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Differential effect of aphidicolin on adenovirus DNA synthesis and cellular DNA synthesis

Marijke M.Kwant and Peter C.van der Vliet†

Laboratory for Physiological Chemistry, State University of Utrecht, 3521 GG Utrecht, The Netherlands

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ABSTRACT

There is strong evidence for a participation of DNA polymerase γ in the replication of adenovirus (Ad) DNA. To study a possible additional role of DNA polymerase α we measured the effect of aphidicolin on viral DNA replication.

In intact cells, aphidicolin inhibits Ad DNA synthesis weakly. The drug concentration required for 50% inhibition of Ad DNA replication was 300-400 fold higher than for a similar effect on cellular DNA synthesis. Such a differential inhibition was also observed in AGMK cells doubly infected with SV40 and the simian adenovirus SA7. No evidence was found for modification of aphidicolin in infected cells or for a change in aphidicolin sensitivity of DNA polymerase α after infection.

The extent of inhibition of purified DNA polymerase α was dependent upon the dCTP concentration. The same situation was observed when DNA synthesis was studied in isolated nuclei from uninfected cells. However, in nuclei from Ad infected cells no effect of dCTP on aphidicolin sensitivity was found. These results were taken as evidence that DNA polymerase α does not participate in the replication of adenovirus DNA.

INTRODUCTION

Aphidicolin is a tetracyclic terpenoid isolated from the fungus Cephalosporium Aphidicola (1). The drug inhibits cell division, cellular DNA synthesis and repair replication in a number of mammalian cells without affecting RNA synthesis or protein synthesis (2, 3, 4). The multiplication of herpes virus, vaccinia virus and SV40 is also sensitive to aphidicolin (1, 4, 20). Recently it has been reported that DNA polymerase α from various sources is strongly inhibited by low concentrations of aphidicolin while DNA polymerase β and γ are resistant to drug concentrations up to 10^{-3} M (2, 3, 4, 5, 17, 20). The sensitivity of both cellular or SV40 DNA replication and DNA polymerase α supports the hypothesis that DNA polymerase α is required for papovavirus DNA synthesis and DNA synthesis in uninfected mammalian cells.

The replication of the linear DNA of the human adenoviruses type 2 and

type 5 (Ad2, Ad5) occurs in the nucleus of permissive cells by a mechanism that differs from that of cellular or papovavirus DNA. Adenovirus DNA replication starts at one of the molecular ends and proceeds unidirectionally by a strand displacement mechanism whereas papovavirus DNA replicates bidirectionally, both parental strands being duplicated almost synchronously (review, see 7, 8).

Other differences include the absence of a tight coupling of DNA synthesis and protein synthesis, as is observed in uninfected cells (9) and the absence of histones in intracellular replicating adenovirus DNA (10). The nucleosome structure of parental Ad2 DNA differs from that of cellular DNA (22). Moreover, Ad5 DNA synthesis is strongly inhibited by 2'3'-dideoxythymidine triphosphate (ddTTP), a nucleotide analogue which does not affect cellular or papovavirus DNA replication. The latter observation has led to the hypothesis that DNA polymerase γ, which is very sensitive to ddTTP, is required for Ad5 DNA synthesis (11, 12). However, these experiments did not exclude an additional function of DNA polymerase α, which enzyme is insensitive to ddTTP. Both DNA polymerases α and γ have been found in replication complexes extracted from adenovirus infected nuclei (18, 19). Therefore we set out to study the sensitivity of Ad5 DNA synthesis to aphidicolin as a means to investigate the possible role of DNA polymerase α in Ad5 DNA replication. The present report shows that adenovirus DNA synthesis is at least 300 fold more resistant to this drug than cellular DNA replication.

The inhibition of Ad5 DNA synthesis *in vitro* by aphidicolin is independent of the dCTP concentration in contrast to the behaviour of purified DNA polymerase α or cellular DNA replication in isolated nuclei. These results argue against a function of DNA polymerase α in the replication of adenovirus DNA.

