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INDUCTION OF DNA POLYMERASE IN *TETRAHYMENA PYRIFORMIS*

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SUMMARY: A number of different treatments will induce DNA polymerase in *Tetrahymena pyriformis*. In the present paper we have studied the induction of DNA polymerase by the inhibitor of DNA synthesis methotrexate plus uridine (M+U) and UV irradiation (after incorporation of bromodeoxyuridine (BUdR) into DNA) in synchronized *Tetrahymena* populations. We have found that M+U must be present in the nuclear S-period in order to induce the polymerase, and that a dose of UV irradiation which is too low to induce DNA polymerase will do so if the damaging effect of the irradiation is enhanced by incorporation of BUdR into DNA.

Induction of a high activity of DNA polymerase in *Tetrahymena pyriformis* after treatment with the inhibitors of DNA synthesis M+U was first described by Westergaard and Pearlman (1). Since then several other treatments have been found to be equally effective inducers of polymerase activity (irradiation with UV light or electrons (2), ethidium bromide (3,4) and chloramphenicol (5). The mechanisms behind the induction is still obscure, but the diversity of the inducers indicate that several routes are possible. It has been suggested that the induced enzyme has a repair function in the mitochondria(2,3,4). However, no repair synthesis of DNA could be detected neither in mitochondrial DNA from ethidium bromide treated cells nor in bulk DNA from M+U treated cells (6,7,8). Therefore, repair synthesis can hardly be the only function of the enzyme.

However, the different inducing treatments seem to have in common that they cause inhibition of DNA synthesis. M+U seems

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to inhibit DNA synthesis only, leaving RNA and protein synthesis unaffected (9), whereas chloramphenicol (5) and ethidium bromide (10) also interfere with the synthesis of other macromolecules. Irradiation may - depending on the dose - inhibit RNA and protein synthesis as well as DNA synthesis. The effect of the UV irradiation on DNA synthesis can be enhanced by incorporation of BUdR into DNA prior to irradiation.

In the present paper we describe induction of DNA polymerase after M+U treatment or UV irradiation of synchronously growing *Tetrahymena* cultures. We show 1) that M+U has to be present in part of the S-period in order to induce the DNA polymerase, and 2) that a dosis of UV irradiation which is too low to induce DNA polymerase in normal cells, will do so in cells which have incorporated BUdR into bulk DNA.

MATERIALS AND METHODS: *Tetrahymena pyriformis*, amicronucleate strain GL, was cultivated and synchronized as previously described (11). Methotrexate (gift from Cyanamid Overseas Corporation, U.S.A., Copenhagen Division) and uridine were added under sterile conditions to the culture to a final concentration of 0.05 and 20 mM, respectively (12). DNA was labeled with the thymidine analogue 5-BUdR which was added to the cell suspension to a final concentration of 0.8 mM. Enzyme preparation and assay procedures were as described previously (2). The ionic conditions were optimal for the inducible enzyme (3). Protein was determined by the method of Lowry *et al.* (13). For irradiation of the cells the UV light source was two 15 Watt germicidal lamps (Philips) which emit more than 85% of their rediation at 254 nm. 100 - 150 ml cell suspension was irradiated in a 1 mm layer with stirring. The irradiation dose is given as the dose at the surface of the medium (measured by an intensity measurer for UV irradiation, 220 - 230 nm, mrk. Hanovia). The irradiation intensity varied less than 5% over the radiation area. At 254 nm the optical density of the medium was 1.25.

RESULTS: Addition of M+U to an exponentially growing culture of *Tetrahymena* leads to a rapid induction of DNA polymerase. Two hours after the addition of M+U an increase of 300-400% has been measured (1). However, if a synchronously growing population is treated, the level of DNA polymerase activity after two hours depends on the time in the cell cycle at which M+U is added. The

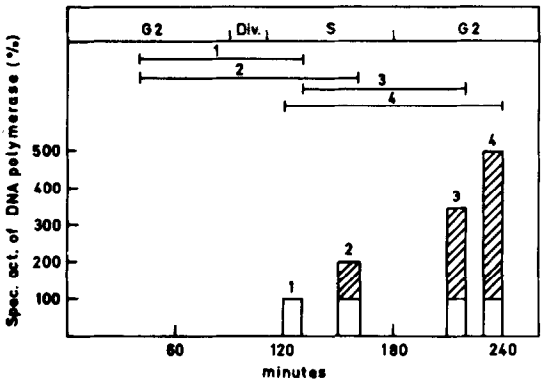

Fig.1. Induction of DNA polymerase by M+U at various times of the cell cycle. The cell cycle is indicated at the top of the figure with the heat shock No. 6 beginning at 160 min. In four subpopulations M+U was present in the time intervals represented by the horizontal bars, marked 1-4. In each population the specific activity of DNA polymerase was estimated between 1.5 and 2 hours after addition of M+U. The results are shown in the lower part of the figure. The abscissa shows minutes after the end of a synchronizing heat shock. The ordinate shows the specific activity of the polymerase in percentage of the control value in untreated cells (=100%). It may be seen that substantial induction of DNA polymerase was found only when M+U was added in the nuclear S-period, inhibiting DNA synthesis in progress.

results in figure 1 show that presence of M+U in 1.5 or 2 hours during the last part of G2 period and during the first part of the S-period has no or only a slight effect on the specific activity of DNA polymerase, whereas addition of M+U in the nuclear S-period induces the enzyme. The specific activity reaches a level 400-500% of the control value within 1.5 - 2 hours. It should be mentioned that synchronizing heat shocks as such have no influence on the level of polymerase activity (14).

