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Functional roles of DNA polymerases β and γ

(neurons/UV-induced DNA repair/synaptosomes/mitochondrial DNA replication)

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ABSTRACT **The physiological functions of DNA polymerases (deoxynucleosidetriphosphate:DNA deoxynucleotidyltransferase, EC 2.7.7.7) β and γ were investigated by using neuronal nuclei and synaptosomes isolated from rat brain. UV irradiation of neuronal nuclei from 60-day-old rats resulted in a 7- to 10-fold stimulation of DNA repair synthesis attributable to DNA polymerase β which, at this developmental stage, is virtually the only DNA polymerase present in the nuclei. No repair synthesis could be elicited by treating the nuclei with *N*-methyl-*N*-nitrosourea, but this was probably due to the inability of brain tissue to excise alkylated bases from DNA. The role of DNA polymerase γ was studied in synaptosomes by using a system mimicking *in vivo* mitochondrial DNA synthesis. By showing that, under these conditions, DNA replication occurs in mitochondria, and exploiting the fact that DNA polymerase γ is the only DNA polymerase present in mitochondria, evidence was obtained for a role of DNA polymerase γ in mitochondrial DNA replication. Based on these results and on the wealth of literature on DNA polymerase α, we conclude that DNA polymerase α is mainly responsible for DNA replication in nuclei, DNA polymerase β is involved in nuclear DNA repair, and DNA polymerase γ is the mitochondrial replicating enzyme. However, minor roles for DNA polymerase α in DNA repair or for DNA polymerase β in DNA replication cannot be excluded.**

The direct assignment of functions to the three mammalian DNA polymerases (deoxynucleosidetriphosphate:DNA deoxynucleotidyltransferase, EC 2.7.7.7) (α, β, and γ) in DNA replication and repair is hampered by the lack of conditional mutants defective in DNA synthesis. To date, one is forced to admit that the evidence presented is at best circumstantial. Despite these reservations, some progress has been made concerning the function of DNA polymerase α. A wealth of information indicates that this enzyme has a major role in DNA replication (reviewed in refs. 1 and 2). Recently, this idea has been further strengthened by the finding that DNA polymerase α is the major polymerase associated with replicating simian virus 40 chromosomes (3–5). In addition, all aspects of *in vitro* replicative synthesis by these chromosomes (3) or HeLa cell lysates (6) were found to be resistant to 2′,3′-dideoxythymidine 5′-triphosphate (d_2TTP), a specific inhibitor of DNA polymerases β and γ but not of DNA polymerase α (3, 6). Conversely, aphidicolin, a selective inhibitor of DNA polymerase α, prevents mitotic division of sea urchin embryos, which requires replicative DNA synthesis, but it has no effect on meiotic division in starfish oocytes, which is not dependent on DNA replication (7). Taken together these observations strongly indicate that DNA polymerase α is the nuclear replicating enzyme.

In contrast, the physiological functions of DNA polymerases β and γ are not understood. DNA polymerase β is often considered to be a repair enzyme, more for a need of a repair polymerase, in analogy to bacteria, than on the basis of facts. The best evidence for its role in DNA repair comes from the work of Bertazzoni *et al.* (8) who found that in phytohemagglutinin-stimulated lymphocytes the major increase in DNA polymerase β coincides with a peak of DNA repair capacity and minimal DNA replication. Recently, in an artificial system, DNA polymerase β has been shown to elongate the endogenous primer sequences of D loop mtDNA (9); however, this has little relevance to the *in vivo* function of this enzyme and, in view of its strictly nuclear location, DNA polymerase β cannot be inferred to be the mtDNA replicase. This function should be attributed to DNA polymerase γ, which is the only DNA polymerase present in mitochondria (10–12), but definitive proof of this function remains to be adduced.

We have approached the question of the physiological roles of DNA polymerases β and γ by exploiting some unique properties of brain neurons which, in adult rats, contain organelles that not only can be isolated easily but, more important, harbor a single DNA polymerase. Thus, nuclei from adult nondividing neurons contain virtually no DNA polymerase other than β (β, 99.2%; γ, 0.8%) (13); by stimulating repair-type synthesis with UV light or chemical carcinogens, the possible involvement of polymerase β in DNA repair can be studied. Similarly, DNA polymerase γ is the only DNA polymerase present in neuronal mitochondria (12), affording the possibility of unambiguous identification of its role in mtDNA synthesis. Using rat neurons we show in this work that DNA polymerase β is able to repair UV-damaged nuclear DNA, whereas DNA polymerase γ is responsible for mtDNA replication.