MATERIALS AND METHODS

Aphidicolin was kindly supplied by Drs. S. Spadari (Pavia), A.H. Todd and B. Hesp (ICI). The drug was dissolved to 18 mg/ml in dimethyl sulfoxide (DMSO) and diluted into buffer before use. The final DMSO concentration in each experiment was less than 1%. Control experiments showed that at this concentration DMSO was not inhibitory.

KB or HeLa cells were grown in suspension culture in Eagles minimal essential medium supplemented with 5% calf serum, 0.05% glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin sulfate. The cells were mycoplasm free as tested via the standard agar plate growth procedure. At a concentration of 3×10^5 cells per ml the cells were infected with Ad5 at a multiplicity of 10 PFU per

cell and harvested 16 hrs post infection. African Green Monkey Kidney (AGMK) cells were grown as described (11). Simian Adenovirus SA7 was obtained from Drs. F. Puvion-Dutilleul and P. Tournier (Villejuif, France).

DNA synthesis

DNA synthesis *in vivo* was monitored in 2 ml cell suspensions to which 10 mM N-2 hydroxyethyl piperazin-N'-2-ethane sulfonic acid (HEPES), pH 7.2 was added to stabilize the pH. After addition of 1 μC /ml [^{3}H]thymidine (specific activity 52 C/mmole) and incubation for 30 min at 37^o C, the cells were centrifuged, washed twice with 0.01 M Tris-HCl - 0.01 M EDTA pH 7.5 and suspended in 1 ml of this buffer.

Total cellular DNA synthesis in uninfected cells was measured after precipitation with 10% trichloroacetic acid (TCA) containing 0.01 M sodium pyrophosphate (PPi). The precipitate was collected on glass fiber filters and washed extensively with 1% TCA containing 0.001 M PPi followed by 96% ethanol. The filters were dried and counted.

Infected cells were carefully lysed by digestion with pronase (0.5 mg/ml) in the presence of 0.1% sodium dodecyl sulfate (SDS) for 30 min at 32^o C. The SDS concentration was raised to 2% and NaCl was added to 1 M. After 4-16 hrs at 4^o C cellular DNA was removed by centrifugation at 10,000 x g for 30 min at 4^o C. As measured from ^{14}C-thymidine prelabeled cells, this procedure removes 98% of the cellular DNA. From the supernatant, containing more than 90% of the viral DNA, aliquots were spotted on Whatmann 3 MM filter pads. The filters were dried, soaked in 10% TCA containing 0.01 M PPi, washed 3 times with 1% TCA - 1 mM PPi, once with 96% ethanol and once with acetone, dried and counted.

Nuclei from Ad5 infected cells were isolated and nuclear DNA synthesis was performed as described (13). The nuclei were incubated for 30 min at 32^o C in the presence of [^{3}H]thymidine triphosphate (specific activity 10 C/mmole). After incubation the nuclei were directly suspended in 1 ml 0.01 M Tris-HCl - 0.01 M EDTA pH 7.5 and DNA synthesis was determined as described above.

For the study of cellular DNA synthesis in isolated nuclei exponentially growing cells were centrifuged, washed once with phosphate buffered saline, once with 10 mM Na-K-PO_4 (pH 8.0) - 2 mM dithiothreitol (DTT) 2 mM $MgCl_2$ - 1 mM EDTA and suspended in this buffer at a concentration of 10^8 cells per ml (24). The cells were Dounce homogenized and the lysate was diluted 5 times with isotonic HEPES buffer (13). The nuclei were centrifuged for 10 min at

2,500 rpm, suspended to 10^8/ml in isotonic HEPES buffer and used for DNA synthesis similarly as nuclei from infected cells (13).

Sedimentation analysis was performed in isokinetic 5-27% sucrose gradients containing 0.01 M Tris-HCl pH 7.5 - 0.001 M EDTA - 1 M NaCl - 0.1% sarkosyl in the SW41 rotor. Centrifugation was for 16 hr at 24,000 rpm, at 4° C. Fractions were collected on paper filters and the DNA was precipitated with TCA and processed as described.

DNA polymerases

DNA polymerase α was isolated from KB cells or Ad5 infected KB cells 16 hr post infection. The enzymes were purified by DEAE cellulose chromatography and DNA cellulose chromatography as described (14). The enzymes were characterized by their sedimentation value (8.8 S), sensitivity to N-ethylmaleimide and resistance to ddTTP (11).

