ISSN 0006-2979, Biochemistry (Moscow), 2010, Vol. 75, No. 7, pp. 905-911. © Pleiades Publishing, Ltd., 2010.
Original Russian Text © A. A. Kazakov, E. E. Grishina, V. Z. Tarantul, L. V. Gening, 2010, published in Biokhimiya, 2010, Vol. 75, No. 7, pp. 1031-1039.

Effect of Human Cell Malignancy on Activity of DNA Polymerase ι

A. A. Kazakov[1], E. E. Grishina[2], V. Z. Tarantul[1]*, and L. V. Gening[1]

[1]Institute of Molecular Genetics, Russian Academy of Sciences, pl. Kurchatova 2, 123182 Moscow, Russia; fax: (499) 196-0221; E-mail: tarantul@img.ras.ru
[2]Ophthalmological Clinical Hospital, Moscow Department of Health, Mamonovsky Pereulok 7, 103001 Moscow, Russia; fax: (495) 650-1822; E-mail: eyelena@mail.ru

Received December 30, 2009
Revision received February 19, 2010

Abstract—An increased level of mutagenesis, partially caused by imbalanced activities of error prone DNA polymerases, is a key symptom of cell malignancy. To clarify the possible role of incorrect DNA polymerase ι (Pol ι) function in increased frequency of mutations in mammalian cells, the activity of this enzyme in extracts of cells of different mouse organs and human eye (melanoma) and eyelid (basal-cell skin carcinoma) tumor cells was studied. Both Mg^{2+}, considered as the main activator of the enzyme reaction of *in vivo* DNA replication, and Mn^{2+}, that activates homogeneous Pol ι preparations in experiments *in vitro* more efficiently compared to all other bivalent cations, were used as cofactors of the DNA polymerase reaction in these experiments. In the presence of Mg^{2+}, the enzyme was active only in cell extracts of mouse testicles and brain, whereas in the presence of Mn^{2+} the activity of Pol ι was found in all studied normal mouse organs. It was found that in cell extracts of both types of malignant tumors (basal-cell carcinoma and melanoma) Pol ι activity was observed in the presence of either Mn^{2+} or Mg^{2+}. Manganese ions activated Pol ι in both cases, though to a different extent. In the presence of Mn^{2+} the Pol ι activity in the basal-cell carcinoma exceeded 2.5-fold that in control cells (benign tumors from the same eyelid region). In extracts of melanoma cells in the presence of either cation, the level of the enzyme activity was approximately equal to that in extracts of cells of surrounding tumor-free tissues as well as in eyes removed after traumas. The distinctive feature of tissue malignancy (in basal-cell carcinoma and in melanoma) was the change in DNA synthesis revealed as Mn^{2+}-activated continuation of DNA synthesis after incorrect incorporation of dG opposite dT in the template by Pol ι. Among cell extracts of different normal mouse organs, only those of testicles exhibited a similar feature. This similarity can be explained by cell division blocking that occurs in all normal cells except in testicles and in malignant cells.

DOI: 10.1134/S0006297910070138

Key words: uveal eye melanoma, basal-cell carcinoma, DNA polymerase ι, TLS

During replication of DNA, enzyme systems of all organisms have to overcome regions damaged in response to internal and external factors. The inability of high-precision replication DNA polymerases to catalyze synthesis in such regions can result in cell death caused by cessation of replication. The key role in this case belongs to the recently discovered specialized DNA polymerases of X and Y families. Unlike the high-precision enzymes carrying out the DNA replication proper, these DNA polymerases more easily overcome different local changes in DNA structure, which allows them to carry out synthesis on damaged DNA regions during synthesis called translesion synthesis (TLS) [1]. In this case these enzymes, unlike replication enzymes, allow high frequency errors in experiments *in vitro* upon copying undamaged template. However, due to ability to incorporate correct nucleotides opposite modified analogs, they overcome replication blocking and help maintain genome stability [1].

