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The Roles of DNA Polymerases $\alpha$, $\beta$, and $\gamma$ in DNA Repair Synthesis Induced in Hamster and Human Cells by Different DNA Damaging Agents*

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The involvement of DNA polymerases $\alpha$, $\beta$, and $\gamma$ in DNA repair synthesis was investigated in subcellular preparations of cultured hamster and human cells. A variety of DNA damaging agents, including bleomycin, neocarzinostatin, UV irradiation, and alkylating agents, were utilized to induce DNA repair. The sensitivity of repair synthesis, as well as replicative synthesis and purified DNA polymerase $\beta$ activity, to inhibition by the DNA polymerase inhibitors dideoxythymidine triphosphate, aphidicolin, cytosine arabinoside triphosphate, and N-ethylmaleimide was determined. No evidence was obtained for a major role of polymerase $\gamma$ in any type of repair synthesis. In both hamster and human cells, the sensitivity of bleomycin- and neocarzinostatin-induced repair synthesis to ddTTP inhibition was essentially identical with that observed for purified polymerase $\beta$, indicating these repair processes proceeded through a mechanism utilizing polymerase $\beta$. Repair synthesis induced by UV irradiation and alkylating agents was not sensitive to ddTTP, indicating repair of these lesions occurred through a pathway primarily utilizing a different DNA polymerase; presumably polymerase $\alpha$. However, replicative synthesis was much more sensitive to polymerase $\alpha$ inhibitors than was repair synthesis induced by UV irradiation or alkylating agents. Neither the amount of DNA damage nor the amount of induced repair synthesis influenced the degree to which the different DNA polymerases were involved in repair synthesis. The possibility that “patch size” or the actual type of DNA damage determines the extent to which different polymerases participate in DNA repair synthesis is discussed.

There are three DNA polymerases, $\alpha$, $\beta$, and $\gamma$, in mammalian cells which can be differentiated by their size, subcellular location, substrate specificities, and susceptibility to specific inhibitors (reviewed in Refs. 1 and 2). The role of each polymerase in DNA metabolism has been the subject of many studies. Most studies indicate that polymerase $\alpha$ is solely responsible for nuclear DNA replication (1, 2) as well as the replication of SV40 and polyoma virus DNA (3). DNA polymerase $\gamma$ is responsible for replicating mitochondrial DNA (1, 4) and is involved in the replication of adenovirus DNA (3). However, which DNA polymerase(s) is responsible for DNA repair synthesis has been the subject of recent controversy. Earlier indirect (1, 2) as well as more recent direct studies (5-8) implicate DNA polymerase $\beta$ in repair synthesis; however, other studies (9-11) indicate that polymerase $\alpha$ is responsible for repair synthesis. A number of subcellular systems have been developed to study DNA repair synthesis (5-7, 9-12). One advantage of subcellular systems is that the effect of DNA polymerase inhibitors which would not rapidly penetrate intact cells (i.e. nucleotides) can be investigated. Using subcellular preparations of human cells in 0-40 mM NaCl and UV irradiation or alkylating agents to induce DNA repair synthesis, polymerase $\alpha$ was reported to be responsible for repair synthesis (9-11). On the other hand, polymerase $\beta$ has been implicated in DNA repair synthesis using subcellular preparations of human and rodent cells in 80-120 mM NaCl and UV irradiation (5) or bleomycin (6, 7) to induce repair synthesis. We have been attempting to reconcile these conflicting reports and to clarify the roles of the different DNA polymerases in repair synthesis. The effect of the salt concentration in situ on the involvement of DNA polymerases $\alpha$ and $\beta$ in repair synthesis has been reported (13). This study reports the effect of DNA polymerase inhibitors on DNA repair synthesis in subcellular preparations of hamster and human cells, using alkylating agents, UV irradiation, neocarzinostatin, and bleomycin to damage DNA. The effect of the amount of DNA damage on the involvement of the different DNA polymerases in repair synthesis is also investigated.

