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Induction of β-Polymerase mRNA by DNA-Damaging Agents in Chinese Hamster Ovary Cells

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Only a few of the genes involved in DNA repair in mammalian cells have been isolated, and induction of a DNA repair gene in response to DNA damage has not yet been established. DNA polymerase β (β-polymerase) appears to have a synthetic role in DNA repair after certain types of DNA damage. Here we show that the level of β-polymerase mRNA is increased in CHO cells after treatment with several DNA-damaging agents.

Studies of mammalian cells with DNA polymerase inhibitors have suggested that β-polymerase has a role in DNA repair (1, 3, 13, 18, 24), and the in vitro activity of purified β-polymerase points to an in vivo role in resynthesis of short gaps after damaged residues have been excised (4, 9, 15, 19, 21, 22). Lesions produced by several DNA-damaging agents are repaired by single-base excision or short-gap excision followed by resynthesis to fill the gap (8). It is unknown whether regulation of β-polymerase gene expression is correlated with DNA repair, although it is clear that levels of β-polymerase mRNA do not correlate with stages of the cell cycle or with replication of genomic DNA (25a). A rodent cDNA for β-polymerase was isolated recently (26), and we have used this DNA as a probe to study regulation of β-polymerase mRNA in Chinese hamster ovary (CHO) cells after treatment with a variety of DNA-damaging agents.

We found that the level of β-polymerase mRNA in exponentially growing CHO cells is elevated three- to fivefold after treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), methyl methanesulfonate (MMS), or *N*-acetoxy-2-acetylaminofluorene (AAAF) (Fig. 1). The results of quantitative RNA dot blot analyses (6, 7) after treatment with high levels of these and other agents are summarized in Table 1. For reference, dot blots also were probed with rodent cDNAs for β-actin (Zmudzka et al., in press), heat shock protein 70 (HSP70) (6), and heterogeneons nuclear ribonucleoprotein complex protein A1 (2). Table 1 shows the abundance of each transcript in poly(A)$^+$ RNA isolated 4 h after irradiation (5) or treatment with MNNG, MMS, AAAF, nitrogen mustard (HN_2), *cis*-Pt(II) diamminedi-chloride, or bleomycin; treatment time for H_2O_2 and adriamycin was 1 h. The elevation in level of β-polymerase transcript after treatment with MNNG was 3.6-fold and was slightly lower with MMS, AAAF, and H_2O_2. The other DNA-damaging agents examined did not cause an increase in the amount of β-polymerase transcript, and likewise, β-polymerase mRNA was not induced by an alternate type of cell stress, heat shock. The levels of β-actin mRNA or A1 mRNA were not elevated after the various treatments; this was true also for HSP70 mRNA, except in the case of heat shock, as expected.

Changes in overall cellular poly(A)$^+$ RNA content did not

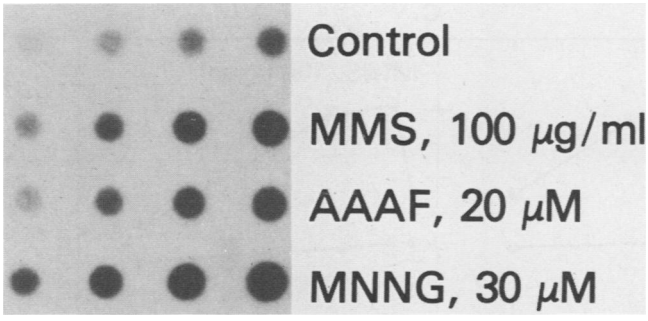

FIG. 1. Autoradiogram of quantitative RNA dot blot analysis of β-polymerase mRNA in CHO cells after treatment with MNNG, MMS, or AAAF. From left to right, 0.05, 0.1, 0.2, and 0.4 μg of poly(A)$^+$ RNA was added to the dot blots.

TABLE 1. Comparison of β-polymerase and other mRNA levels in CHO cells after treatment with DNA-damaging agents

DNA-damaging agent	Dose	Relative mRNA level (SD)[a]				Poly(A)$^+$ RNA (μg)
		β-Polymerase	α-Actin	A1	HSP70	
MNNG	30 μM	3.6 (0.4)	0.9	0.9	0.5	71.3
MMS	100 μg/ml	3.5 (0.3)	0.8	1.0	0.8	75.9
AAAF	20 μM	2.9 (0.3)	0.8	1.0	0.9	77.9
H_2O_2	400 μM	2.3 (0.4)	1.1	1.3	0.7	77.1
UV light	14 J/m^2	0.6 (0.1)	1.0	1.1	0.4	66.9
Near-UV light	300 J/m^2	0.8 (0.3)	1.2	1.1	0.4	65.4
HN_2	40 μM	1.2 (0.2)	1.0	1.0	1.0	71.7
cis-Pt	45 μg/ml	0.9 (0.1)	0.8	0.8	1.1	71.4
X-ray	40 Gy	1.1 (0.3)	1.1	—	0.9	75.0
Adriamycin	400 ng/ml	1.0 (0.1)	1.1	1.1	1.0	43.0
Bleomycin	50 μg/ml	1.1 (0.2)	0.9	—	1.0	70.0
Heat shock	9 min at 45.5°C	0.9 (0.1)	0.7	1.2	26.2	82.9
Control		1	1	1	1	73.8