To prevent mutagenic effect of specialized DNA polymerases in normal cells, there are a number of post-translational regulatory mechanisms that strictly control activity of such polymers and restrict their access to a replication fork. Mechanisms that regulate activity of the Y family DNA polymerases are rather complex and include protein modification and formation of multienzyme complexes based on protein–protein interactions. Therefore, studying DNA polymerase activities in

Abbreviations: BCC, basal-cell carcinoma; DTT, dithiothreitol; PNK, polynucleotide kinase; Pol ι, DNA polymerase ι; TLS, translesion synthesis.

* To whom correspondence should be addressed.

extracts of nuclei and cells influenced by regulatory factors gives more adequate information compared to that obtained from investigation of DNA polymerase gene expression at the transcription level and from *in vitro* analysis of activity of homogeneous enzyme preparations.

Products of the overwhelming majority of DNA polymerases working in a mixture are difficult to discern because all these enzymes are able to incorporate complementary nucleotides opposite DNA template. The distinctive feature of DNA polymerase ι (Pol ι), owing to which its products can be identified, is the ability to incorporate mainly dG opposite dT template even in the presence of dATP [2]. In our previous work [3] we described a method for testing Pol ι activity in crude cell extracts based on this property. We have applied this method for determination of Pol ι activity in cell extracts of some mouse organs and human uveal eye melanoma using Mg^{2+} as the cofactor of the DNA polymerase reaction [4, 5].

Although Mg^{2+} is traditionally considered as the activator of DNA replication, it has been recently shown that not only Pol ι of the DNA polymerase Y family, but a number of other enzymes of the X family, in particular DNA polymerases Pol μ [6] and Pol λ and Pol β [7, 8] are more efficiently activated by Mn^{2+} than by Mg^{2+} in experiments *in vitro*. However, in this case the precision of DNA synthesis is decreased [7, 8]. In this work Mn^{2+} was used as cofactor for investigation of Pol ι activity in human tumor tissues. We found that under our conditions only Pol ι is able to incorporate dG opposite dT in DNA template, and then we studied its activity in tumor cell extracts. Our data show that the enzyme activity in malignant skin cells significantly differs from that in normal and benign tumor cells.

MATERIALS AND METHODS

Tissue samples. Two mouse lines (C57BL and 129J) were used for investigation of DNA polymerase activity in normal tissues. The activity was checked for six organs (liver, kidney, heart, lung, brain, and testicle). Mouse line 129J was used as control for Pol ι activity because a nonsense mutation that blocks synthesis of the full-value protein exists in the second exon of the encoding gene in this line [9].

In our experiments cell extracts of three species of human malignant tumors were analyzed: uveal eye melanoma, basal-cell skin carcinoma, and MALT-lymphoma (according to WHO classification of 2001) taken from the eyelid. Benign tumors of different origin removed from the same eyelid regions of different patients free of basal-cell carcinoma (BCC) were used as controls for BCC. Samples of normal eye uvea taken from the tumor-carrying eyes, as well as samples obtained from tumor-free eyes removed from different patients after traumas, were used as controls for melanoma.

DNA polymerase activity in tumor tissues of 40 patients was analyzed. Of all the samples, eight of skin BCC and 22 of uveal eye melanoma were of malignant tumors. Benign tumors (10 samples) such as papilloma (5 patients), chalazion (2), eyelid cyst (1), molluscum contagiosum (1), and keratoma (1) served as controls for BCC. Controls for uveal melanoma were 22 samples of choroid (vascular eye membrane) taken from tumor-affected eyes and 11 samples of choroid taken from tumor-free eyes.

Preparation of extracts. Tumor extracts were prepared from fresh tumor samples (from different patients) that were homogenized on ice in a Teflon homogenizer in 0.14 M Na-phosphate buffer, pH 7.4 (PBS; Helicon, Russia), containing 80% glycerol. Buffer volume of 1 μl was taken for 1 mg of homogenized tissue. The homogenate was centrifuged at 4°C for 4 min at 14,000*g*. The supernatant was used as enzyme preparation. Protein concentration was measured using Protein Assay Reagent (BioRad, USA). In this case protein content in extracts in different experiments was in the range from 15 to 30 mg/ml.