MATERIALS AND METHODS

Neuronal Nuclei and Synaptosomes. Neuronal nuclei (14) and synaptosomes (15) were prepared from the forebrain cortex of 60-day-old and 14-day-old rats (SIV-50 strain), respectively. The preparations were frozen immediately at −70°C until use. Nuclei were counted by using a flow cytophotometer (Phywe, Göttingen, Germany), and protein concentrations were determined by the method of Lowry *et al.* (16). Repair DNA synthesis in neuronal nuclei and synaptosomes was assayed as described in Table 1.

***In Vitro* System Supporting mtDNA Synthesis in Synaptosomes.** Synaptosomes were suspended in 0.32 M sucrose/1 mM KH_2PO_4, pH 7.5/0.1 mM EDTA and were made perme-

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Abbreviations: BrdUTP, 5-bromo-2′-deoxyuridine 5′-triphosphate; d_2TTP, 2′,3′-dideoxythymidine 5′-triphosphate; MNU, *N*-methyl-*N*-nitrosourea.

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able by storage on ice for 5 min in the presence of 0.5% (vol/vol) Brij 58 (Serva). Unless otherwise stated, 20 μl of permeable synaptosomes (0.5–1 mg of protein) was added to a reaction mixture containing 50 mM Tris·HCl (pH 8.5), 1 mM ATP, 120 mM KCl, 1 mM dithiothreitol, 2 mM $MnCl_2$, and 0.2 mg of bovine serum albumin per 0.1 ml final volume. After preincubation at 37°C for 15 min (in order to deplete the endogenous pool of deoxyribonucleoside triphosphates) [*methyl*-^{3}H]dTTP (1000 cpm/pmol, 30 μM; Amersham) and dATP, dGTP, and dCTP (50 μM each; Calbiochem) were added and DNA synthesis was allowed to proceed at 37°C for 1 hr. This was followed by either procedure (*i*) or (*ii*) as described below.

(*i*) For determination of [^{3}H]dTTP incorporation, the reaction was stopped by the addition of 1.9 ml of 1 M $NaClO_4$ and 0.1 ml 10% sodium dodecyl sulfate, and the mixture was then extracted with 2 ml of phenol/chloroform, 1:1 (vol/vol). To 1.5 ml of the aqueous phase was added 5 ml of 10% (wt/vol) trichloroacetic acid containing 1% (wt/vol) $Na_4P_2O_7$, and the precipitate was collected onto GF/C filters for liquid scintillation counting (17). (*ii*) For isolation of mtDNA the reaction was stopped by adjusting to 150 mM EDTA, 300 mM NaCl, and 2.25% (wt/vol) sodium dodecyl sulfate. The mixture was heated to 50°C for 5 min, incubated with predigested (18) proteinase K (1 mg/ml; Merck) at 50°C for 2 hr, and dialyzed against 15 mM NaCl/1.5 mM sodium citrate, pH 7.0. CsCl (Merck) was added to a density of 1.715 g/cm^3 and the solution was centrifuged in a Spinco 65 fixed-angle rotor at 35,000 rpm for 60 hr at 20°C. Fractions (150–200 μl) were collected from the bottom and analyzed for acid-precipitable radioactivity (17) and density. The fractions containing radioactivity were pooled, dialyzed against 15 mM NaCl/1.5 mM sodium citrate, precipitated with ethanol, and subjected to the analytical procedures described below.

Analytical Ultracentrifugation. DNA in CsCl/15 mM NaCl/1.5 mM sodium citrate (ρ, 1.690 g/cm^3) was spun in a Beckman model E centrifuge at 44,000 rpm for 16 hr at 25°C. The density of the DNA was calculated (19) by reference to added poly(dA-dT) (ρ, 1.679 g/cm^3).

Restriction Endonuclease Analysis. DNA was digested with *Hin*dIII and electrophoresed on agarose gels (20).