MATERIALS AND METHODS

Reagents—Bleomycin and neocarzinostatin were kindly supplied by Bristol Laboratories, Syracuse, NY. MNNG* was from Aldrich, ddTTP was from P-L Biochemicals, Inc. Aphidicolin was supplied by Imperial Chemical Industries, Cheshire, England. [Methyl-$^3$H]TTP (10-20 Ci/mmol), [A^-3H(N)]dATP (10-20 Ci/mmol), [methyl-$^3$C]TdR (48 mCi/mmol), [methyl-$^3$H]Tdr (20 Ci/mmol), and Biofluor scintillation fluid were purchased from New England Nuclear. ddTTP was the gift of Dr. B. Tseng. All other reagents were from Sigma. Scintillation counting was performed in a Beckman LS-9000 scintillation counter.

Growth of Cells—CHO cells were grown in suspension culture and growth arrested in the G1 period as previously described (12). Diploid HF cells, designated Hs0027F, were obtained from the Naval Biociences Laboratory, Oakland, CA and were the same cell line used by Ciarrocchi et al. (9). HF cells were propagated in Dulbecco’s modifi-

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1 The abbreviations used are: MNNG, N-methyl-N'-nitro-N-nitrosoguanidine; CHO, Chinese hamster ovary; HF, human fibroblasts; araCTP, cytosine arabinoside 5'-triphosphate; ddTTP, 2',3'-dideoxythymidine 5'-triphosphate; NEM, N-ethylmaleimide; NMU, N-nitrosomethyl urea; BrdUTP, bromodeoxyuridine 5'-triphosphate.
cation of Eagles medium (Gibco, Grand Island, NY) with 15% newborn calf serum (Microbiological Associates). Growth-arrested (G1) phase cells were obtained by placing confluent cultures in medium containing 0.25% newborn serum for 48 h.

In Situ DNA Synthesis—DNA replicative and DNA repair synthesis were measured in in situ preparations of CHO and HF cells, following treatment of the cells with lysocetamin to render the cells permeable to dNTPs (8, 12, 14). Details for treating CHO cells with lysocetamin have been described (12, 13). HF cells were removed from 100-mm culture plates with trypsin-EDTA (Gibco), followed by washing 2 times at 4 °C in solution A (35 mM 4-[(2-hydroxyethyl)-1-piperazine]ethanesulfonic acid, pH 7.4; 150 mM sucrose; 5 mM potassium phosphate, pH 7.4; 5 mM MgCl2; 0.5 mM CaCl2) containing 0.2 mM a-bungarotoxin and 0.1 mM fluoride, suspended in solution A (0 °C) at 8 × 10^7 cells/ml and permeabilized by the addition of lysocetamin (Sigma, Type I) to a final concentration of 0.5 mg/ml. DNA replicative and repair synthesis were then measured in lysocetamin-treated cells as indicated below.

Exponentially growing CHO and HF cells were permeabilized and used to measure replicative DNA synthesis, whereas growth arrested (G1) cells were used for DNA repair studies. For replicative synthesis, cells were incubated at 2 × 10^6 cells/ml in solution A containing 1.25 mM ATP, 5 mM phosphoenolpyruvate, 0.1 mM CTP, GTP, UTP, 12.5 mM [3H]dATP (8.0 Ci/mmol), and 250 μM dCTP, dGTP, dUTP at 37 °C for 30 min. For DNA repair synthesis, G1 cells were treated with repair-inducing agents, before or after permeabilization as described, and incubated in the same solution described for DNA replication. Incorporation of [3H]dUTP into DNA was determined as described (12).

CsCl Gradients—To characterize DNA synthesis in situ, permeable cells were incubated in the DNA synthesis solution with BrdUTP (250 μM) substituted for TTP; [3H]dATP (12.5 μM) was the labeled nucleotide. Following incubation at 37 °C, the DNA was isolated, sheared, denatured, and centrifuged to equilibrium in CsCl gradients, as described (15).

Sucrose Gradients—The amount of DNA damage induced by various agents was estimated by determining the size of DNA in alkaline sucrose gradients. Exponential HF cells were treated with 3 μM [3H]dTTMP (0.3 Ci/mmol) for 24 h, allowed to grow to confluence in [3H]dTTMP-free medium and growth arrested in medium containing 0.25% newborn calf serum for 48 h. The labeled cells were treated with DNA damaging agents before or after permeabilization, as indicated, and the DNA was isolated and centrifuged on 5-20% alkaline sucrose gradients in a SW41 rotor at 30,000 RPM for 10 h, 15 °C, as described (15).