Irradiation with ultraviolet light (UV) has previously been shown to induce DNA polymerase activity in exponentially growing cultures (2). However, figure 2 shows that induction will occur only if the irradiation dose is higher that a threshold value of 150 ergs/mm^2.

Figure 3 shows the DNA polymerase activity after UV irradiation of synchronized cells exposed to BUdR for 1 hour periods

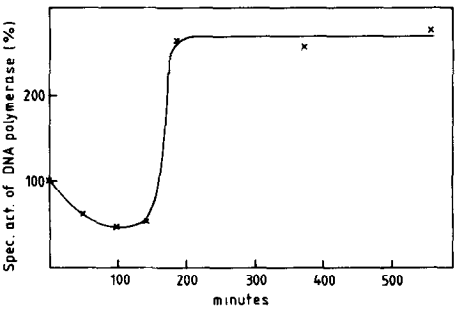

Fig.2. The effect of various doses of UV irradiation on the specific activity of DNA polymerase in an exponentially growing population. The abscissa indicates the irradiation dose (ergs/mm^2). The ordinate shows the specific activity of DNA polymerase in percentage of the unirradiated control (=100%). The specific activity of DNA polymerase was measured 2 hours after the irradiation. It appears that the induction of DNA polymerase occurs only when the UV dose exceeds 150 ergs/mm^2.

at various times in the cell cycle. The UV irradiation dose used (100 ergs/mm^2) was below the threshold value (cf.fig.2). When BUdR is present for 1 hour after the end of the shock period (0-60 min.) no BUdR is incorporated into nuclear DNA and no enzyme induction is observed after the UV irradiation. When BUdR is present in the next hour of the cell cycle (60-120 min.), BUdR is incorporated into DNA during the first 20 min. of the macronuclear S-perion, and an increase to about 200% of the control value is obtained in polymerase activity after UV irradiation. When BUdR is present during the last hour of the macronuclear S-period (120-180 min.) the effect of the UV irradiation at 180 min. gives an increase of the polymerase activity to 400-500% of the control value. Incorporation of BUdR into DNA *per se* has no effect on the polymerase activity.

DISCUSSION: It has been shown that M+U has to be present in the nuclear S-period in order to induce DNA polymerase, and it seems reasonable therefore to correlate the induction of DNA polymerase with the inhibition of bulk DNA synthesis. The induction of DNA

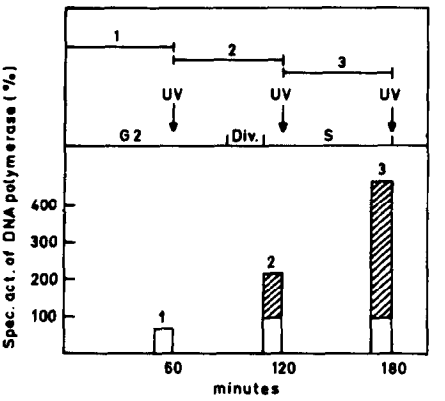

Fig.3. The induction of DNA polymerase in cells first treated with BUdR then irradiated with UV. The plan of the experiment may be seen in the upper part of the figure. BUdR was present in the synchronized population in the periods indicated by the horizontal bars marked 1-3, and the cells were irradiated at the times indicated. The results are shown in the lower part of the figure. The polymerase activity was measured 2 hours after the irradiation. The abscissa shows minutes after the end of a synchronizing heat shock. The ordinate shows the polymerase level in percentage of the control (=100%). It may be seen that incorporation of BUdR into DNA for 1 hour during the nuclear S-period followed by UV irradiation near the end of the S-period increased the DNA polymerase activity almost five times, whereas the presence of BUdR and UV irradiation outside the nuclear S-period showed no induction of the enzyme.

polymerase by low dosis of UV preceded by BUdR treatment also points at nuclear DNA as the primary target for the induction treatment. The number of single strand breaks introduced by a given dosis of UV, is much higher and the rate of production of breaks by UV of wavelength 254-280 nm is at least 2-3 orders of magnitude greater in BUdR containing DNA than in normal DNA (15,16) Therefore, only those *Tetrahymena* cells treated with BUdR during the nuclear S-period will be sensitized to irradiation. As it appears these cells also induce DNA polymerase activity rapidly. On the other hand, when cells are grown in BUdR without irradiation, no DNA damage has been observed and these cells show a normal level of DNA polymerase.

Thus DNA polymerase seems to be induced both when nuclear DNA synthesis is inhibited without DNA damage (M+U) and when

nuclear DNA synthesis is inhibited and DNA damaged (BUdR + UV). The enzyme synthesis might then be interpreted as an attempt to overcome the inhibition of DNA synthesis.

The cellular localisation of the induced enzyme is at present unclear. Westergaard *et al.* (3) found, that the enzyme induced in the presence of M+U was preferentially associated with the mitochondrial fraction. Also ethidium bromide has been shown to induce this polymerase (4). On the other hand it has been reported more lately (17,18) that the enzyme induced by ethidium bromide has properties similar to the major enzyme from untreated exponentially growing cells, and it was suggested that the major enzyme was not of mitochondrial origin (19).

In our view the major enzyme activity induced is most likely the enzyme - or part of the enzyme complex - which replicates nuclear DNA *in vivo*. The activity of this enzyme fluctuates through the cell cycle and shows a maximum in the middle of the S-period (5). Its synthesis is probably turned on and off by factors which control the DNA replication in relation to the cell cycle. In this context induction of DNA polymerase in cells where DNA replication is arrested and/or the DNA damaged might be viewed as an attempt to compensate for a status of the cells which normally requires a *de novo* synthesis of the enzyme.

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