Electron Microscopy. DNA in 30 mM triethanolamine·HCl, pH 7.9/100 mM sodium acetate/1 mM EDTA was prepared for electron microscopy as described (21).

RESULTS AND DISCUSSION

DNA Polymerase β Repairs UV-Damaged DNA in Neuronal Nuclei. Neuronal nuclei from the forebrain cortex of adult rats are ideal for testing the hypothesis (8) that DNA polymerase β is a repair enzyme because they contain virtually no DNA polymerase other than the β (13). Neuronal nuclei were investigated for their repair capacity after irradiation with UV light or treatment with the chemical carcinogen *N*-methyl-*N*-nitrosourea (MNU) (22). As a negative control, neuronal mitochondria confined within synaptosomes (for a definition of the term "synaptosome," see below) were treated in the same way. No stimulation of deoxyribonucleoside triphosphate incorporation by UV irradiation was expected in this case because mitochondria are known to lack the ability to excise pyrimidine dimers (23).

Incorporation of [^{3}H]dTTP in nuclei was stimulated approximately 7- to 10-fold by irradiation with UV light (Table 1). This stimulation was suppressed when, after irradiation, DNA synthesis was allowed to proceed in the presence of d_2TTP, a specific inhibitor of DNA polymerases β and γ (3, 6). No evidence of DNA repair synthesis was obtained in nuclei incubated with MNU or in synaptosomes after UV or MNU treatment.

These results strongly indicate that DNA polymerase β repairs UV-damaged DNA. However, this polymerase clearly performs no repair synthesis on MNU-treated neuronal DNA. This is probably related to the known inability of brain tissue to excise alkylated bases from DNA (24), a process thought to involve specific *N*-glycosylases (25). Because MNU is a direct carcinogen that requires no metabolic activation (26), its failure to stimulate DNA synthesis in nuclei cannot be attributed to a lack of reactivity in our experimental system.

Synaptosomes did not respond to UV light or to the carcinogen treatment, suggesting that mitochondria are deficient in the enzymes necessary for pyrimidine dimer excision (23) and in those for removal of alkylated bases; alternatively, the mitochondrial DNA polymerase γ (10–12) may not be able to perform repair DNA synthesis.

DNA Polymerase γ Replicates mtDNA. In order to study the role of DNA polymerase γ, a quasi *in vivo* DNA synthesizing system was developed using permeable synaptosomes. The term "synaptosomes" (27) applies to discrete particles that derive from pinched-off nerve endings during the homogenization of brain tissue. They are surrounded by a resealed membrane and contain residual cytoplasm, mitochondria, and transmitter-filled synaptic vesicles (13, 15, 27). Because, of the three mammalian DNA polymerases, only DNA polymerase γ exists in mitochondria (10–12), this enzyme can conveniently be studied in synaptosomes. There are two advantages of using synaptosomes instead of isolated mitochondria (13): (*i*) synaptic nerve endings in the brain are anatomically separated from the cell nuclei and the danger of contamination by nuclear DNA polymerases is therefore minimized, so that mtDNA synthesis can be studied in the absence of interfering nuclear DNA synthesis; and (*ii*) the packaging of synaptosomal mitochondria in membrane-bounded bags filled with cytoplasm helps to preserve their integrity during isolation and allows them to be studied in their quasi natural environment.

Synaptosomes Synthesize DNA *In Vitro*. Under the conditions used, synaptosomes incorporated [^{3}H]dTTP into acid-insoluble material *in vitro* (Table 2). The reaction was linear for about 15 min and leveled off after 1 hr. The complete system was required for optimal incorporation. Without permeabilization and preincubation, incorporation decreased to 12%. Omission of unlabeled dATP, dGTP, and dCTP only decreased incorporation by 33%. By using [^{3}H]dTTP of different specific activities, we found that the endogenous deoxyribonucleoside triphosphate pool was not totally depleted by 15 min of preincubation; however, longer preincubation decreased the amount of template DNA available for the reaction. The reaction was strongly dependent on ATP but not on the other three ribonucleoside triphosphates. There was no need for an ATP-regenerating system at ATP concentrations above 1 mM. Boiled synaptosomes did not incorporate [^{3}H]-dTTP.

