Biochem. J. (1981) **199**, 453–455
Printed in Great Britain

Aphidicolin does not inhibit DNA repair synthesis in ultraviolet-irradiated HeLa cells

A radioautographic study

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(*Received 23 June 1981/Accepted 10 July 1981*)

A radioautographic examination of nuclear DNA synthesis in unirradiated and u.v.-irradiated HeLa cells, in the presence and in the absence of aphidicolin, showed that aphidicolin inhibits nuclear DNA replication and has no detectable effect on DNA repair synthesis. Although the results establish that in u.v.-irradiated HeLa cells most of the DNA repair synthesis is not due to DNA polymerase α, they do not preclude a significant role for this enzyme in DNA repair processes.

Aphidicolin is a tetracyclic diterpene tetraol (Brundret *et al.*, 1972) that inhibits DNA synthesis and the growth of eukaryotic cells. *In vitro* aphidicolin specifically inhibits the replicative (Bollum, 1975; Falaschi & Spadari, 1978; Weissbach, 1979) DNA polymerase α, or α-like DNA polymerase of plants (Amileni *et al.*, 1979), with no effect on DNA polymerases β and γ (Bucknall *et al.*, 1973; Ikegami *et al.*, 1978; Pedrali-Noy & Spadari, 1979; Krokan *et al.*, 1979; Pedrali-Noy *et al.*, 1980; Kwant & van der Vliet, 1980; Sugino & Nakayama, 1980; Sala *et al.*, 1980, 1981; Huberman, 1981; and references therein). Direct and unequivocal demonstration of selective inhibition of nuclear DNA replication by aphidicolin in both animal (Geuskens *et al.*, 1981) and plant cells (Sala *et al.*, 1981) has been achieved by electron-microscope radioautography. The synthesis of organellar (mitochondrial and chloroplast) DNA is resistant to aphidicolin (Zimmermann *et al.*, 1980; Geuskens *et al.*, 1981; Sala *et al.*, 1981) and correlates with the intrinsic resistance of mitochondrial DNA polymerase γ and chloroplast γ-like DNA polymerase (Sala *et al.*, 1980) to this drug. These studies have clearly shown that DNA polymerase α (or the α-like polymerase in plants) is essential in nuclear DNA replication and is not involved in the replication of organellar DNA, although they do not, of course, preclude the involvement of other DNA polymerases, in addition to polymerase α, in nuclear DNA replication.

DNA polymerase β, which is thought to be involved in repair of damaged nuclear DNA (Coetzee *et al.*, 1978; Hübscher *et al.*, 1978, 1979; Weissbach, 1979; and references therein), is resistant to aphidicolin when assayed *in vitro*. However, conflicting observations on the effect of aphidicolin on DNA repair synthesis *in vivo* have been reported (Berger *et al.*, 1979; Ciarrocchi *et al.*, 1979; Hanaoka *et al.*, 1979; Pedrali-Noy & Spadari, 1980; Seki *et al.*, 1980; Giulotto & Mondello, 1981; Snyder & Regan, 1981).

The present paper reports a radioautographic demonstration of DNA repair synthesis in HeLa cells in the presence of aphidicolin.

Experimental

Cell growth

HeLa cells were grown in RPMI medium with 10% foetal calf serum with a generation time of about 18–20h. Cells were allowed to grow until confluence, then collected and resown in the same medium for 24h before u.v. irradiation.

Ultraviolet-light irradiation

Cells attached to glass slides were irradiated with a Philips Mineralight lamp with maximum energy output at a wavelength of 254nm. Doses were measured by a Latarjet dosimeter. The slides were then incubated with fresh medium containing [^{3}H]-thymidine (25Ci/mmol) at a final concentration of 20μCi/ml with or without 15μM-aphidicolin. After 1h incubation, the medium was removed and the cells were washed with phosphate-buffered saline (Dulbecco & Vogt, 1954) containing 1mg of unlabelled thymidine-ml and, where appropriate, 15μM-

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aphidicolin, washed again with phosphate-buffered saline and fixed with ethanol/acetic acid (3:1, v/v) for 20min at 20°C and 20min at 4°C. After several washes with distilled water and unlabelled thymidine, the cells were rinsed with ethanol and prepared for radioautography as previously described (Hardt *et al.*, 1980).

Cell counts

The percentage of cells in S phase was estimated by examining 300 cells. DNA repair synthesis was estimated by counting the number of grains in the nuclei of 300 labelled cells, after subtracting the background value (grains present in unirradiated cells). The standard deviation did not exceed 30%.

Chemicals

[*methyl*-^{3}H]Thymidine (25 Ci/mmol) was from The Radiochemical Centre, Amersham, Bucks., U.K. Aphidicolin was kindly supplied by Dr. A. Todd, Imperial Chemical Industries, Macclesfield, Cheshire, U.K.