DNA polymerase γ was isolated according to Knopf et al. (15) and purified by DEAE cellulose, phosphocellulose, hydroxylapatite and DNA cellulose chromatography. The enzyme was essentially free from contaminating DNA polymerase α or β as indicated by its sensitivity to N-ethylmaleimide and 99% inhibition by ddTTP under conditions which do not inhibit DNA polymerase α or β (11). The DNA polymerase γ preparation was 4-fold more active with its preferred template, poly(rA)·oligo(dT), than with activated DNA.

DNA polymerase α activity was assayed in 10 mM Tris-HCl pH 8.5 - 10 mM $MgCl_2$ - 1.0 mM DTT - 50 μM dATP, dCTP, dGTP, 5 μM [^{3}H]dTTP (2 C/mmol) with 200 μg/ml of activated calf thymus DNA. The assay for DNA polymerase γ in the presence of poly(rA)·oligo(dT) was in 50 mM Tris-HCl pH 7.5 - 100 mM KCl - 0.5 mM $MnCl_2$ - 2.5 mM DTT - 0.5 mg/ml bovine serum albumin (BSA) and 1 μM [^{3}H]dTTP (10 C/mmol). Poly(rA)·dT_{12-18} was present at 25 μg/ml and was prepared freshly before each assay by hybridization of poly(rA) and dT_{12-18} (2:1 w/w) in 50 mM Tris-HCl pH 7.5 - 100 mM KCl for 5 min at 70° C followed by slow cooling.

When DNA polymerase γ was assayed with activated DNA as template the reaction mixture consisted of 50 mM Tris-HCl pH 8.5 - 7.5 mM $MgCl_2$ - 0.5 mM DTT - 50 mM dATP, dCTP, dGTP and 1 μM [^{3}H]dTTP (10 C/mmol) in the presence of 0.5 mg/ml BSA and 100 μg/ml activated DNA. In all assays the values obtained after incubation at 0° C were subtracted.

RESULTS

The DNA synthesis in exponentially growing KB or HeLa cells was

measured as a function of the aphidicolin concentration. Fig. 1 demonstrates that cellular DNA synthesis is very sensitive to low concentrations of the drug. KB cell DNA synthesis was 50% inhibited at a concentration of 8×10^{-8} M aphidicolin ($C_{50} = 8 \times 10^{-8}$ M) and the inhibition was almost complete (99%) at 3×10^{-6} M concentration. When HeLa cells were assayed, the dose response curve was almost identical and a value for $C_{50} = 1.0 \times 10^{-7}$ M was found.

In contrast to these results, Ad5 DNA replication in infected KB cells was not inhibited significantly at these aphidicolin concentrations. Inhibition started only above 10^{-6} M and 50% inhibition was attained at 3.2×10^{-5} M aphidicolin (Fig. 1), which is 300-400 fold higher than required for inhibition of DNA synthesis in uninfected cells. Similar results have recently been obtained by others (17, 20).

Several explanations are possible for the relative resistance of adenovirus DNA synthesis to aphidicolin. Since it is generally assumed that aphidicolin inhibits cellular DNA replication by interfering with the activity of DNA polymerase α, infected cells might contain a modified DNA polymerase α with a reduced sensitivity to aphidicolin. Major changes in DNA polymerase α have not been found in Ad5 infected cells (14), but minor modi-