To demonstrate that [^{3}H]dTTP incorporation was physically associated with the synaptosomes and that these particles remained largely intact during incubation, reaction mixtures were recentrifuged on density gradients identical to those originally used for the isolation of the synaptosomes (Fig. 1). Most of the radioactivity was recovered at the boundaries between 9 and 12% and 12 and 16% Ficoll, as expected for undamaged synaptosomes (15). Thus, these experiments give strong support to the view that [^{3}H]dTTP incorporation occurs in physical association with the intact particles. No radioactivity was pelleted to the bottom, ruling out the presence of a significant number of free mitochondria released from disrupted synaptosomes (15). However, the presence of a few damaged synaptosomes can not be totally excluded because a small shoulder

Table 1. Repair DNA synthesis in neuronal organelles after UV irradiation or treatment with MNU

Organelle	UV irradiation [^{3}H]dTTP incorporation* Control	Irradiated	Irradiated + d_2TTP†	Irradiated/ control	*N*-Methyl-*N*-nitrosourea Carcinogen, mM	[^{3}H]dTTP incorporation	Treated control
Nuclei	0.43‡	3.98	0.019	9.3	0 (control)	0.24	—
	0.41‡	3.37	0.021	8.8	0.18	0.25	1.04
	0.42‡	3.09	0.012	7.4	0.90	0.16	0.67
					1.80	0.25	1.04
Synaptosomes	0.104	0.088	0.032	0.84	0 (control)	0.070	—
	0.094	0.097	0.017	1.04	0.18	0.055	0.79
	0.080	0.084	0.012	1.05	0.90	0.066	0.87
					1.80	0.054	0.76

For UV irradiation (40 J/m^2), nuclei were suspended in 0.32 M sucrose/2 mM $MgCl_2$/1 mM KH_2PO_4, pH 7.5, and synaptosomes were suspended in 0.32 M sucrose/0.1 mM EDTA/1 mM KH_2PO_4, pH 7.5. Then, the organelles were centrifuged, resuspended in 50 mM $NaHCO_3$, pH 9.0/1.5 mM dithiothreitol and incubated for DNA repair synthesis as described by Ciarrocchi and Linn (22). For MNU treatment, MNU in dimethyl sulfoxide was added to nuclei and synaptosomes suspended as above. After incubation at 0°C for 4 hr, the organelles were recovered by centrifugation, resuspended in 50 mM $NaHCO_3$, pH 9.0/1.5 mM dithiothreitol and assayed for DNA repair synthesis (22).

* Shown as pmol [^{3}H]dTTP incorporated per 15 min per 10^8 nuclei or pmol [^{3}H]dTTP incorporated per 15 min per mg of synaptosomal protein.

† After irradiation the organelles were incubated in the usual assay mixture except for the inclusion of d_2TTP, a specific inhibitor of DNA polymerases β and γ (3, 6), at a final concentration of 50 μM and a d_2TTP/dTTP ratio of 100:1.

‡ An incorporation of 0.42 pmol of [^{3}H]dTTP/15 min per 10^8 nuclei corresponds to 5.8 pmol of total deoxyribonucleoside triphosphates per hr/3 × 10^6 pmol of DNA nucleotides or 2 ppm of the total input DNA per hr.

of radioactivity at the 0–9% Ficoll interface might indicate their presence.

Finally, the labeled product of the *in vitro* reaction was identified as DNA by digestion with pancreatic DNase I. This rendered all radioactivity acid soluble.

DNA Synthesized by Synaptosomes Is mtDNA. In order to prove that the labeled DNA synthesized *in vitro* by synaptosomes was of mitochondrial origin, labeled DNA was isolated from a preparative CsCl gradient and analyzed by analytical ultracentrifugation, restriction endonuclease digestion, and electron microscopy. In agreement with literature data on rat mtDNA, the isolated DNA banded at a density of 1.701 g/cm^3 (28), gave six bands (molecular weights, 4.1, 2.7, 1.7, 1.4, 0.51, and ≈0.1 × 10^6) upon digestion with *Hin*dIII (29), and was circular (Fig. 2) with a circumference of approximately 5 μm (28).

Newly Synthesized DNA in Synaptosomes Arises by Replication. In order to determine whether, during incubation of synaptosomes *in vitro*, mtDNA was being replicated or repaired, the following studies were performed.