DNA Polymerase β—DNA polymerase β was purified to near homogeneity from hamster livers according to Kunkel et al. (16) through the hydroxyapatite step. Polymerase β assays were performed in solution A containing 250 μM dATP, dCTP, dGTP, 1.25 mM [3H]dTTP (16 Ci/mmol), 100 μM CTP, GTP, UTP, 1.25 mM ATP, 5.0 mM of phosphoenolpyruvate, and 160 μg/mL of DNA-activated DNA. Following incubation at 37 °C for 60 min, incorporation of [3H]dTTP into DNA was determined as described (17).

RESULTS

In Situ DNA Synthesis—DNA replication and repair synthesis have been characterized in lysocetamin-treated CHO cells (12, 13). To characterize DNA synthesis in permeable HF cells, DNA synthesized in situ was density labeled with BrdUTP and analyzed on CsCl gradients (17), as described under "Materials and Methods." In the absence of DNA-damaging agents, a small but detectable level of DNA synthesis could be observed in permeable CHO and HF (not shown) cells were permeabilized and incubated in the DNA replication solution containing BrdUTP to density label DNA. CsCl gradient analysis demonstrated that essentially all of the labeled DNA banded at much higher than normal density. Typical values for the amount of DNA synthesized in permeable HF cells, observed as picomoles of [3H]dUTP incorporated/5 × 10^6 cells/30 min were: 15 for exponential cells, 0.08 for G1 cells, 1.6 for G0/G1 cells treated with bleomycin, 1.2 for G1 cells treated with neocarzinostatin, 0.45 for G1 cells treated with MNNG, 0.43 for G1 cells treated with NMU, and 0.42 for G1 cells treated with UV irradiation. Similar values were obtained with CHO cells (13). These studies established that normal replicative synthesis can be studied in permeable preparations of exponential CHO and HF cells and that exposing G1-CHO cells and G1-HF cells to DNA damaging agents induces typical DNA repair synthesis which can be studied in situ.

Effect of DNA Polymerase Inhibitors on DNA Replication and Repair Synthesis—Mammalian DNA polymerases α, β, and γ can be differentiated by their sensitivities to specific inhibitors. Polymerase α is inhibited by aphidicolin, araCTP, and NEM; polymerase β is inhibited by ddTTP; and polymerase γ is inhibited by ddTTP and NEM. The effect of the concentration of DNA polymerase inhibitors on DNA replication and repair synthesis in situ, as well as on the activity of purified hamster liver polymerase β, was determined. In order to compare the effect of polymerase inhibitors on in situ replicative synthesis, in situ repair synthesis induced by different DNA damaging agents and polymerase β activity, results were expressed as per cent inhibition of DNA synthesis by each polymerase inhibitor. In situ repair synthesis, results were corrected for the effect of DNA polymerase inhibitors on DNA synthesis in G1-CHO and -HF cells in the absence of DNA damaging agents. Figs. 2–5 show the inhibition of the various types of DNA synthesis studied by ddTTP, aphidicolin, araCTP, and NEM, respectively. The concentration of each polymerase inhibitor which decreased the different types of DNA synthesis 50% was determined.

Figs. 2–5 are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry. 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. DTM-D255, cite authors, and include a check or money order for $2.00 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverley Press.
from Figs. 2-5 and is indicated in Table I. Repair synthesis induced by NMU and MNNG in CHO and HF cells is inhibited to the same extent by DNA polymerase inhibitors (13). To simplify Figs. 2-5, the data obtained with NMU is presented in CHO cells, and data obtained with MNNG is presented in HF cells. In CHO cells, repair synthesis induced by bleomycin was inhibited 50% by 115 μM ddTTP, whereas 1900 μM ddTTP was required to produce 50% inhibition of repair synthesis induced by methylating agents and of replicative synthesis (Fig. 2A and Table I). Inhibition of DNA polymerase β and of bleomycin repair synthesis in CHO cells exhibited very similar sensitivities to inhibition by ddTTP. In HF cells, repair synthesis induced by bleomycin and neocarzinostatin were inhibited 50% by 116 and 8 μM ddTTP, respectively; however, repair synthesis induced by methylating agents and UV irradiation required much higher concentrations of ddTTP (>900 μM) to attain 50% inhibition. These studies demonstrate that DNA repair synthesis induced by bleomycin or neocarzinostatin is much more sensitive to ddTTP inhibition than is repair synthesis induced by NMU, MNNG, or UV irradiation.