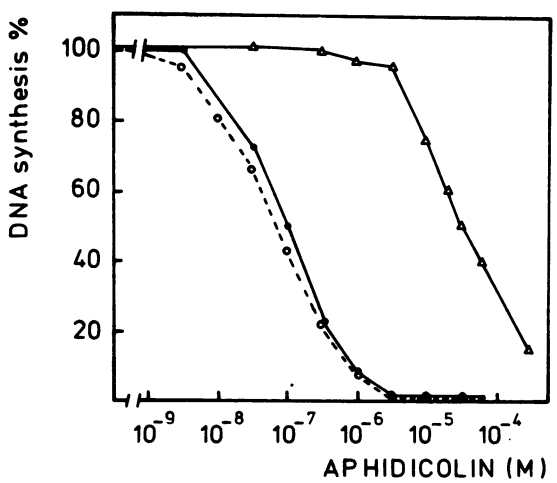

Fig. 1 Effect of aphidicolin on cellular or adenovirus DNA synthesis *in vivo*. Uninfected KB cells (o), HeLa cells (•) or Ad5 infected KB cells (Δ) were labeled for 30 min with [^{3}H]thymidine in the presence of various concentrations of aphidicolin. The incorporation of radioactivity in cellular (o, •) or viral (Δ) DNA was measured. 100% = 35,627 cpm per 10^6 KB cells, 18,873 cpm per 10^6 Hela cells and 28,706 cpm per 10^6 Ad5 infected KB cells.

fications are not excluded. Therefore, we isolated DNA polymerase α from infected as well as uninfected cells and assayed their sensitivity to aphidicolin (Fig. 2). However, both enzymes were almost equally inhibited with C_{50} = 2.9 μM for DNA polymerase α from uninfected cells and C_{50} = 2.7 μM for the enzyme isolated from infected cells (see Table II). The complete inhibition at high aphidicolin concentrations makes the presence of a resistant DNA polymerase α fraction in infected cells not likely. The C_{50} values for KB cell DNA polymerase α correspond well to the results obtained for DNA polymerase α from sea urchin embryo's, regenerating rat liver and HeLa cells (2, 3, 4, 17).

We could also exclude a modification in aphidicolin sensitivity for DNA polymerase γ after infection. Both DNA polymerase γ from infected and uninfected cells were immune to high concentrations of aphidicolin, up to 3×10^{-3} M (Fig. 2). This result was independent of the assay conditions used: neither in the presence of the preferred template poly(rA)·oligo(dT), nor with activated DNA as template did aphidicolin have any effect.

Another possible explanation for the 300-400 fold difference in sensitivity is that uninfected cells metabolize aphidicolin to a more active com-

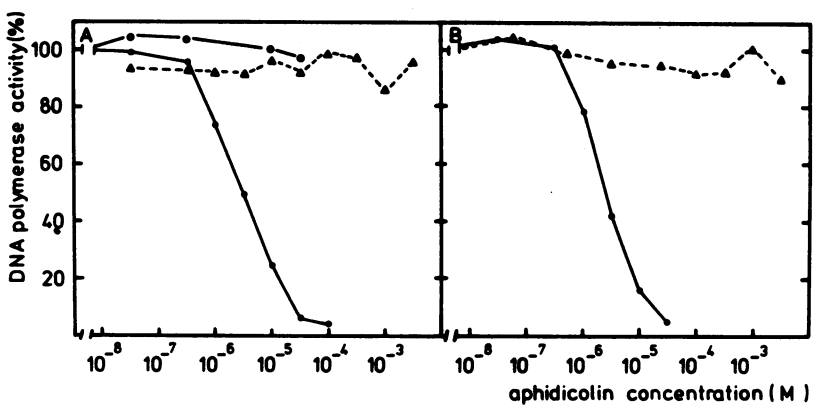

Fig. 2 Effect of aphidicolin on purified DNA polymerases from uninfected (A) or Ad5 infected (B) KB cells. A. DNA polymerase α activity (•) was measured for 30 min at 37° C with activated DNA as template. DNA polymerase γ was measured either with poly(rA)·oligo(dT) for 30 min at 30° C (Δ) or with activated DNA for 30 min at 37° C (o). 100% activity for each of the DNA polymerases was 9.8 pmol (α), 5.4 pmol (γ with poly(rA)·oligo(dT) and 1.2 pmol (γ with activated DNA). B. DNA polymerase α (•) and γ (Δ) purified from Ad5 infected KB cells 16 hrs post infection. 100% activity was 15.5 pmol for DNA polymerase α and 1.15 pmol for DNA polymerase γ with poly(rA)·oligo(dT) as template.

pound while infected cells do not, or, alternatively, that infected cells somehow inactivate aphidicolin. An attempt to test these possibilities was made. Uninfected or Ad5 infected KB cells were preincubated in the presence of 3 x 10^{-6} M aphidicolin for 30 min at 37° C. The cells were centrifuged and the supernatant was mixed with either infected or uninfected cells. As can be seen in Table I, preincubation of aphidicolin with Ad5 infected cells did not diminish its inhibitory potential in uninfected KB cells. Preincubation of aphidicolin with uninfected cells made the drug only slightly more reactive towards adenovirus DNA synthesis (78%, compared to 90% without preincubation, Table I). We also tested the activity of aphidicolin, extracted with 60% methanol from the preincubated cells. Again, no evidence was found for extensive intracellular modification (not shown).