(*i*) The extent of *in vitro* DNA synthesis was evaluated from the measured [^{3}H]dTTP incorporation rates and compared with values available for DNA repair synthesis and replication. Calculations showed that permeabilized synaptosomes incorporated a total amount of deoxyribonucleoside triphosphates equivalent to 5–10% of input DNA in 1 hr (Table 2). Calculations (30) based upon the 0.015 g/cm^3 increase in density of DNA observed by banding mtDNA in CsCl after incubation of synaptosomes with [^{3}H]BrdUTP instead of dTTP (Fig. 3) gave similar results. We believe that the DNA synthesis in synaptosomes is replicative synthesis because the rate of DNA synthesis (5–10% of the input DNA in 1 hr) is at least 25,000-fold greater than the rate of repair synthesis (2 ppm of input DNA in 1 hr) found in control neuronal nuclei (Table 1, footnote ‡). Considering that our synaptosomes were not synchronized and that our preincubation treatment may have led to an underestimation of the rate of DNA synthesis in synaptosomes, our observed *in vitro* rate of DNA synthesis probably corresponds

Table 2. Incorporation of [^{3}H]dTTP by synaptosomes *in vitro*

	Incorporation pmol*	%
Complete	62.2	100
− Brij 58; − preincubation	7.5	12
− Brij 58	51.0	82
− preincubation	39.2	63
− dATP, dGTP, dCTP	41.7	67
− ATP	5.6	9
+ GTP, CTP, UTP (50 μM each)	61.6	99
− $MnCl_2$; + $MgCl_2$ (5 mM)	21.3	35
+ creatine kinase (1 mg/ml) + creatine phosphate (0.1 mg/ml)	61.6	99
− synaptosomes; + boiled (10 min) synaptosomes	0.5	<1

* Of total deoxyribonucleoside triphosphates incorporated in 1 hr per mg of protein; 1 mg of synaptosomal protein corresponds to 0.21–0.41 μg of DNA.

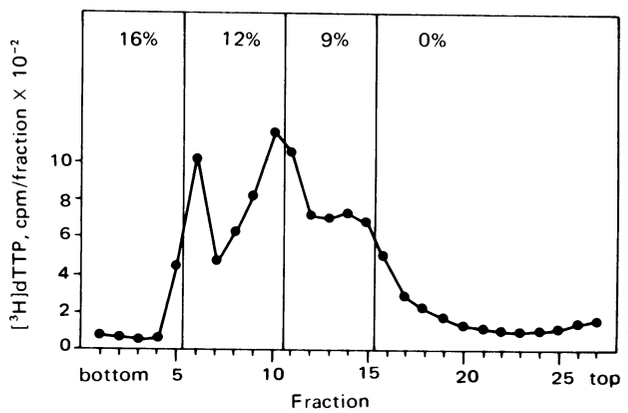

FIG. 1. Reisolation of synaptosomes by density gradient centrifugation after permeabilization and incubation with [^{3}H]dTTP. Permeabilized synaptosomes (200 μl) were incubated and then centrifuged on a discontinuous Ficoll (Pharmacia) gradient (wt/vol percentages shown) identical to the one originally used for their isolation. Most of the radioactivity was recovered at the 9–12% and 12–16% (wt/vol) Ficoll interfaces. This was interpreted to mean that [^{3}H]dTTP incorporation occurred in physical association with intact synaptosomes.

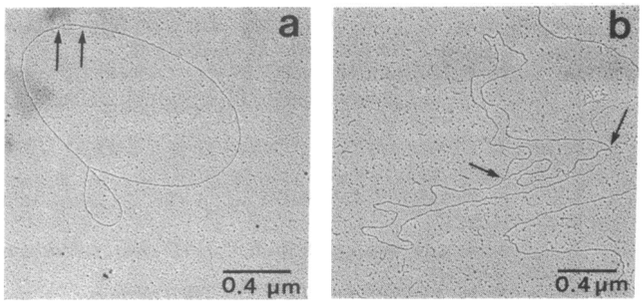

FIG. 2. Electron micrographs of mtDNA isolated from synaptosomes after incubation for DNA synthesis *in vitro*. Typical replication structures (arrows) are seen in the form of displacement loops (*a*) and expanded displacement loops (*b*).

closely to the *in vivo* rate [total genome or 100% of input DNA replicated in 1 hr (31)].