[a] Hybridization was quantified by densitometry; values in the linear range of the hybridization curve were used. Relative RNA abundance was determined by normalizing to RNA from untreated control cells in the same experiment. Values represent the means of four dot blot determinations with independent samples of RNA (2×10^8 cells) plus or minus the standard deviation. Cell survival determined by plating was <1% for MMNG, MMS, AAAF, HN_2, and cis-Pt; 4% for UV treatment; 15% for near-UV treatment; 31% for adriamycin; and >90% for heat shock.

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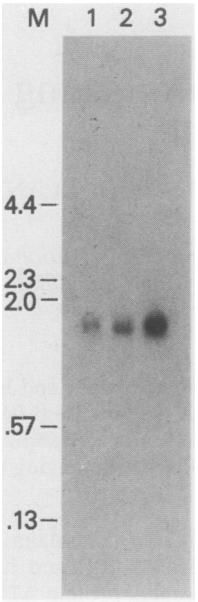

FIG. 2. Autoradiogram of Northern blot analysis of β-polymerase mRNA in CHO cells after treatment with MNNG. Equal amounts of poly(A)$^+$ RNA from untreated cells (lane 1) and from cells treated for 4 h with 10 (lane 2) or 30 μM (lane 3) MNNG were separated, transferred to a nylon filter, and hybridized with labeled β-polymerase cDNA, pUC9-10F (16). Numbers indicate kilobases.

account for the increase in β-polymerase mRNA, since the yield of poly(A)$^+$ was similar in all samples (~35 μg/10^8 cells; Table 1). Cell growth effects also could be excluded, since induction occurred rapidly compared with the doubling time of the cells, and it is known that β-polymerase mRNA is not growth phase responsive in CHO cells (25a). Finally, Northern (RNA) blot analysis of poly(A)$^+$ RNA from cells treated with 10 μM or 30 μM MNNG was conducted (Fig. 2). The results indicated that the induction seen with MNNG corresponds entirely to the 1.4-kilobase β-polymerase transcript (17, 25a, 26).

The β-polymerase mRNA induction by the alkylating agents MMS and MNNG was examined further (Fig. 3). β-polymerase mRNA level was highest 4 h after exposure of cells to MMS and remained elevated for several hours after removal of the drug. Similar results were obtained with MNNG (not shown). When transcription was blocked with actinomycin D, no induction of β-polymerase mRNA was observed, suggesting that induction required active transcription. In the presence of actinomycin D, the level of β-polymerase mRNA decreased only slightly, suggesting an mRNA half-life of >4 h. The results with actinomycin D were identical when MMS treatment was omitted (not shown), suggesting that the stability of the β-polymerase mRNA is not altered by the inducing agent. The data in Fig. 3 show that there was an optimum dose for MMS and MNNG induction. Therefore, we conducted dose-response and time experiments with the other agents used in Table 1. No β-polymerase mRNA increase was found; an example of this with UV irradiation is shown in Table 2. Finally, we examined the effect of MNNG and MMS treatment on the level of β-polymerase protein in the crude cell extract by using activity gel analysis (12, 14). During the 12-h period after the addition of MNNG (Fig. 4) or MMS (not shown), there was no significant difference in the level of β-polymerase protein in the cells.

TABLE 2. β-Polymerase mRNA levels after UV irradiation[a]

UV dose (J/m^2)	Time after irradiation (h)	Relative mRNA level (SD)	
		β-Polymerase	β-Actin
0		1	1
7	4	1.1 (0.2)	1.0
14	4	0.9 (0.2)	1.1
21	4	0.8 (0.1)	0.9
28	4	0.8 (0.3)	1.0
14	2	1.0 (0.1)	1.1
14	4	0.7 (0.4)	1.1
14	8	0.6 (0.2)	1.2
14	16	1.4 (0.3)	1.1

[a] Relative RNA abundance was determined as in Table 1. The dose-response and time course experiments were performed on different days.