Results

Radioautographic demonstration of DNA repair synthesis in the presence of aphidicolin

The results obtained with exponentially growing HeLa S3 cells are shown in Plate 1. In unirradiated control cultures in the absence of aphidicolin (Plate 1*a*), [^{3}H]thymidine was incorporated only into nuclei of cells that were in the S phase of the cell cycle (approx. 30% of the cell population). The addition of aphidicolin (Plate 1*c*) inhibited completely the incorporation of thymidine into nuclear DNA, as is expected from the specific inhibition of the replicative polymerase α by aphidicolin. A very small number of grains were found in some cells, probably corresponding to those that had been in S phase.

When cells were exposed to a u.v. dose of 15 J·m^{-2} before incubation with [^{3}H]thymidine in the absence of aphidicolin (Plate 1*b*), the amount of DNA synthesis occurring in S-phase cells was essentially unaltered (note that the radioautographic exposure time had been shortened from 18 to 4 days to allow counting of grains in non-S-phase cells), but u.v. irradiation induced incorporation of [^{3}H]-thymidine in all cells in other stages of the growth cycle. This radiation-stimulated DNA synthesis is indicative of a repair process and agrees with the reported ability of cells outside the S phase to repair u.v. damage (Giulotto *et al.*, 1978).

When aphidicolin was added to irradiated cells (Plate 1*d*), the heavily labelled cells that were in the S phase at the time of incubation disappeared because the replicative polymerase α is inhibited by the drug, but all cells showed a uniform light labelling comparable with that observed in the cells (see Plate 1*b*) that were not in the S phase, i.e. u.v.-induced DNA repair synthesis.

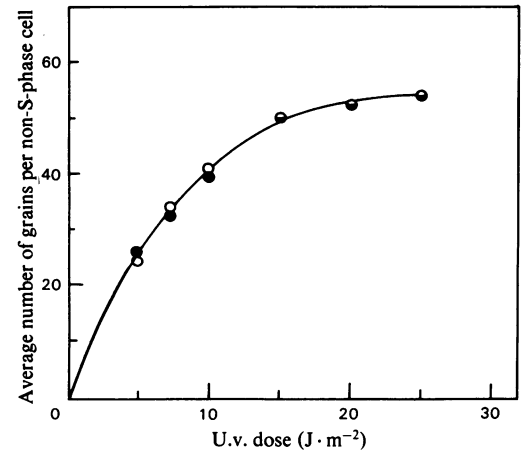


Fig. 1. *U.v.-dose-dependence of grain count in control* (○) *and aphidicolin-treated* (●) *HeLa-cell cultures*
For details see the Experimental section.

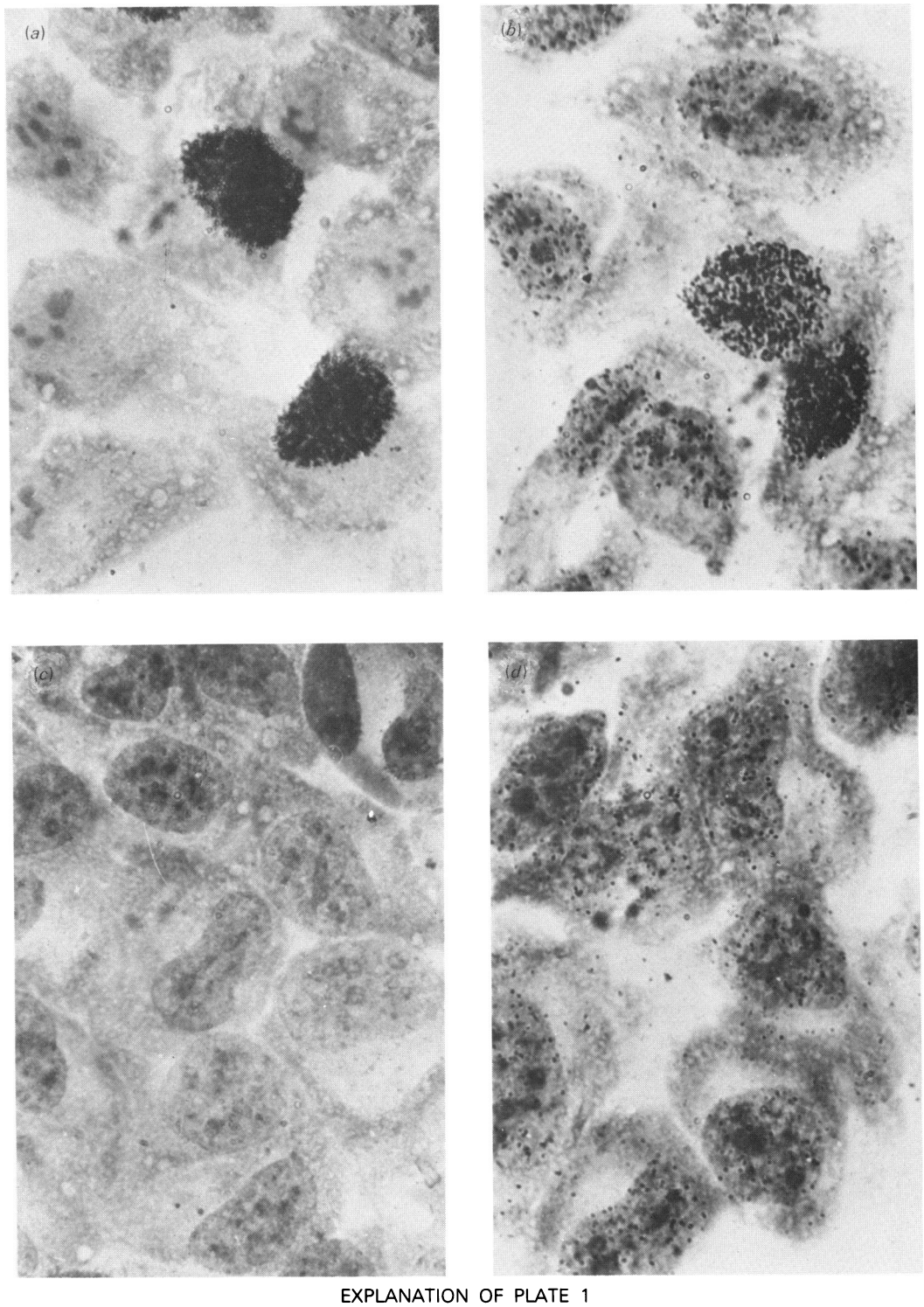
Dose-dependence of u.v.-induced DNA repair synthesis in control and aphidicolin-treated cells