(*ii*) The mtDNA present after incubation of the synaptosomes *in vitro* in the DNA synthesizing system was isolated and analyzed by electron microscopy for typical replication structures (32). These were present in the form of displacement loops and expanded displacement loops (Fig. 2). To show that these replication structures originated during the incubation *in vitro*, DNA synthesis was allowed to proceed in the presence of d_2TTP, an inhibitor of the mtDNA polymerase (see below). As is evident from Table 3, this resulted in a highly significant decrease in replication structures (χ^2 test, $P < 0.01$), indicating that replication can indeed take place *in vitro*.

(*iii*) Newly synthesized DNA in synaptosomes was examined by electron microscopy as an additional means of discriminating between DNA replication and repair. For this purpose, synaptosomes were incubated in the presence of mercurated dCTP, the mtDNA was isolated, and the mercurated sequences were detected by staining with thiolated tRNA. As reported (13), a considerable number of mtDNA molecules were found

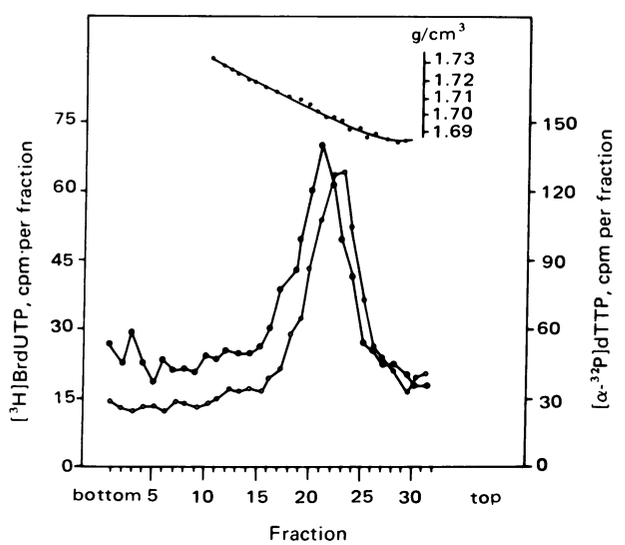

FIG. 3. Neutral CsCl gradient centrifugation of mtDNA from synaptosomes labeled *in vitro* with either [α-^{32}P]dTTP (○) or [^{3}H]BrdUTP (●). Synaptosomes were permeabilized, and two aliquots (50 μl) were incubated in parallel with either the light or the heavy deoxyribonucleoside triphosphate. Incubations were as described in *Materials and Methods* except that [^{3}H]dTTP was replaced by [α-^{32}P]dTTP (Amersham) or [6-^{3}H]BrdUTP (New England Nuclear). After addition of the stop solution, both samples were combined and processed for CsCl centrifugation. Analysis of the gradient revealed a density shift of the bromosubstituted DNA amounting to 0.015 g/cm^3.

Table 3. Replication structures present in mtDNA after incubation of synaptosomes *in vitro* with or without d_2TTP

	Without d_2TTP		With d_2TTP	
	No.	%	No.	%
Fully duplex circular DNA molecules	144	72.0	177	86.3
Duplex circular DNA molecules containing replication structures	56	28.0	28	13.7
Total	200	100	205	100

Synaptosomes (250 μl) were permeabilized and preincubated, and two 100-μl aliquots were incubated in parallel for *in vitro* DNA synthesis except that to one of the samples d_2TTP was added to a final concentration of 300 μM and a d_2TTP/dTTP ratio of 10:1. After incubation the DNA was isolated from both samples and circular molecules were counted by electron microscopy. The proportions of mtDNA molecules containing typical replication structures (displacement loops or expanded displacement loops, see Fig. 2) were compared in both samples and were analyzed statistically (χ^2 test). This showed a highly significant ($P < 0.01$) reduction of replication structures in the d_2TTP-treated sample.

to carry tRNA molecules arranged in a single long continuous stretch, indicative of DNA replication rather than repair. In the latter case one would have expected DNA repair synthesis to have been characterized by the appearance of clusters of tRNA molecules randomly distributed over the entire mitochondrial genome. The fact that such an arrangement was never observed suggests that most, if not all, newly synthesized mtDNA arose by DNA replication.