Two features of the β-polymerase mRNA induction were similar to damage-induced mRNA elevations for DNA repair enzymes in lower organisms (8, 11, 20). Induction was relatively rapid, and induction was in the range of three- to fivefold only. The agents examined in this study varied in the types of DNA damage they produced and in the corresponding DNA repair. For example, MNNG, MMS, and H_2O_2 produced single-base damage repaired by excision and in-

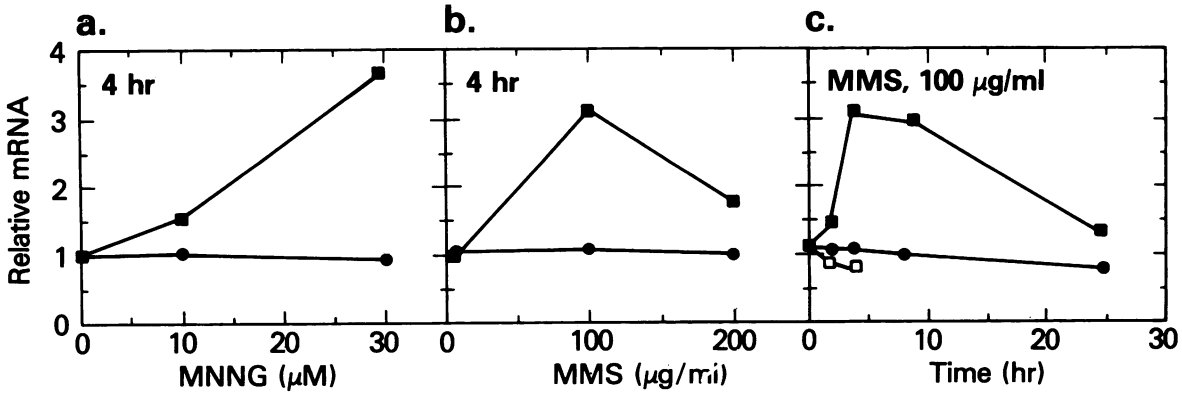

FIG. 3. Dose-response and time course for β-polymerase induction by alkylating agents. The relative RNA abundance of β-polymerase (■) and β-actin (●) transcripts was determined. (a and b) Dose-response experiments. CHO cells were treated with the indicated concentrations of alkylating agents for 4 h. (c) Time study. MMS (100 μg per ml) was added at the start of the experiment and removed after 2 h for the 2-h time point or after 4 h for the other samples; values on the ordinate are relative to untreated controls. β-Polymerase mRNA in cells also treated with 5 μg of actinomycin D per ml added 10 min before MMS is also indicated (□).

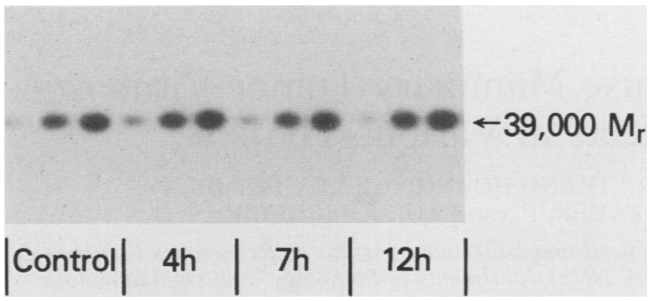

FIG. 4. Autoradiogram of activity gel showing relative levels of β-polymerase in CHO cells treated with MNNG. A portion corresponding to the middle of the 10% gel is shown; enzymatic activity of renatured enzyme is used as the indicator of the number of protein molecules (12). Cells cultured in medium alone (control) or exposed to 30 μM MNNG for 4 h and then cultured in medium without alkylating agent (7 and 12 h) were collected, sonicated, and extracted with buffer containing 0.5 M NaCl. After centrifugation, clear homogenates were obtained, and portions of each homogenate with 3.3, 6.6, or 10 μg of protein were analyzed (13).

sertion of one to several new nucleotides, i.e., short-patch repair (8). UV irradiation mainly produced pyrimidine dimers repaired by excision and insertion of ~100 new nucleotides per lesion, i.e., long-patch repair (8). AAAF produced single-base damage, but this was repaired by the long-patch excision mechanism in human cells (8). The induction of β-polymerase mRNA appears to correlate with high levels of single-base damage.

In addition to future studies of β-polymerase gene regulation, the results reported here point to the existence of an inducible DNA repair response in Chinese hamster cells. Regulatory elements have been found in the promoter for the β-polymerase gene (22a, 23, 25), and it will be possible to determine if these elements are involved in response to DNA damage. Also, studies of β-polymerase protein synthesis rate after MNNG or MMS treatment may yield interesting information on control mechanisms for this enzyme. The observation that an increase in β-polymerase level does not accompany the increase in mRNA level suggests that post-transcriptional steps play a regulatory role in expression of the gene after DNA damage. Interestingly, yeast DNA ligase mRNA increases after DNA damage, yet there is little or no increase in enzymatic activity level (10).

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