DNA repair synthesis was also studied as a function of u.v. irradiation (Fig. 1), and the average number of grains per non-S-phase cell was found to be the same for both control cells and cells treated with aphidicolin.

The average number of grains increased with increasing doses of u.v. light, but, as was seen in earlier studies (Rommelaere *et al.*, 1974), the dose–response curve is only linear at low u.v. doses.

Discussion

Our radioautographic analysis of nuclear DNA synthesis in unirradiated and u.v.-irradiated HeLa cells, in the presence and in the absence of aphidicolin, shows that this drug is a specific inhibitor of nuclear DNA replication in eukaryotes and has no detectable effect on DNA repair synthesis. This result correlates with the sensitivity of DNA polymerase α and the resistance of DNA polymerase β to aphidicolin *in vitro* and is consistent with the proposed role of polymerase β in DNA repair synthesis. Our results are consistent with earlier experiments which failed to detect an effect of aphidicolin on DNA repair synthesis, as measured by biochemical methods (Pedrali-Noy & Spadari, 1980; Seki *et al.*, 1980) or by radioautography of u.v.-irradiated chromosomes (Guilotto & Mondello, 1981). However, some other studies have reported



EXPLANATION OF PLATE 1

Radioautographs of HeLa cells incubated with [^3H]thymidine

(*a*) Unirradiated cells incubated in the absence of aphidicolin. Radioautographs were developed after 18 days. (*b*) Irradiated ($15\,J\cdot m^{-2}$) cultures incubated in the absence of aphidicolin. Radioautographs were developed after 4 days in order to allow counting of grains in the nuclei of non-S-phase cells. (*c*) Unirradiated cells in the presence of $15\,\mu M$-aphidicolin. Radioautographs were exposed for 18 days. (*d*) Irradiated ($15\,J\cdot m^{-2}$) cultures in the presence of $15\,\mu M$-aphidicolin. Radioautographs were exposed for 4 days as in (*b*). Magnification ×810.

that aphidicolin does inhibit DNA repair synthesis (Hanaoka *et al.*, 1979; Berger *et al.*, 1979; Ciarrocchi *et al.*, 1979). The different results found in those studies may be due either to the involvement of DNA polymerase α in DNA repair synthesis in these systems, or, possibly, to the procedures needed to isolate nuclei (Hanaoka *et al.*, 1979) or to permeabilize cells (Berger *et al.*, 1979; Ciarrocchi *et al.*, 1979) in order to allow the entrance of exogenously supplied labelled deoxyribonucleoside triphosphates.

Snyder & Regan (1981) have investigated the persistence of low-molecular-weight DNA and of pyrimidine dimers in u.v.-irradiated human fibroblasts. They found that aphidicolin significantly inhibited DNA repair, as judged by these criteria. Our results show that the majority of DNA repair synthesis is not inhibited by aphidicolin and is therefore not due to DNA polymerase α. Although these results do not preclude a significant role for DNA polymerase α in DNA repair processes, they establish that in u.v.-irradiated HeLa cells the extent of DNA repair synthesis by DNA polymerase α must be small. Physiological conditions and cellular differentiation are known to affect activity of DNA polymerase α, and their effects on DNA repair have yet to be adequately studied.

We thank Dr. O. Doubleday for helpful discussions and help with the final version of the manuscript, and C. Bruck for supplying HeLa-cell cultures. The technical assistance of R. Legas and A. Rebuzzini is acknowledged. This work was partly supported by the Programma Finalizzato 'Controllo della crescita neoplastica' del Consiglio Nazionale delle Ricerche, Italy, and by the Belgian Government and Université Libre de Bruxelles (Actions de Recherches concertées).

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