The effect of aphidicolin on DNA synthesis in situ is shown in Fig. 3 and Table I. In both CHO and HF cells, replication was much more sensitive to aphidicolin than was repair synthesis induced by any agent. At all concentrations of aphidicolin tested (1-20 μg/ml), purified DNA polymerase β was unaffected.

As observed with aphidicolin (Fig. 3), DNA replication in both CHO and HF cells was inhibited by much higher concentrations of araCTP (50% inhibition at ≈35 μM araCTP) than was repair synthesis induced by any of the DNA-damaging agents tested (Fig. 4 and Table I). Purified DNA polymerase β was significantly inhibited by high concentrations of araCTP; 1.1 mM araCTP inhibited polymerase β >30% (Fig. 4A).

The effect of NEM on DNA synthesis in permeable CHO and HF cells is shown in Fig. 5 and Table I. In both cell lines, bleomycin-induced repair synthesis was least sensitive to NEM; 50% inhibition occurred at 1600 μM NEM. Replication in both cells was inhibited 50% by 290 μM NEM. In CHO agents tested (Fig. 4 and Table I). Purified DNA polymerase β was significantly inhibited by high concentrations of araCTP; 1.1 mM araCTP inhibited polymerase β >30% (Fig. 4A).

Using the data presented in Figs. 2-5, the concentrations of ddTTP, aphidicolin, araCTP, and NEM which decrease replication or repair synthesis induced by different agents 50% in CHO and HF cells are indicated below. The concentration of inhibitors which reduce the activity of purified hamster liver DNA polymerase β 50% is also indicated. N.E. indicates no effect, and * indicates the highest concentration of inhibitor tested reduced DNA synthesis 30%.

TABLE I

| DNA synthesis | ddTTP | Aphidicolin | araCTP | NEM |
|---------------|-------|-------------|--------|-----|
|               | μM    | μg/ml       | μM     | μM  |
| Hamster       |       |             |        |     |
| Replication   | 800   | 6.9         | 35     | 280 |
| Bleomycin     | 15    | 18          | 1100   | 550 |
| NMU repair    | 1000  | 5           | 1100   | 90  |
| Polymerase β  | 10    | N.E.        | 1100*  | N.E.|
| Human         |       |             |        |     |
| Replication   | 270   | 0.6         | 35     | 300 |
| Bleomycin     | 16    | 14          | 1100*  | 650 |
| Neocarzinostatin | 20     | 20          | 1100   |     |
| MNNG repair   | 700   | 20          | 750    | 170 |
| UV repair     | 1100  | 6           | 1100   | 80  |

"M. R. Miller and D. N. Chinault, unpublished information."
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Fig. 6. Alkaline sucrose gradient analysis of HF cell DNA following exposure to different concentrations of bleomycin and MNNG. Intact HF cells were treated with 0.25 (A) or 0.05 mM MNNG (C) for 1 h, then permeabilized with lysolecithin and incubated at 37°C for 15 min. Permeable HF cells were treated with 5 (B) or 0.75 μg/ml of bleomycin (D). The DNA was then isolated and analyzed on 5-20% alkaline sucrose gradients as described above. "Materials and Methods." Centrifugation was in an SW4a rotor for 10 h, at 31,000 rpm, 15°C. Fractions were collected from the top and acid precipitated onto Whatman GF/A filters as described (17). [3H]DNA applied to each gradient was between 800,000 and 850,000 cpm, and >90% of applied tritium was recovered. The Beckman LS-9000 liquid scintillation counter was programmed for digital integration and automatic quench corrections and the normalized plots derived from the scintillation counter are shown above. Arrows indicate the position of fd DNA.

