounts of DNA synthesis, because no change in the nuclease sensitivity was observed when the amount of DNA synthesized in the absence of aphidicolin in UV-irradiated cells was reduced to the level of DNA synthesis in the presence of aphidicolin by shortening the incubation time for DNA synthesis.

DISCUSSION

It has been shown that the inhibition of DNA polymerase α by aphidicolin is influenced by dNTP concentrations (or pool sizes) and DNA polymerase α levels in the assay system, and that the inhibition rate is reduced by the presence of the higher concentrations of dNTPs and DNA polymerase α (1, 15). Snyder and Regan (16) showed that inhibition of DNA repair in human cells by aphidicolin was very efficient in confluent resting cells but not in rapidly cycling cells and suggested that differences in dNTP pools between cycling and confluent resting cells determined the different efficacies of the agent in these two situations. A similar phenomenon was observed by some other investigators, although the explanations of the phenomenon were not necessarily identical (9, 17-19).

We compared aphidicolin sensitivities between replicative DNA synthesis and UV-induced UDS *in vivo* in some rapidly cycling mammalian cells. UV-induced UDS was partially inhibited by aphidicolin but was less sensitive to aphidicolin than replicative DNA synthesis in all types of cells tested. The sensitivity difference between replicative DNA synthesis (occurring in S-phase) and UDS (occurring in all phases of the cell cycle) was not likely due to the differences in dNTP pools and DNA polymerase α levels in cells, because both dNTP and DNA polymerase α levels in cells are known to be higher in S-phase than in G_1-phase in the cell cycle (20-22), and these cells (doubling time : about 24 h for HeLa and HEp-2 cells) were thought to be largely in either G_1- or S-phase. The above idea was also supported by the autoradiographical finding that UV-induced UDS was less sensitive to aphidicolin than was replicative DNA synthesis in almost all cells rather than in a limited population of them. The present results indicate that both aphidicolin-sensitive DNA polymerase (DNA polymerase α) and aphidicolin-resistant DNA polymerase (non-α DNA polymerase) are involved in UV-induced UDS in mammalian cells.

Involvement of aphidicolin-resistant DNA polymerase in UV-induced UDS was more strongly suggested in HEp-2 cells in the present experiment. DNA synthesis in UV-irradiated HEp-2 cells was maximally inhibited by aphidicolin at 5-10 μg/ml and reached about 50 % of activity of the aphidicolin-free control.

No further inhibition occurred with increasing aphidicolin concentrations. Hydroxyurea treatment of cells increased the sensitivity to aphidicolin, as reported previously (16). About 80 % of UV-induced UDS in HEp-2 cells was inhibited maximally by aphidicolin in the presence of hydroxyurea. The increase of about 30 % in the maximum inhibition rate due to the presence of hydroxyurea could not simply be explained by the hydroxyurea effect of reducing dNTP levels, because the rate of inhibition by aphidicolin of UDS measured without hydroxyurea did not approach 80 % with increasing concentrations of aphidicolin, which competes with dNTPs. Other unknown effects of hydroxyurea on UDS (23) may be involved in the difference.

The partial sensitivity to aphidicolin of UV-induced UDS was also shown in permeable HEp-2 cells, in which concentrations of dNTPs and inhibitors were manipulated easily. Characterization of the non-α DNA polymerase involved in UV-induced UDS was tried by using the permeable cell system. UDS was highly sensitive to 1 mM N-ethylmaleimide which preferentially inhibits DNA polymerases α and γ, and was slightly sensitive to 20 μM ddTTP which preferentially inhibits DNA polymerases β and γ. Although the result confirmed the involvement of DNA polymerase α in UV-induced UDS, involvement of either DNA polymerase β or γ was not clearly demonstrated. A few previous reports suggested the involvement of both DNA polymerases α and β in UDS (3-7). Among them Dresler and Lieberman (7) reported the similar high sensitivity to N-ethylmaleimide of UV-induced UDS, as we reported in the present paper, and suggested that a N-ethylmaleimide sensitive factor was involved in polymerase β-mediated repair synthesis. Although further studies are required to ascertain DNA polymerase β as the non-α DNA polymerase involved in UV-induced UDS, it is possible that both DNA polymerases α and β are involved cooperatively in UV-induced UDS, as suggested in bleomycin-induced UDS (5).

To clarify further the involvement of both DNA polymerases α and β in UDS, it is important to know their functional difference and possible cooperation in UDS. The finding that DNA synthesized in UV-irradiated HEp-2 cells in the absence of aphidicolin was more resistant to staphylococcal nuclease than that in the presence of aphidicolin suggested that DNA repair synthesis in the intranucleosomal region occurred more easily with than without involvement of DNA polymerase α in UDS.

