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Involvement of DNA polymerase β in proliferation of rat liver induced by lead nitrate or partial hepatectomy

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We have studied the expression pattern of DNA polymerase β in two different models of in vivo cell proliferation. Both mRNA levels and enzyme activity of DNA polymerase β markedly increased before and/or during DNA synthesis in proliferating hepatocytes in mitogen-treated and partially hepatectomized rats. The time-courses of the expression of the gene coding for DNA polymerase β were significantly different in the two cell systems. A 5-fold increase in DNA polymerase β mRNA was observed 8 h after lead nitrate administration, i.e. well before the onset of DNA synthesis. In the regenerative liver cells a 3-fold increase in the amount of mRNA was observed 24–48 h after partial hepatectomy, the event being coincident with extensive DNA synthesis. In both systems, the increase of mRNA levels was always paralleled by an increase in enzyme activity, suggesting that DNA polymerase β activity may be regulated at a pre-translational level.

DNA polymerase β; Regenerating liver; Lead nitrate

1. INTRODUCTION

The structural conservation of DNA polymerase β through evolution suggests the importance of this enzyme [1]. DNA polymerase β seems to be one of the most important enzymes involved in the repair of damaged DNA because of its preference for short gaps formed during excision repair [2]. Recent studies show a different distribution of DNA polymerase β activity, as well as of the levels of its mRNA, in tissues of mouse [3] and rat [4]. The 5′ flanking regions of the DNA polymerase β gene are characterized by (i) G–C rich sequences, (ii) putative methylation sites, (iii) SP1 binding sites localized near the transcriptional start site, and (iv) silencer regions [5,6]. A recent report [6] indicates that the above mentioned tissue-specific expression is primarily controlled by the silencer elements. Moreover, another promotor element that mediates a positive response to certain types of DNA-damaging agents has been recently identified [7]. In fact, in CHO cells an increase of DNA polymerase β mRNA levels was found after treatment with a variety of DNA-damaging agents [8]. In this case the increase in mRNA levels was detected early and required active transcription.

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It is therefore evident that the expression of DNA polymerase β is regulated by a complex mechanism, and its activity may vary with the process of cell proliferation.

Only a slight fluctuation of DNA polymerase β mRNA levels has been described during the cell cycle. In particular, the activity of the DNA polymerase β promotor was found to be the same in growing and quiescent fibroblast AG-1522 cell lines [9] and the mRNA levels in synchronized HeLa cells remains almost constant during all stages of the cell cycle [9].

We recently reported [10] quite different results. When human peripheral blood mononuclear cells were stimulated by phytohemagglutinin (PHA), both DNA polymerase β activity and mRNA levels increase rapidly, reaching a maximum during DNA synthesis. Further experiments with actinomycin D suggested that the increase in mRNA levels may be regulated at the transcriptional level.

The present study was designed to determine whether variations either in activity or in mRNA levels of DNA polymerase β might occur in vivo during cell proliferation induced by two-thirds partial hepatectomy [11] or by the liver mitogen, lead nitrate [12].

In vivo and in vitro studies on the effects of lead are few and often contradictory. In fact, while lead nitrate is able to induce in vivo liver hyperplasia in the absence of hepatic cell necrosis [13], it was, however, found to induce mutations at the HPRT locus in V79 cells at concentrations that do not cause direct DNA damage [14]. Furthermore, lead may interfere with DNA syn-

thesis and/or repair. In particular, lead chloride has been shown to decrease the accuracy of DNA polymerases in vitro, an event which, if mimicked in vivo, would result in mutations in cellular DNA [15]. Other in vitro studies have demonstrated that concentrations of lead nitrate in the μM range inhibit DNA polymerase β activity [16]. In spite of the above information, the mechanism(s) by which lead compounds act are still unknown.

In the present work we found an early increase in mRNA levels and enzymatic activity of DNA polymerase β after lead nitrate administration to male Wistar rats. Additionally, the same experiments were carried out by following compensatory liver cell regeneration after partial hepatectomy. Similar results were found in that both enzymatic activity and mRNA levels of DNA polymerase β increased after stress, however the time-course of stimulation was significantly delayed after hepatectomy compared to the lead nitrate administration.

2. MATERIALS AND METHODS

2.1. *Animals*

Male Wistar rats (220 g), obtained from Charles River, Milan, Italy, were fed a semi-synthetic diet (Ditta Piccioni, Brescia, Italy) and had free access to drinking water. The animals were either subjected to two-thirds partial hepatectomy [11] under light ether anesthesia or treated with an aqueous solution of lead nitrate given through the femoral vein at a dose of 100 μmol/kg body weight. Rats were sacrified 1, 4, 8, 24, 48, 72, 96 and 120 h after treatment.