Determination of DNA polymerase activity. Two complementary deoxyribooligonucleotides were used as substrates for determination of DNA polymerase activity: 17-membered primer 5′-GGAAGAAGAAGTATGTT-3′ and 30-membered template 5′-CCTTCTTCATTCTAACATACTTCTTCTTCC-3′, which form upon hybridization duplex with protruding 5′-end.

The primer was labeled from the 5′-end using T4 polynucleotide kinase (PNK) and [γ-^{32}P]ATP in 70 mM Tris-HCl buffer, pH 7.6, containing 10 mM $MgCl_2$ and 5 μM dithiothreitol (DTT) (BioRad) at 37°C for 30 min. Then the enzyme was inactivated at 70°C for 10 min.

The substrate for the enzymic reaction was obtained after annealing at 73°C for 3 min and following cooling to room temperature in 50 μl of 300 pmol of labeled primer with 350 pmol of template in PNK buffer containing 100 mM NaCl. The reaction was carried out in 10 μl of reaction mixture containing 300 nM substrate (in 50 mM Tris-HCl, pH 8.0, 5 mM $MgCl_2$ or 0.2 mM $MnCl_2$, 1 μM DTT), dATP and dGTP, 2 μl extract of appropriate sample, at 37°C for 10 min. The dATP and dGTP concentration in all experiments was 1 mM. The reaction was stopped by cooling in ice with subsequent addition of an equal volume of 95% formamide, 0.05% xylene cyanole, and 0.05% bromphenol blue mixture.

The reaction products were electrophoresed in 18% polyacrylamide gel (length 25 cm, thickness 0.2 mm, acrylamide/*bis*-acrylamide 30 : 1) with 7 M urea in Tris-borate buffer at 30 mA (to the beginning of bromphenol blue release from the gel). After electrophoresis the gel was kept for 20 min in fixing solution containing 10% acetic acid, 30% ethanol, and 1% glycerol, and then dried and autoradiographed for 24 h. The autoradiograph was scanned on a Storm 840 Phosphorimager (Amersham

Biosciences, USA). The data were analyzed using the Image Quant 5.2 program. The Pol ι activity was calculated using the formula MoG = (dG × 100%)/(4*a* + 3*b* + 2*c* + *d*) (MoG, misincorporation of G). To estimate the amount of products of Pol ι reaction on primer completion to 19-, 20-, and 21-membered nucleotide, the following formula was used: MoG = ((*d*G + *c*G + *b*G + *a*G) × 100%)/(4*a* + 3*b* + 2*c* + *d*), where *a*, *b*, *c*, and *d* are intensities of electrophoretic bands associated with 21-, 20-, 19-, and 18-nucleotide fragments, respectively.

RESULTS

Scheme of primer completion in oligonucleotide substrate. DNA polymerase activities in different organ and tissue cell extracts were registered by elongation of ^{32}P-labeled primer. Structures of oligonucleotide substrate consisting of 30-membered template and 17-membered primer used in the reaction are shown in "Materials and Methods" and in Fig. 1. The reaction mixture contained two nucleotide triphosphates (dATP and dGTP) and Mg^{2+} (5 mM) or Mn^{2+} (0.2 mM) as activators. Optimal concentrations of bivalent cations providing for the most efficient incorporation of G opposite template T by extracts of mouse testicles and brain where chosen experimentally. For Mn^{2+} this concentration was within the optimum limits for homogeneous Pol ι preparations, while for Mg^{2+} it appeared to be significantly higher [10].

Possible variants of primer completion by DNA polymerases of different cell extracts observed in our experiments under described conditions are shown in Fig. 1. Variant *1* is the 17-membered primer completion by complementary bases to 21-membered oligonucleotide. Cessation of further synthesis is observed due to the absence of dTTP from the reaction mixture. Electrophoretic separation of the product mixture results in a pattern (I in Fig. 1) containing bands corresponding to 17-membered primer, 21-membered product of comprehensive completion, and to incomplete 18- to 20-membered synthesis products.