Table II

| Repair synthesis | Inhibition of repair synthesis |
|------------------|-------------------------------|
|                  | ddTTP | Aphidicolin | araCTP | NEM |
| 0.75 μg/ml of bleomycin | 0.56±1.33 | 70 | 49 | 37 | 0 |
| 0.05 mM MNNG | 0.62±0.82 | 64 | 56 | 29 | 0 |
| 0.05 mM MNNG | 0.38±0.42 | 27 | 62 | 61 | 64 |
| 0.25 mM MNNG | 0.21±0.44 | 32 | 67 | 57 | 60 |

Although the DNA polymerase inhibitors used in this study are not absolutely specific, the differential effect of the inhibitors on DNA repair synthesis induced by different agents helps clarify the role of polymerases α, β, and γ in DNA repair synthesis. A primary role of DNA polymerase γ in repair synthesis would be indicated by inhibition of repair synthesis by relatively low concentrations of ddTTP as well as NEM; however, such inhibition was not observed in repair synthesis induced by any of the agents studied (Table I). Polymerase γ, therefore, appears to play a minor, if any, role in DNA repair synthesis.
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 repair synthesis in mammalian cells. Repair synthesis induced by MNNG, NMU, or UV irradiation was relatively insensitive to ddTTP, whereas repair induced by bleomycin or neocarzinostatin was inhibited by much lower concentrations of ddTTP (Fig. 2). This observation suggests that different DNA polymerases are involved in repairing different types of DNA damage. The similarity of ddTTP inhibition of neocarzinostatin and bleomycin repair to the ddTTP inhibition of purified polymerase β (Fig. 2) indicated that polymerase β was primarily responsible for repair synthesis induced by these two agents. Although very high concentrations of araCTP inhibited repair synthesis induced by bleomycin and neocarzinostatin, similar concentrations of araCTP also inhibited polymerase β (Fig. 4). It is difficult to explain why repair synthesis induced by neocarzinostatin and bleomycin was inhibited by high levels of aphidicolin, but purified polymerase β was not inhibited by aphidicolin (Fig. 3). This observation may indicate that: 1) polymerase β is required for one step in bleomycin or neocarzinostatin repair synthesis while polymerase α is required for a different step, 2) polymerase β alone resynthesizes most of the DNA areas damaged by bleomycin and neocarzinostatin, and polymerase α alone resynthesizes other areas or 3) in addition to polymerase α, aphidicolin interacts with another cellular protein(s) in such a manner to reduce polymerase β activity in situ. Indeed, decreased sensitivity of polymerase α to aphidicolin during purification has been interpreted as loss of a polymerase α accessory protein which interacts with aphidicolin (25, 26).

Repair synthesis induced by MNNG, NMU, and UV irradiation were inhibited only by high concentrations of ddTTP, which also inhibits polymerase α (Fig. 1).4 The lack of sensitivity of repair synthesis induced by methylyating agents and UV irradiation to ddTTP indicated that neither polymerase β nor α participated in these repair synthesis processes. However, MNNG, NMU, and UV-induced repair were much less sensitive to both aphidicolin and araCTP than was replication. A strong correlation between sensitivity of polymerase α and sensitivity of repair synthesis induced by MNNG, NMU, and UV irradiation to polymerase α inhibitors has not been established. Thus, while polymerase β appears to be involved in repair of bleomycin or neocarzinostatin damage, we can only conclude that a ddTTP-insensitive polymerase is responsible for repairing MNNG, NMU, and UV radiation damage. Our results may indicate that different forms of DNA polymerase α (28, 29), with different sensitivities to aphidicolin and araCTP, participate in DNA replication and repair synthesis or that a novel polymerase is involved in MNNG-, NMU-, and UV-induced repair synthesis.

Interpretation of NEM inhibition of DNA synthesis in situ is difficult since, in addition to polymerases α and γ, any other proteins requiring a sulfhydryl group for activity would be inactivated. Nonetheless, repair synthesis induced by MNNG, NMU, and UV irradiation were much more sensitive to NEM inhibition than was repair synthesis induced by bleomycin (Fig. 3). Our results explain conflicting reports in the literature (5–11). The similar sensitivity of repair synthesis in rodent (CHO) and human (HF) cells to DNA polymerase inhibitors (Figs. 2–5) indicates that differences in cell species was not the origin of the conflicting reports. Differences in salt concentrations employed in situ has also been shown not to alter the extent to which polymerase inhibitors reduce repair synthesis (13). The extent of involvement of different polymerases in DNA repair synthesis appears to be related to the agent used to damage DNA. The only exception to our results, of which we are aware, is a report by Hubscher et al. (5), indicating polymerase β was responsible for UV-induced repair synthesis in human neuronal nuclei. These cells did not contain polymerase α and may be viewed as atypical or special cells.