These experiments, although not conclusive, suggest that extensive metabolic conversion of aphidicolin can not explain the differential inhibition of Ad5 and cellular DNA synthesis.

TABLE I

Preincubation of aphidicolin with infected or uninfected cells does not change its inhibitory potential.

Cell tested	Preincubation conditions of supernatant	% DNA synthesis
KB	–	100
KB	aphidicolin (3 μM)	3
KB	Ad5 infected KB cells	95
KB	Ad5 infected KB cells + aphidicolin (3 μM)	4
Ad5 infected KB	–	100
Ad5 infected KB	aphidicolin (3 μM)	90
Ad5 infected KB	KB cells	99
Ad5 infected KB	KB cells + aphidicolin (3 μM)	78

Uninfected or Ad5 infected KB cells, 16 hr post infection, were preincubated for 30 min at 37° C with or without $3 \cdot 10^{-6}$ M aphidicolin. The cells were centrifuged and to the supernatant new KB cells or Ad5 infected KB cells were added to obtain a suspension of the original density ($2 \cdot 10^6$ cells/ml). [^{3}H]thymidine (1 μCi/ml) was added for 30 min at 37° C and viral or cellular DNA synthesis was determined. 100% = 39,531 cpm per 10^6 uninfected cells and 32,633 cpm per 10^6 Ad5 infected KB cells.

Differential inhibition of SA7 and SV40 DNA replication

As another approach to study possible metabolic conversion or pool effects we investigated the effect of aphidicolin on the DNA synthesis of two different viruses which replicate concomitantly in the same host cell. We used monkey kidney (AGMK) cells doubly infected both with SV40 and simian adenovirus SA7.

Since adenovirus infection shuts off SV40 DNA synthesis (27) the cells were infected with SV40 24 hrs prior to addition of SA7. At 16 hrs after SA7 infection the cells were labeled with [^{3}H]thymidine in the presence or absence of 3 µM aphidicolin and total viral DNA was analyzed by sucrose gradient centrifugation (Fig. 3). In the control, without aphidicolin, two peaks are observed at 30 S (SA7 DNA) and 21 S (SV40 DNA). The SV40 synthesis is three-fold reduced compared to cells singly infected with the same m.o.i., indicating that the cells are indeed doubly infected (results not shown). In the presence of aphidicolin only SA7 DNA is synthesized (Fig. 3B) and SV40 synthesis is blocked completely. The SA7 DNA replication itself is 45% reduced by aphidicolin while in singly infected cells a 30% reduction was observed. Thus, SV40 co-infection slightly increased the sensitivity of SA7 DNA, while SV40 DNA replication is completely blocked both in singly and doubly infected cells.