Variant *2* is synthesis with involvement of Pol ι in which G is incorporated opposite template T positioned immediately opposite the 3′-end of the primer, after which synthesis ceases due to the enzyme-characteristic T-stop. This property described for homogeneous Pol ι preparations is cessation of synthesis after incorporation of G opposite template T [2]. Pattern II shown in Fig. 1 represents a mixture of products formed after the first and second variants of synthesis, because it contains not only Pol ι products but products formed by other DNA polymerases as well. As a result, a double band is formed in the region of 18-membered oligonucleotide, where the Pol ι product containing dG at the 3′-end moves more slowly compared to 18-membered products of other DNA polymerases containing dA at their 3′-ends.

Variant *3* is synthesis that shows the existence of activity of primer completion after dG is incorrectly incorporated by Pol ι. As a result, pattern III is a mixture of products simultaneously synthesized in three variants of synthesis. The distribution of electrophoretic bands corresponding to all products of DNA polymerase reaction was confirmed by electrophoresis of corresponding synthetic oligonucleotides, each of which was labeled by ^{32}P using polynucleotide kinase (data not shown).

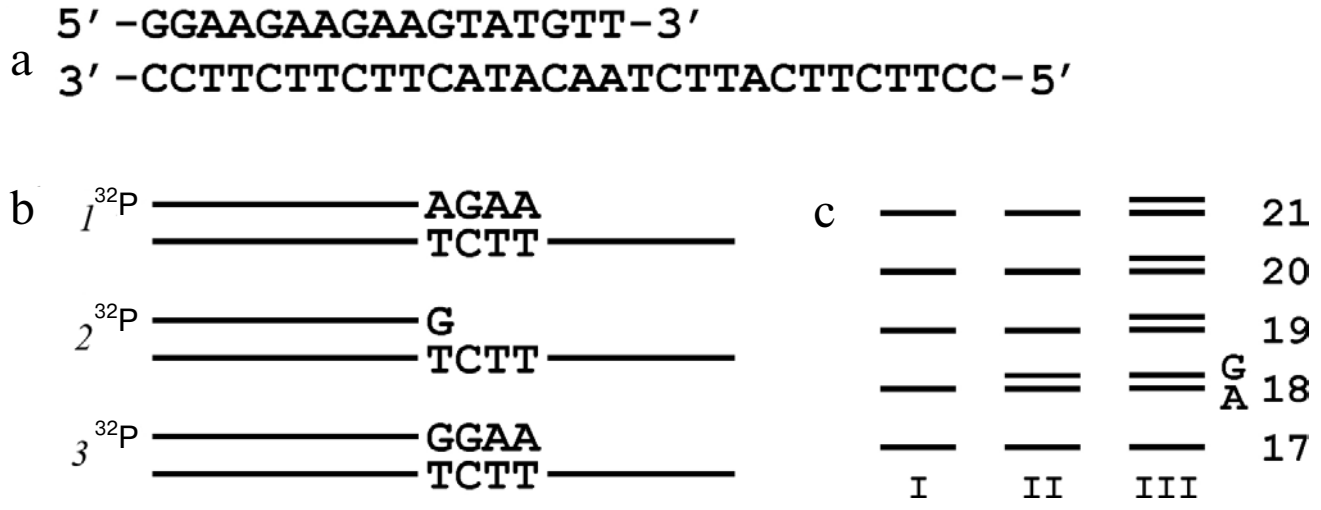

Fig. 1. Variants of primer completion by cell extracts and patterns characterizing their DNA polymerase activity. a) Oligonucleotide substrate on which DNA polymerase reaction is carried out. b) Scheme of three main synthesis variants possible on the given substrate: *1*) faultless synthesis; *2*) synthesis with Pol ι involvement when the enzyme incorporates dG opposite template dT and stops synthesis; *3*) synthesis with involvement of C when the latter incorporates dG opposite template dT and then further synthesis proceeds from this dG. c) Pattern types observed on electrophoregrams using different cell extracts.