2.2. *Northern hybridization analysis*

Total RNA was extracted from the liver by the guanidine thiocyanate method [17]. Samples of 50 μg of RNA were separated by electrophoresis on a 1% agarose gel containing 6% formaldehyde, blotted onto a Hybond-N membrane and hybridized with cDNA for rat DNA polymerase β [18], that was labeled with the Multiprime DNA labeling system (Amersham, 0.5–2 $\times 10^9$ cmp/μg). DNA polymerase β mRNA was located by autoradiography. The mRNA levels were measured with a Bio-Rad Model 620 video densitometer.

2.3. *Assay for RNA polymerase β activity*

An activity gel for DNA polymerase β was carried out as described previously [10]. Crude extracts containing 50 μg proteins were separated on a 10% SDS-polyacrylamide gel containing sonicated salmon sperm DNA (150 μg/ml). After electrophoresis, the gel was washed in renaturation buffer (50 mM Tris-HCl, pH 8.0, 3 mM β-mercaptoethanol), soaked in renaturation buffer containing 6 M guanidine-HCl and then soaked again in renaturation buffer. The gel was incubated in 50 mM Tris-HCl, pH 7.5, containing 0.1 mM EDTA and 1 mM DTT for 20 min, followed by incubation of the gel in 50 mM Tris-HCl, pH 7.5, 7 mM $MgCl_2$, 1 mM EDTA, 1 mM DTT, 12 mM each of dATP, dGTP and dTTP and 10 mCi/ml [^{32}P]dCTP (3,000 Ci/mmol) for 12 h at 37°C. It was then washed with 5% trichloroacetic acid, dried and autoradiographed using Kodak XAR-5 film. Quantitation was performed by scanning densitometry.

2.4. *Protein determination*

Proteins were estimated according to Bradford [19] using bovine serum albumin as the standard.

3. RESULTS

On the basis of the afore-mentioned data, we wanted to examine the relationship between activity and mRNA levels of DNA polymerase β during cell proliferation in vivo. Either in rat liver hyperplasia induced by mitogens, such as lead nitrate, or in compensatory cell proliferation occurring after partial hepatectomy, the liver behaves as a synchronized culture of hepatocytes. This was suggested by data on histone H3 gene expression (Figs. 1–4) and by [^{3}H]thymidine incorporation into DNA (data not presented).

Fig. 1B shows a marked enhancement of DNA polymerase β mRNA levels at 4 h after lead nitrate administration to rats. At 8 h mRNA levels reached a maximum, corresponding to a 5-fold increase over basal levels (Figs. 1B and 2). This phenomenon preceded the increase of histone H3 mRNA levels, which displayed a maximum at 48 h (Figs. 1B and 2).

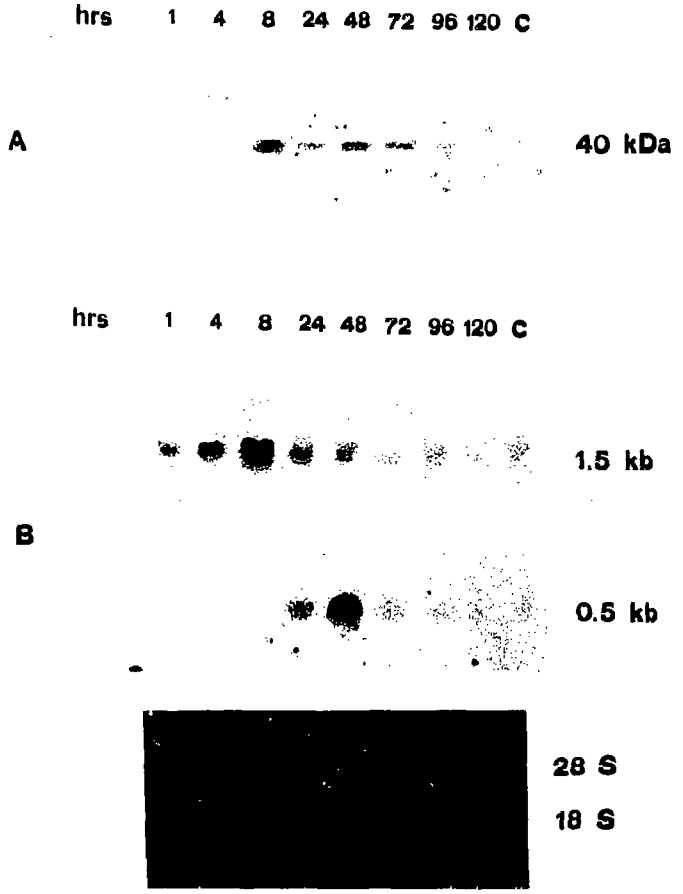

Fig. 1. Time-course of DNA polymerase β enzyme activity and its mRNA levels in lead nitrate-treated rat liver. Samples of rat liver were analysed as described in Materials and Methods collected after lead nitrate treatment: (A) activity of DNA polymerase β (40 kDa), (B) mRNA levels of DNA polymerase β gene (1.5 kb) and histone H3 gene (0.5 kb). The amount of ribosomal RNAs in the agarose gel quantified by ethidium bromide staining is shown in the lower part of panel B. The positions of ribosomal RNAs (28 S and 18 S) are indicated on the right. C denotes control animals.