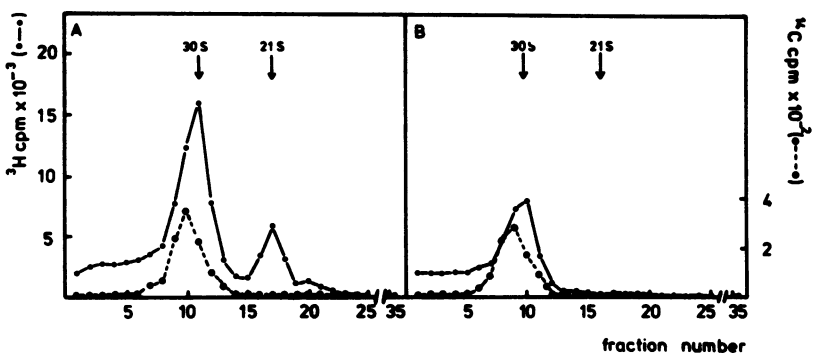

Fig. 3 Differential inhibition of SV40 and SA7 DNA synthesis by aphidicolin in doubly infected AGMK cells. Confluent plates of AGMK cells were infected with SV40 (10 PFU/cells) followed by addition of simian adenovirus SA7 after 24 hr. 16 hr later the cells were labeled for 2 hr with 20 µCi/ml of [^{3}H]thymidine in the absence (A) or presence (B) of 3 µM aphidicolin. Viral DNA was extracted and analysed on an isokinetic 5-27% sucrosegradient ^{14}C-Ad5 DNA was added as an internal marker (31 S). Sedimentation was for 16 hr at 25,000 rpm at 4° C in the SW41 rotor.

Effects of dCTP variation

To test further whether DNA polymerase α was functioning in viral DNA replication we made use of the recent observation (21, 28, Pedrali-Noy and Spadari, personal communication) that the aphidicolin inhibition of DNA polymerase α is competitive with respect to the dCTP concentration. We tested the aphidicolin sensitivity of KB DNA polymerase α as a function of the dNTP concentration (Fig. 4) and confirmed that only the dCTP concentration has a strong influence on the inhibition by aphidicolin, and not any of the other dNTP's. No such relation exists for DNA polymerase γ: both at 50 μM dCTP and 1 μM dCTP DNA polymerase γ remained immune to aphidicolin (data not shown).

Whatever the mechanism of this dCTP effect, it enables us to test the possible involvement of DNA polymerase α in adenovirus DNA replication by manipulation of the dCTP concentration in an *in vitro* system of isolated nuclei.

Nuclei from Ad5 infected KB cells can elongate and terminate pre-existing replicative intermediates but are defective in the initiation of

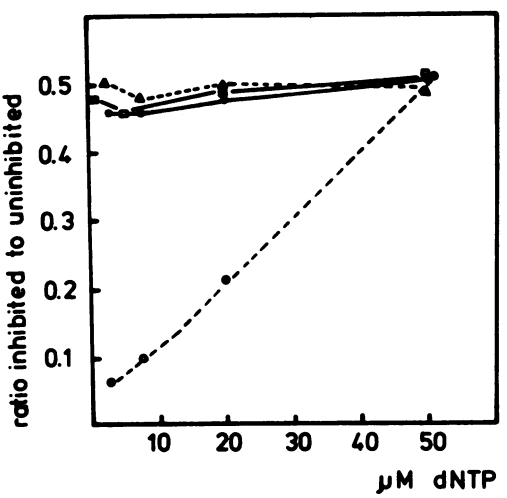

Fig. 4 DNA polymerase α inhibition by aphidicolin is dependent upon the dCTP concentration. The inhibition of DNA polymerase α by 2.5 μM aphidicolin was tested as a function of the concentration of the four dNTP's. For variation of dATP, dGTP and dCTP, ^{3}H-TTP (5 μM, 10 Ci/mmol) was added as label while for variation of TTP both ^{3}H-TTP of variable specific activity, as ^{3}H-dGTP (5 μM, 10 Ci/mmol) was used, with similar results.

●—● dATP x---x dGTP

□—□ dTTP o---o dCTP

new rounds of replication (16). In the presence of 50 µM dCTP, the normal incubation condition, again a differential inhibition was observed between cellular and viral DNA synthesis (Fig. 5), although the difference is only tenfold (Table II) instead of the 300-400 fold difference observed in intact cells. In the presence of 1 µM dCTP cellular DNA synthesis shows an increased sensivitity towards aphidicolin while adenovirus DNA synthesis does not (Fig. 5). At these low dCTP concentrations we observed C_{50} = 0.08 µM for cellular DNA synthesis compared to 0.8 µM with 50 µM dCTP. A value of 0.08 µM is close to the sensitivity obtained in intact cells. For Ad5 DNA synthesis omission of dCTP slightly decreased the drug sensitivity from C_{50} = 8 µM to C_{50} = 11 µM. When dATP was omitted instead of dCTP no difference in inhibition was found, neither for Ad5 DNA nor for cellular DNA. Omission of dTTP, studied in the presence of [^{3}H]dGTP as radioactive label, increased the sensitivity of Ad5 DNA synthesis without effect on cellular DNA synthesis or DNA polymerase α activity (not shown).