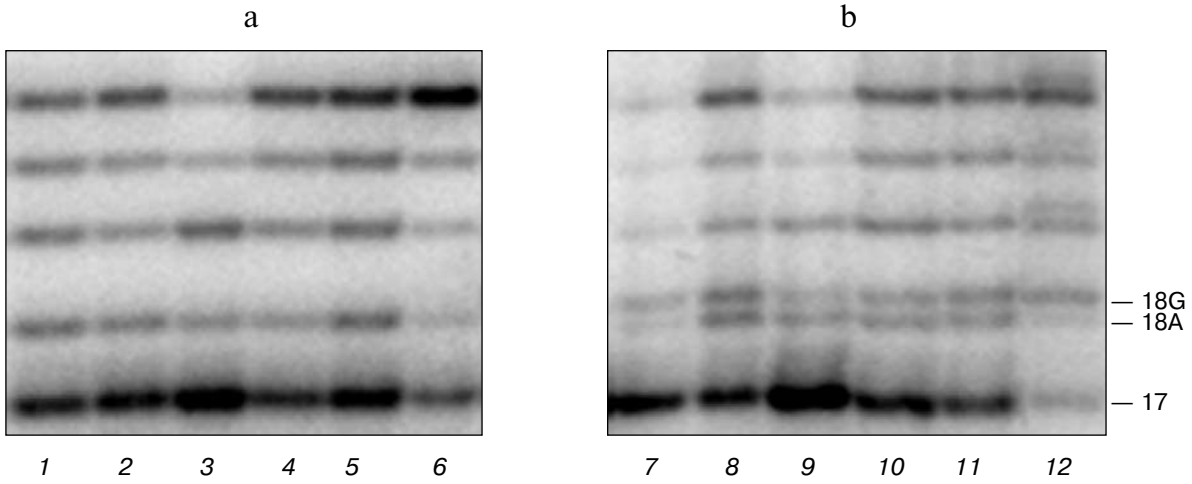

Fig. 2. Activity of DNA polymerases in cell extracts from different organs of mouse lines 129J (a) and C57BL (b) in the presence of Mn^{2+}: *1*, *7*) kidney; *2*, *8*) liver; *3*, *9*) heart; *4*, *10*) lung; 5, *11*) brain; *6*, *12*) testicle.

DNA synthesis by mouse cell extracts. Figure 2 shows that in cell extracts of all analyzed organs of 129J line mice carrying nonsense mutation in the gene encoding Pol ι [9] a set of products corresponding to pattern I is formed (Fig. 1). This is indicative of the absence of Pol ι activity in these extracts. However, analysis of cell extracts from C57BL line mice in lanes *7*-*11* reveals distribution of products corresponding to pattern II and shows that these extracts contain Pol ι activity restricted by T-stop. Lane *12* (Fig. 2) shows the electrophoregram of products of the DNA polymerase reaction carried out by the C57BL testicle extract. In this case T-stop is overcome, because this electrophoregram is similar to pattern III (Fig. 1).

Determination of Pol ι activity level in tumor cell extracts. Results obtained during investigation of DNA polymerase activities in mouse cell extracts in the presence of different cofactors allowed us to begin studying the DNA-synthesizing activity in human tumor cell extracts. Since the structure and biochemical properties of DNA polymerases in different mammalian species are very similar, the approach can be considered as appropriate.

Relative Pol ι activity in tumor cell extracts and in those of control human samples was determined on electrophoregrams as the ratio of intensity of bands corresponding to Pol ι products to total intensity of bands corresponding to products of other DNA polymerases. The formula for calculations is given in "Materials and Methods". Since it was shown earlier that Pol ι activity in cell extracts is strongly dependent on the type of analyzed tissue [3, 4], we used as control for tumor cells the same tissues not affected by tumor (normal or with benign tumors). Results of analysis of relative Pol ι activity in tumor cell extracts are given in the table. It is seen that the enzyme activity in extracts of melanomas affecting eye membrane is comparable with its activity in the cells used as control in this case (eye choroids). However, in cell extracts of eyelid BCC in the presence of Mn^{2+} as cofactor the enzyme activity approximately 2.5-fold exceeded that in extracts of the eyelid benign tumors used as control. In