At least three factors may be involved in determining which polymerase participates in repair synthesis 1) the amount of DNA damage, 2) the “patch size” of repaired DNA and 3) the actual type of damage being repaired. Data in this report (Fig. 6 and Table II) indicate that the amount of DNA damage does not significantly alter the involvement of polymerase β or the ddTTP-insensitive polymerase. The size of DNA synthesized after damage by some agents, such as X irradiation, is relatively small (1–5 nucleotides) and is termed “short patch,” whereas longer areas of DNA (=100 nucleotides) are synthesized in response to agents such as UV irradiation (23) and alkylating agents (22). Because DNA polymerase α requires a gap ranging from 25–50 nucleotides to initiate synthesis, while polymerase β is active on DNA with smaller gaps, Grossman (30) and Cleaver (31) have hypothesized that the patch size may influence which polymerase participates in repair synthesis. We offer an alternative explanation of our findings. During repair of many types of DNA damage, incision polymerases create 3’ and 5’ termini on damaged DNA strands which are substrates for typical repair nucleases. The repair nucleases create gaps (“short” or “long”) which are acted on by a ddTTP-insensitive polymerase. On the other hand, bleomycin and neocarzinostatin create breaks in DNA which may not be susceptible to the same repair nucleases. After bleomycin treatment, the 3’ end of the broken strand is “blocked” by a CH2-CH-COOH group (26), and following neocarzinostatin treatment, the 5’ end appears to be “blocked” by a residual sugar moiety (32). Such blocked termini may not be substrates for typical repair nucleases. Repair of damaged DNA containing blocked termini may require excision of blocked termini by a polymerase β-associated nuclease, followed by resynthesis primarily, but not necessarily exclusively, with polymerase β. An example of such a nuclease is DNase V, described by Mosbaugh and Meyer (33). Studies are in progress to test this hypothesis.

Another factor which may contribute to our results is that bleomycin- and neocarzinostatin-induced repair synthesis were initiated in permeable cells, whereas MNNG, NMU, and UV repair synthesis were initiated in intact cells. Preparation of in situ cell systems may alter initiation of normal repair processes in some fashion, causing DNA polymerases to participate differently in repair synthesis. It will be imperative to investigate this possibility to determine whether in situ systems are valid models for studying DNA repair synthesis.

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REFERENCES

1. Weissbach, A. (1979) Arch. Biochem. Biophys. 198, 386–396
2. Hanawalt, P. C., Cooper, P. K., Ganesan, A. K., and Smith, C. A. (1979) Annu. Rev. Biochem. 48, 783–836
3. Krokan, H., Schaffer, P., and DePammphilis, M. L. (1979) Biochemistry 18, 4431–4443
4. Zimmermann, W., Chen, S. M., Bolden, A., and Weissbach, A. (1980) J. Biol. Chem. 255, 11847–11852
5. Hubscher, U., Kuenzle, C. C., and Spadari, S. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 2316–2320
6. Castellot, J. J., Jr., Miller, M. R., Lehtomaki, D. M., and Pardee, A. B. (1979) J. Biol. Chem. 254, 6904–6908
7. Seki, S., Oda, T., and Ohashi, M. (1980) Biochem. Biophys. Acta 610, 413–420
8. Warwa, E., and Dolejs, I. (1979) Nucleic Acids Res. 7, 1675–1686
9. Ciarrocchi, G., Jose, J. G., and Linn, S. (1979) Nucleic Acids Res. 7, 1205–1219
10. Hizasaoka, F., Kato, H., Ikegami, S., Ohashi, M., and Yamada, M. A. (1979) Biochem. Biophys. Res. Commun. 87, 575–580
Roles of DNA Polymerases α, β, and γ in DNA Repair Synthesis