Replicating Enzyme in Mitochondria Is DNA Polymerase γ. In view of the arguments proposed above for mtDNA replication in synaptosomes and the previous demonstration that DNA polymerase γ is the only DNA polymerase occurring in these particles (12), it is hard to escape the conclusion that mtDNA is replicated by DNA polymerase γ. To validate this assumption further, the polymerase inhibitor d_2TTP (3, 6) was tested for its effect on both [^{3}H[dTTP incorporation by synaptosomes *in vitro* and activity of purified homologous DNA polymerase γ. Both systems were inhibited to virtually the same extent (Fig. 4), indicating that mtDNA synthesis in synaptosomes was indeed performed by DNA polymerase γ.

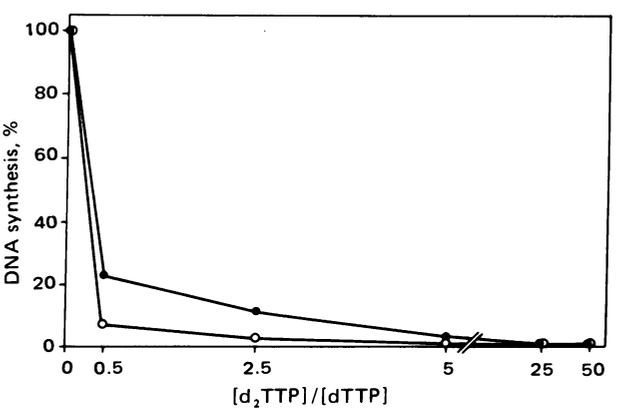

FIG. 4. Inhibition by d_2TTP of mtDNA synthesis by synaptosomes *in vitro* (●) and by purified (1300 units/mg) homologous DNA polymerase γ (○). Permeable synaptosomes (20 μl) were incubated with [^{3}H]dTTP (30 μM) in the presence of increasing concentrations of d_2TTP, and the incorporation of [^{3}H]dTTP was measured. The activity of purified DNA polymerase γ (0.04 unit) isolated from synaptosomal mitochondria (12) was measured by using an assay specific for this enzyme (33), and the effect of increasing concentrations of d_2TTP was determined. For each system, DNA synthesis was expressed as percentage of that of the uninhibited reaction.

CONCLUSIONS

The data reported in this communication provide solid evidence for an involvement of DNA polymerase β and γ in DNA repair and replication, respectively. With a single exception (8), previous functional assignments have been largely speculative (1, 2).

It is now clear that DNA polymerase β is able to repair UV-damaged DNA in neuronal nuclei. Because the neuronal β enzyme has recently been shown by physicochemical and catalytic properties to be closely similar to β polymerases from other tissues (34), it seems likely that a role in DNA repair might be common to all β polymerases. Although DNA polymerase β does not repair MNU-treated neuronal DNA, this is probably due to the inability of brain tissue to excise alkylated bases (24). DNA repair synthesis by DNA polymerase β has been demonstrated after UV-irradiation and after alkylation treatment of mammalian cultured cells in which DNA replication had been inhibited by aphidicolin, a specific inhibitor of DNA polymerase α both *in vitro* and *in vivo* (ref. 35; unpublished data).

DNA polymerase γ evidently is capable of supporting mtDNA replication as demonstrated by the facts that DNA polymerase γ is the only DNA polymerase occurring in mitochondria and that mitochondria do replicate their DNA in isolated synaptosomes. The question of whether DNA polymerase γ also may function in DNA repair has not been answered; even if the answer were yes, DNA repair synthesis would not have been detected because mitochondria, in general, lack the enzymes necessary for pyrimidine dimer excision (23), and mitochondria from brain may share this tissue's inability to remove alkylated bases from DNA (24).

Summarizing all the evidence presently available for the respective roles of the three mammalian DNA polymerases, we conclude that DNA polymerase α is mainly responsible for DNA replication in nuclei, DNA polymerase β is involved in nuclear DNA repair, and DNA polymerase γ is the mitochondrial replicating enzyme, although minor roles for DNA polymerase α in DNA repair or for DNA polymerase β in DNA replication cannot be excluded.

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