Pol ι activity in tumor tissues

Number of patients	Tissue type	Average Pol ι activity		T-Stop overcoming
		Mg^{2+}	Mn^{2+}	Mn^{2+}
11	choroid of tumor-free eye (control 1)	14.92 ± 6.89	21.06 ± 6.63	no
22	choroid of tumor-affected eye (control 2)	12.88 ± 3.77	12.92 ± 3.97	no
22	uveal eye melanoma	11.20 ± 5.26	16.07 ± 8.00	yes
10	eyelid benign tumors (control)	0	8.90 ± 3.31	no
8	eyelid BCC	6.40 ± 2.32	21.50 ± 5.61	yes

this case in the presence of Mg^{2+} Pol ι activity was not detected at all in cell extracts of the eyelid benign tumors, while in the eyelid BCC cell extracts it was three times lower than that determined in the presence of Mn^{2+}.

Overcoming T-stop in tumor cell extracts. The study of homogeneous Pol ι preparations showed that after noncanonical dG incorporation opposite template dT no further synthesis is observed, i.e. T-stop emerges [2].

Results of investigation of DNA-synthesizing activity of individual extracts for different species tumor tissues and tissues used as control are given in Fig. 3. They show that cell extracts of all analyzed malignant tumors generate similar sets of oligonucleotide products. In the case of DNA polymerase reaction in the presence of Mg^{2+} this set corresponded to pattern II (Fig. 1) and with Mn^{2+} as cofactor the product set corresponded to pattern III. This means that Mg^{2+} activates Pol ι, while Mn^{2+} additionally activates primer completion after incorrectly incorporated dG. Among cell extracts of normal mouse organs only testicle cell extracts exhibited the same property in the presence of Mn^{2+} (lane *12* in Fig. 2). The Pol ι activity in the mouse testicle cell extracts in the presence of Mg^{2+} was also studied in our previous works where it was shown that these extracts, like extracts of malignant tumor cells, generate a set of oligonucleotide products characteristic of pattern II [3]. Figure 3 shows that pattern II is generated by cell extracts of the tissue, surrounding melanoma, as well as by extracts of cells of the injured eye fragments. In this case the benign tumor cell extracts are characterized by overall low level of DNA synthesis. Although in this case Mn^{2+} also activates Pol ι, no T-stop overcoming is observed, like in normal cells. In the presence of Mn^{2+} the T-stop overcoming was also stimulated by the MALT-lymphoma cell extract analyzed by us in a single case (lane *12* in Fig. 3).

DNA synthesis after dG using primer mimicking Pol ι product. Additional experiments were performed to determine whether any DNA polymerase of the studied cell extracts is able to continue synthesis after dG, incorrectly incorporated by Pol ι. For this purpose, in addition to the usually used labeled 17-membered primer, the labeled 18-membered oligonucleotide that differed from the previous one only by additional dG at the 3′-end was added upon oligonucleotide annealing during preparation of the DNA polymerase reaction substrate. In this case the amount of 18-membered primer added upon annealing was three times lower than that of the 17-membered one, which is most frequent after the action of Pol ι-containing extracts on the usual substrate containing only 17-membered primer. The DNA polymerase reaction was carried out under conditions used for usual DNA synthesis in the presence of various cell extracts as described in "Materials and Methods".

Results of analysis of cell extracts of various organs of the two mouse lines for their ability to complete the modified 18dG primer are given in Fig. 4. It is seen that cell extracts of different organs of both mouse lines are much more efficient in synthesis from 17-membered primer, because dG at the 3′-end of 18-membered oligonucleotide is not complementary to the template. Nevertheless, all cell extracts of C57BL mice are able to carry out synthesis from 18-membered primer and synthesize the 19-membered product, while cell extract of testicles of mice the same line can also synthesize the 20-membered product. Among extracts of 129J line mice only testicle extract synthesized the 19-membered product. Thus, despite the nonsense mutation in the Pol ι gene, testicle extracts of 129J mice are able to carry out synthesis from 18-membered primer, and it can be concluded that this product is synthesized by different enzymes rather than by Pol ι. However, the fact that the 18-membered primer completion by extracts of the C57BL organs is much more efficient is indicative of certain involvement of Pol ι in this process.