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REFERENCES

1. Huberman, J.A.: New views of the biochemistry of eukaryotic DNA replication revealed by aphidicolin, an unusual inhibitor of DNA polymerase α. *Cell* **23,** 647-648, 1981.
2. Ikegami, S., Taguchi, T., Ohashi, M., Oguro, M., Nagano, H. and Mano, Y.: Aphidicolin prevents mitotic cell division by interfering with the activity of DNA polymerase-α. *Nature* **275,** 458-460, 1978.
3. Seki, S., Hosogi, N. and Oda, T.: Participation of DNA polymerases α and β in unscheduled DNA synthesis in mammalian cells. *Acta Med. Okayama* **37,** 213-225, 1983.
4. Miller, M.R. and Chinault, D.N.: Evidence that DNA polymerases α and β participate differentially in DNA repair synthesis induced by different agents. *J. Biol. Chem.* **257,** 46-49, 1982.
5. Seki, S., Ohashi, M., Ogura, H. and Oda, T.: Possible involvement of DNA polymerases α and β in bleomycin-induced unscheduled DNA synthesis in permeable HeLa cells. *Biochem. Biophys. Res. Commun.* **104,** 1502-1508, 1982.
6. Cleaver, J.E.: Structure of repaired sites in human DNA synthesized in the presence of inhibitors of DNA polymerases alpha and beta in human fibroblasts. *Biochim. Biophys. Acta* **739,** 301-311, 1983.
7. Dresler, S.L. and Lieberman, M.W.: Identification of DNA polymerases involved in DNA excision repair in diploid human fibroblasts. *J. Biol. Chem.* **258,** 9990-9994, 1983.
8. Berger, N.A., Kurohara, K.K., Petzold, S.J. and Sikorski, G.W.: Aphidicolin inhibits eukaryotic DNA replication and repair --- implications for involvement of DNA polymerase α in both processes. *Biochem. Biophys. Res. Commun.* **89,** 218 -225, 1979.
9. Bohr, V. and K$\not$ber, L.: The effect of aphidicolin on DNA repair in resting and mitogen stimulated human lymphocytes. *Biochem. Biophys. Res. Commun.* **108,** 797-803, 1982.
10. Miller, M.R. and Chinault, D.N.: The roles of DNA polymerases α, β, and γ in DNA repair synthesis induced in hamster and human cells by different DNA damaging agents. *J. Biol. Chem.* **257,** 10204-10209, 1982.
11. Seki, S. and Oda, T.: DNA synthesis in permeable mouse ascites sarcoma cells. *Cancer Res.* **37,** 137-144, 1977.
12. Smith, C.A. and Hanawalt, P.C.: Repair replication in human cells ; simplified determination utilizing hydroxyurea. *Biochim. Biophys. Acta* **432,** 336-347, 1976.
13. Lampidis, T.J. and Little, J.B.: Enhancement of UV-induced unscheduled DNA synthesis by hydroxyurea. *Exp. Cell Res.* **110,** 41-46, 1977.
14. Seki, S., Watanabe, S. and Oda, T.: Replicative DNA synthesis and unscheduled DNA synthesis in permeable sarcoma cells studied by nuclease digestion. *Biochim. Biophys. Acta* **607,** 36-42, 1980.
15. Oguro, M., Suzuki-Hori, C., Nagano, H., Mano, Y. and Ikegami, S.: The mode of inhibitory action by aphidicolin on eukaryotic DNA polymerase α. *Eur. J. Biochem.* **97,** 603-607, 1979.
16. Snyder, R.D. and Regan, J.D.: Differential responses of log and stationary phase human fibroblasts to inhibition of DNA repair by aphidicolin. *Biochim. Biophys. Acta* **697,** 229-234, 1982.
17. Bermúdez, E.: The inhibition of DNA repair in primary rat hepatocyte cultures by aphidicolin : evidence for the involvement of alpha polymerase in the repair process. *Biochim. Biophys. Res. Commun.* **109,** 275-281, 1982.
18. Collins, A.R.S., Squires, S. and Johnson, R.T.: Inhibitors of repair DNA synthesis. *Nucleic Acids Res.* **10,** 1203-1213, 1982.
19. Van Zeeland, A.A., Bussmann, C.J.M., Degrassi, F., Filon, A.R., Van Kosteren-van Leeuwen, A.C., Palitti, F. and Natarajan, A.T.: Effects of aphidicolin on repair replication and induced chromosomal aberrations in mammalian cells. *Mutation Res.* **92,** 379-392, 1982.

20. Sarngadharan, M.G., Robert-Guroff, M. and Gallo, R.C.: DNA polymerases of normal and neoplastic mammalian cells. *Biochim. Biophys. Acta* **516,** 419-487, 1978.
21. Reichard, P.: From deoxynucleotides to DNA synthesis. *Fed. Proc.* **37,** 9-14, 1978.
22. Skoog, K.L., Bjursell, K.G. and Nordenskjöld, B.A.: Cellular deoxyribonucleotide triphosphate pools levels and DNA synthesis. *Adv. Enzyme Regul.* **12,** 345-354, 1974.