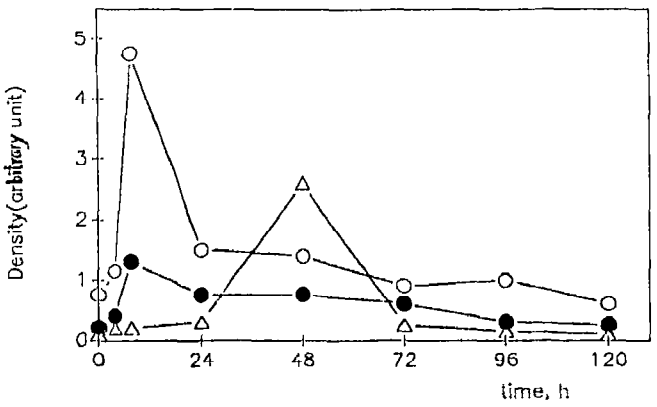

Fig. 2. Quantitative measurement of the enzyme activity and of steady-state levels of DNA polymerase β and histone H3 mRNAs in lead nitrate-induced liver hyperplasia. Symbols represent quantitative data obtained by densitometric scanning of the bands shown in Fig. 1. (●), DNA polymerase β enzyme activity; (○), DNA polymerase β mRNA levels, (△), histone H3 mRNA levels. Control values are not indicated.

The activity of DNA polymerase β increased 8 h after lead nitrate administration (3-fold basal levels); high levels of DNA polymerase β activity were still detectable in the next 24–48 h, when DNA synthesis had already begun (Figs. 1A and 2).

When the regenerative liver cell proliferation system was analysed, a significant increase in the amount of DNA polymerase β mRNA (3-fold the basal level) was observed 24–48 h after partial hepatectomy (Figs. 3B and 4) in parallel with the increase of the histone H3 mRNA levels (Figs. 3B and 4). Similar data were obtained by Nowak et al. [20], who observed a 2-fold increase in DNA polymerase β mRNA levels 18–24 h after partial hepatectomy.

In both systems the increase in enzymatic activity was preceded by an increase in the level of mRNA (Figs. 1–4).

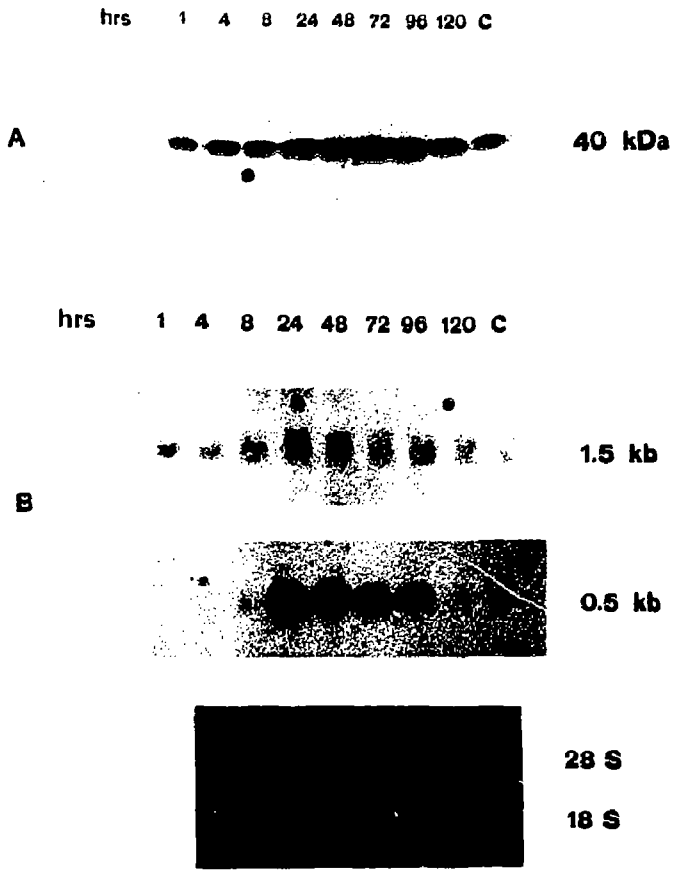

Fig. 3. Time-course of DNA polymerase β enzyme activity and its mRNA levels in compensatory liver cell proliferation after partial hepatectomy. Samples of rat liver were analysed as described in Materials and Methods after the operation: (A) enzyme activity of DNA polymerase β (40 kDa), (B) mRNA levels of DNA polymerase β (1.5 kb) and histone H3 (0.5 kb). The amount of ribosomal RNA measured by ethidium bromide staining is shown in the lower part of panel B. The positions of ribosomal RNAs (28 S and 18 S) are indicated on the right. C denotes control animals.