These data indicate that replication of cellular DNA in isolated nuclei shows a similar effect as purified DNA polymerase α, in contrast to adenovirus DNA synthesis.

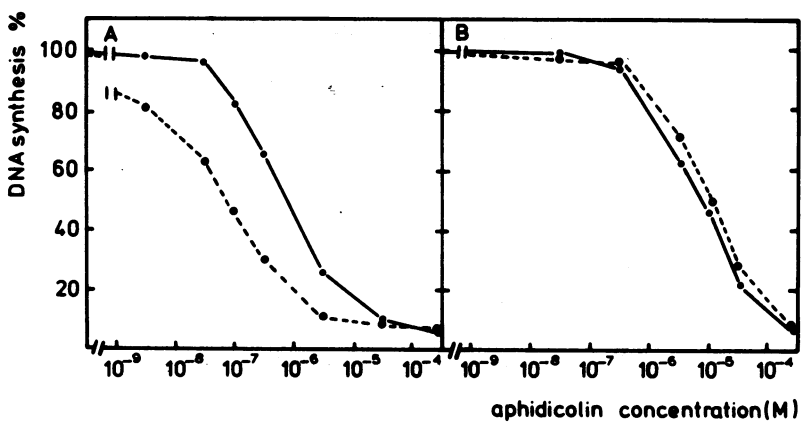

Fig. 5 Effect of a low dCTP concentration on the inhibition of cellular (A) or adenovirus (B) DNA synthesis in isolated nuclei. Nuclei were incubated in the absence (o---o) or presence (●——●) of added dCTP (50 µM) with various concentrations of aphidicolin. In the presence of dCTP, 100% incorporation was 30,430 cpm per 10^6 nuclei for infected cells and 2,661 cpm per 10^6 nuclei for uninfected cells. Omission of dCTP in the reaction mixture reduced the DNA synthesis 69% for Ad5 and 45% for uninfected nuclei. Both with and without added dCTP the synthesized DNA in infected nuclei was of viral nature as judged by sedimentation at 31 S in a sucrose gradient.

TABLE II

Aphidicolin concentration required for 50% inhibition (C_{50})$^{\Delta}$

system	C_{50} (μM)
DNA polymerase α (KB cells)	2.9
DNA polymerase α (Ad5 infected KB cells)	2.7
DNA polymerase γ$^{\square}$ (KB cells)	>300
DNA polymerase γ* (Ad5 infected KB cells)	>300
Cellular DNA synthesis (KB cells)	0.09
Ad5 DNA synthesis (KB cells)	32
Cellular DNA synthesis (nuclei, 50 μM dCTP)	0.8
Cellular DNA synthesis (nuclei, minus dCTP)	0.08
Ad5 DNA synthesis (nuclei, 50 μM dCTP)	8
Ad5 DNA synthesis (nuclei, minus dCTP)	11

Δ All values are the average of at least two experiments

□ Tested both with activated DNA and with poly(rA)·oligo(dT) as template

* Tested only with poly(rA)·oligo(dT) as template

DISCUSSION

The replication of adenovirus DNA requires both viral coded and host proteins, one of which is DNA polymerase. Of the three classes DNA polymerases which can be identified in eukaryotic cells, only α and γ have been found in replication complexes, which can elongate pre-existing replication intermediates (18, 19). Cellular and viral DNA synthesis can be easily distinguished by its sensitivity to ddTTP (11, 12). This study and the results of Longiaru et al. (17) and Krokan et al. (20) show that the DNA polymerase α-specific inhibitor aphidicolin also differentiates between the two types of DNA synthesis. *In vivo* the discrepancy in the concentration of the drug needed for inhibition is 300-400 fold while *in vitro*, in isolated nuclei the difference is 130 fold. The latter value is obtained at low dCTP concentration and is dependent upon the concentration of this nucleotide in the *in vitro* system.