11. Berger, N. A., Kurohara, K. K., Petzold, S. J., and Sikovski, G. W. (1979) Biochem. Biophys. Res. Commun. 89, 218-255
12. Miller, M. R., Castellot, J. J., Jr., and Pardee, A. B. (1978) Biochemistry 17, 1073-1080
13. Miller, M. R., and Chinault, D. N. (1982) J. Biol. Chem. 257, 46-49
14. Miller, M. R., Castellot, J. J., Jr., and Pardee, A. B. (1979) Exp. Cell Res. 120, 421-425
15. Tseng, B. Y., and Goulain, M. (1975) J. Mol. Biol. 99, 317-337
16. Kunkel, T. A., Tcheng, J. E., and Meyer, R. R. (1978) Biochem. Biophys. Acta 520, 302-316
17. Ross, S. L., and Moses, R. E. (1976) Antimicrob. Agents Chemother. 9, 239-246
18. Crooke, S. T., and Bradner, W. T. (1975) J. Med. 7, 333-427
19. Kappen, L. S., and Goldberg, I. H. (1978) Biochemistry 17, 729-734
20. Kappen, L. S., Papier, M. A., Goldberg, I. H., and Samy, T. S. A. (1980) Biochemistry 19, 4760-4785
21. Lawley, P. D., and Thatcher, C. J. (1979) Biochem. Biophys. Res. Commun. 89, 218-255
22. Roberts, J. J. (1978) Adv. Radiat. Biol. 7, 212-248
23. Haseltine, W. A., Gordon, L. K., Lindan, C. P., Grafstrom, R. H., Sharp, N. L., and Grossman, L. (1980) Nature 285, 634-640
24. Gilot, L., Takeshita, M., Johnson, E., Iden, C., and Grollman, A. P. (1981) J. Biol. Chem. 256, 8608-8615
25. Ikegami, S., Taguchi, T., Ohashi, M., Oguro, M., Nagano, H., and Mano, Y. (1978) Nature 275, 458-460
26. Huberman, J. A. (1981) Cell 23, 467-468
27. Holmes, A. M., Besseread, I. F., and Johnston, L. R. (1974) Eur. J. Biochem. 43, 487-499
28. Chen, Y.-C., Bohn, E. W., Planck, S. R., and Wilson, S. H. (1979) J. Biol. Chem. 254, 11678-11687
29. Bhattacharya, P., Smet, I., and Bao, S. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 2933-2937
30. Grossman, L. (1981) Arch. Biochem. Biophys. 211, 511-522
31. Cleaver, J. E. (1981) J. Cell Physiol. 108, 163-173
32. Takeshita, M., Kappen, L. S., Grollman, A. P., Eisenberg, M., and Goldberg, I. H. (1981) Biochemistry 20, 7599-7606
33. Mosbaugh, D. W., and Meyer, R. R. (1980) J. Biol. Chem. 255, 10239-10247

Supplemental Material

The Roles of DNA Polymerases α, β, and γ in DNA Repair Synthesis
Induced in Hamster and Human Cells by Different DNA Damage Agents
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Fig. 1. Effect of dCTP on DNA synthesis in situ and on purified DNA polymerase activity. Results are presented as a function of dCTP concentration. Each DNA synthetic process was measured in CHO (A) and WI-38 (B) cells. Purified DNA polymerase activity is expressed as a percentage of the control. Panel A: CHO cells; Panel B: WI-38 cells.

Fig. 2. Effect of araC on DNA synthesis in situ and on DNA polymerase activity. Results are as described in Fig. 1 and the text. CHO cells: A; WI-38 cells: B. DNA replicative synthesis, C; synuclein repair synthesis, D; MMS repair synthesis, E; UV repair synthesis, F.

Fig. 3. Effect of aphidicolin on DNA synthesis in situ. Results are as described in Fig. 1 and the text. CHO cells: A; WI-38 cells: B. DNA replicative synthesis, C; aphidicolin repair synthesis, D; synuclein repair synthesis, E; MMS repair synthesis, F; UV repair synthesis, G.

Fig. 4. Effect of araC on DNA synthesis in situ and on DNA polymerase activity. Results are as described in Fig. 1 and the text. CHO cells: A; WI-38 cells: B. DNA replicative synthesis, C; chromomycin repair synthesis, D; aminoglycosidic repair synthesis, E; synuclein repair synthesis, F; MMS repair synthesis, G; UV repair synthesis, H.