4. DISCUSSION

A previous report [9] indicated that in a transformed cell line DNA polymerase β gene expression does not change significantly during the cell cycle. In normal lymphocytes, however, a marked increase of both DNA polymerase β activity and mRNA levels was observed after stimulation with PHA [10].

In the present work, DNA polymerase β activity and mRNA levels displayed a cell cycle-dependent phenomenon in vivo.

In both systems, DNA polymerase β activity and hybridizable mRNA levels increased to similar levels. This suggests that DNA polymerase β activity is regulated at a level prior to translation.

It should be noted that the time-course inductions by the two proliferative stimuli were found to be different. Liver hyperplasia induced by the mitogen, lead nitrate, was characterized by an early increase of both the DNA polymerase β mRNA levels and enzyme activity, their maximum values occurring well before DNA synthesis. It is possible that this early activation of DNA polymerase β might be caused by lead through DNA damage or possibly through indirect mechanisms. In particular, lead chloride has been shown to increase the frequency of misincorporation of base pairs into the DNA [15]. Other authors [14] observed mutations in V79 cells exposed to lead nitrate, but they did not detect DNA single-strand breakage as determined by alkaline elution.

On the basis of the data presented here we cannot define the molecular mechanisms by which lead may interfere with DNA synthesis or repair, however, we demonstrated that DNA polymerase β activity and mRNA levels increase after lead nitrate administration, suggesting that what occurs in vivo is quite different from what occurs in vitro where lead inhibits the activity of the enzyme [16].

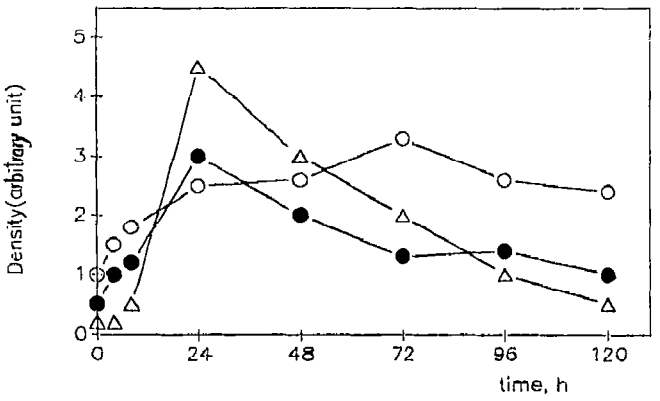

Fig. 4. Quantitative measurement of the enzyme activity and of the steady-state levels of DNA polymerase β and histone H3 mRNAs in compensatory liver cell proliferation. Symbols represent quantitative data obtained by densitometric scanning of the bands shown in Fig. 3. (○), DNA polymerase β enzyme activity; (●), DNA polymerase β mRNA levels, (△), histone H3 mRNA levels. Control values are not indicated.

The increase of DNA polymerase β mRNA in compensatory cell proliferation is in good agreement with results reported by Nowak et al. [20] in the same in vivo model. On the other hand, we found a significant increase in DNA polymerase β activity (2–3-fold the basal level) that is significantly higher than that found by Philippe et al. [21] using a different experimental method. On the basis of the time-course of DNA polymerase β induction in the compensatory cell proliferation we suggest that DNA polymerase β is involved in replicative DNA synthesis.

The differences found in DNA polymerase β induction in the two in vivo systems are not surprising if one takes into account the different mechanisms that characterize the two models. In the compensatory cell proliferation, cell loss is the primary event and cell proliferation occurs to replace the lost cells. In the case of lead nitrate treatment, however, the mitogenic effect is the primary event resulting in liver hyperplasia [13]. Furthermore, the two models exhibit a differential expression of the immediate early genes, c-*fos* and c-*myc* [22], and differential effects on at least two steps of chemical hepatocarcinogenesis [23]. It is also possible that the differences found in the time-course of DNA polymerase β induction may not be due necessarily to the different proliferative stimuli, but rather to the chemical nature of the mitogen used in this study. In this respect it is of interest to note that our preliminary studies indicate that two other liver mitogens, nafenopine and cyproterone acetate, do not induce an early increase in the expression of DNA polymerase β mRNA.

In conclusion, the data obtained in two different cell proliferation systems seems to indicate that the induction of DNA polymerase β is a general phenomenon correlated with the cell cycle and that DNA polymerase β activity is not regulated at the level of translation.

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