The inhibition of adenovirus DNA synthesis at high aphidicolin concentrations has been interpreted to mean that, in addition to DNA polymerase γ, DNA polymerase α plays a role in adenovirus DNA replication, possibly in a single complex of both enzymes (17, 20). Such an interpretation does not explain the 300-400 fold difference in aphidicolin concentration required to

inhibit the two processes, even when they occur in the same cell as in AGMK cells doubly infected with SA7 and SV40. We have neither found any evidence for metabolic conversion or accumulation of aphidicolin, nor did we observe any change in aphidicolin sensitivity for DNA polymerase α or γ after infection of cells with adenovirus.

A major argument against a role of DNA polymerase α comes from variation of the dCTP concentration. Both DNA polymerase α (21) and cellular DNA synthesis *in vitro* show an increased sensitivity at low dCTP concentrations, which are close to the intracellular concentration of dCTP *in vivo*. At these low concentrations a good correlation exists between the sensitivity of DNA polymerase α and DNA synthesis in isolated nuclei or intact cells. This adds further evidence to the role of DNA polymerase α as the "replicative" enzyme. However, no such correlation was observed in viral infected cells (Fig. 5). If DNA polymerase α plays a role in the adenovirus replication fork, similarly as in chromosomal DNA synthesis, one would expect a similar reaction to low dCTP concentrations. We can not completely exclude that a modified micro environment in the replication fork changes the mechanism of aphidicolin or dCTP dependency of DNA polymerase α. Recently, Habara et al. (28) showed that Ad2 DNA synthesis in a replication complex was sensitive to aphidicolin (C_{50} = 8 x 10^{-6} M) when assayed with [^{3}H]-dTTP as radioactive label, but much less when one of the other dNTP's was used. This was in contrast to the results obtained with purified DNA polymerase α. The authors also concluded that the mode of aphidicolin inhibition on DNA synthesis in the replication complex was not coincident with that of purified DNA polymerase α.

As an independent approach we have also studied the effect of neutralizing anti-DNA polymerase α gammaglobulin (obtained from Dr. R.C. Gallo, NIH, ref. 24) on the synthesis of Ad5 DNA in isolated nuclei. No inhibition was observed under conditions where anti-DNA binding protein gammaglobulin, directed against a viral coded protein required for DNA replication, was able to inhibit viral DNA synthesis (13).

DNA polymerase γ is completely resistant to aphidicolin. Thus, the weak sensitivity of adenovirus can not be explained by an interaction with this enzyme. Possibly aphidicolin affects other yet unknown replication factors at high concentrations. These factors may be common to infected and uninfected cells but remain undetected in the uninfected cell due to the high sensitivity of DNA polymerase α.

Alternatively, a modified DNA polymerase α could be present in low amounts which has escaped detection in the infected cells. Further studies on the

TABLE III

Similarities between adenovirus and mitochondrial DNA synthesis and differences with cellular or papovavirus DNA synthesis

	Adenovirus/mitochondrial	Cellular/SV40
Mechanism	Asynchronous duplication of the two strands (displacement)	Synchronous duplication of both strands (Cairns' type)
Direction	Unidirectional	Bidirectional
Nucleosome structure	Aberrant	Normal
Correlation with histone synthesis	No	Yes
Inhibition by cycloheximine	No	Yes
Effect of ddNTP	Strong inhibition	No inhibition
Effect of aphidicolin	Weak inhibition (Ad)	Strong inhibition

mechanism of inhibition by aphidicolin and the isolation of aphidicolin resistant mutants may clarify the effect of the drug on viral DNA replication.

The replication of adenovirus DNA and mitochondrial DNA share a number of properties and differ in many respects from that of SV40 DNA and cellular DNA (see Table III). In view of the identical properties of the nuclear DNA polymerase γ and mitochondrial DNA polymerase it has been suggested (11, 25) that DNA polymerase γ is the enzyme which acts in strand displacement synthesis while DNA polymerase α participates in Cairns' type DNA replication. Dissection of the adenovirus DNA replication machinery using *in vitro* DNA replication systems (26) might help to elucidate this point further.

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†Correspondence to: P.C.van der Vliet, Laboratory for Physiological Chemistry, Vondellaan 24 a, 3521 GG Utrecht, The Netherlands

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