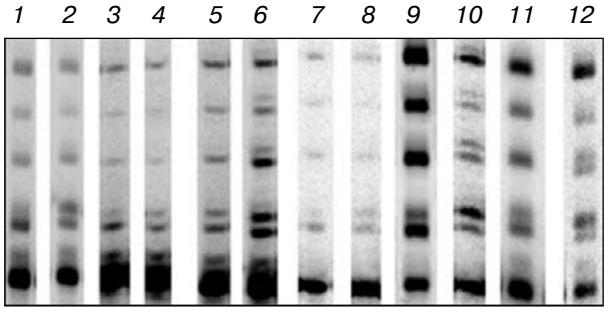

Fig. 3. Pol ι activity in cell extracts of malignant and normal tissues: *1*, *2*) choroid of tumor-free eye (control); *3*, *4*) choroid of tumor-affected eye; *5*, *6*) uveal eye melanoma; *7*, *8*) benign tumor (control); *9*, *10*) skin BCC; *11*, *12*) lymphoma; *1*, *3*, *5*, *7*, *9*, *11*) reaction was performed in the presence of Mg^{2+}; *2*, *4*, *6*, *8*, *10*, *12*) in the presence of Mn^{2+}.

DISCUSSION

Earlier we proposed a method for testing Pol ι activity in crude cell extracts based on the unique ability of the enzyme to incorporate dG opposite template dT [2]. In the presence of Mg^{2+} only cell extracts of mouse testicle and brain exhibited such activity [3]. It has been recently shown that Mn^{2+} many times more efficiently activates purified Pol ι preparations than Mg^{2+}. In this case the range of optimal Mn^{2+} concentrations is far wider than that of Mg^{2+} [10]. The data allowed the authors to suppose that Mg^{2+} might be the enzyme activator in a living cell. Our results also favor this hypothesis because in the presence of Mn^{2+} Pol ι activity was detected and enhanced in extracts of almost all studied organs of normal mice. Although there are significant reasons to assume that *in vivo* just Mn^{2+} plays the role of main Pol ι cofactor, additional data supporting this conclusion are necessary.

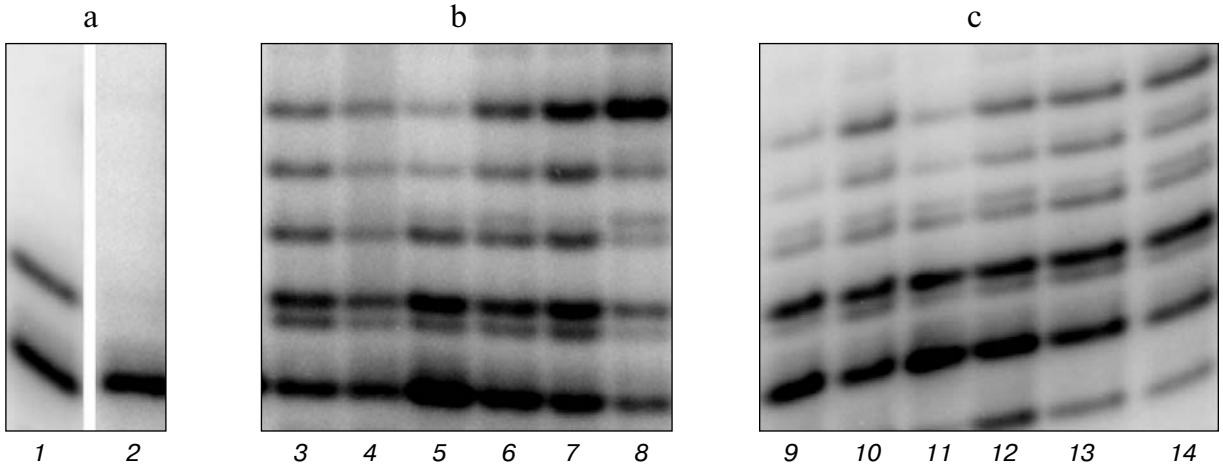

Fig. 4. DNA synthesis after dG using an artificially synthesized primer mimicking Pol ι product. a) Primers used in reaction: *1*) substrate mimicking Pol ι activity and including both normal primer and that containing deliberately incorporated guanine in position 18; *2*) primer without deliberately incorporated guanine in position 18. b, c) Products of DNA polymerase reaction carried out by different cell extracts of 129J and C57BL lines using substrate mimicking Pol ι activity: *3*, *9*) kidney; *4*, *10*) liver; *5*, *11*) heart; *6*, *12*) lung; *7*, *13*) brain; *8*, *14*) testicle.

The absence of activity providing for dG incorporation opposite template dT in extracts of different 129J mouse organs, containing mutant gene encoding Pol ι [9], suggests that Pol ι activity observed by us in cell extracts of other mouse lines belongs just to this enzyme. Besides, the absence of additional bands in patterns produced by cell extracts of different organs of 129J mice in the presence of Mn^{2+} shows that the presence of these ions does not stimulate incorrect activity of other DNA polymerases present in these extracts.

The proposed method was applied in this work for analysis of Pol ι activity in cell extracts of two types of cancer cells (skin BCC and uveal eye melanoma) using Mn^{2+} as cofactor. Measurement of Pol ι activity in different types of cancer cells has shown that for skin BCC it is significantly increased compared to control (table), whereas no such increase was observed in melanomas. In this case both in normal cells of different mouse organs and in human tumors and control cells in the majority of cases Pol ι activity in the presence of Mn^{2+} was higher than in the presence of Mg^{2+}. The only exception is choroid of tumor-affected eye, where the activities of this enzyme were practically identical in the presence of either cofactor (table). Cell extracts of brain of C57BL mice were characterized by similar behavior (data not shown). Evidently, the effect of bivalent cations in different tissues is mediated by certain cofactors whose influence cannot be detected for homogeneous Pol ι preparations.

The data indicate that total Pol ι activity in different types of cancer cells cannot serve as an unambiguous index of malignancy for these cells. In this respect the effect of T-stop overcoming in cancer cells found by us is more demonstrative. It is known that after dG incorporation opposite template dT, carried out by Pol ι, DNA synthesis ceases (T-stop described for homogeneous enzyme preparations [2]). In our experiments T-stop was observed in analysis of cell extracts of most mouse organs (except testicle) (Fig. 2, lanes *7-11*). This was revealed by the absence of elongation of 18-membered Pol ι product having dG at its 3′-end.

Investigation of cell extracts of control human tissues (eye choroids, eyelid benign tumors) also revealed the existence of T-stop in the presence of either cation (Fig. 3, lanes *1-4* and *8*). However, analysis of products of malignant tumor cell extracts revealed oligonucleotides formed during completion of 18-membered Pol ι product, which resulted in electrophoretic distribution of reaction products characteristic of pattern III (Fig. 1).

Thus, it can be concluded that not high Pol ι activity in cell extracts but rather the ability of cancer cell extracts to overcome T-stop can be the characteristic feature of cell malignancy. This can be additionally confirmed by detection of the same ability in lymphoma extract analyzed by us (Fig. 3).

To determine how T-stop can be overcome and whether Pol ι is involved in this process, we used a modified substrate mimicking Pol ι activity that contained dG in position 18 at the 3′-end. The reaction using cell extracts of mouse line 129J as activating agent showed that testicle extracts carry out synthesis from unpaired guanine. These data show that not Pol ι is involved in T-stop overcoming, but rather different DNA polymerases that are able to carry out synthesis from the template-unpaired nucleotide. However, a somewhat different situation was found for the reaction activated by C57BL cell extracts. First, synthesis from erroneous guanine was detected in all analyzed organs; second, this synthesis was more efficient in testicle extract. This shows that although Pol ι itself is

not involved in synthesis, it promotes the process. To a certain extent this resembles the combined overcoming of lesions by two DNA polymerases observed in experiments with homogeneous enzyme preparations [11].

What can cause the T-stop overcoming effect? It is known that DNA polymerases in replication fork work together with each other in a complex exhibiting a complicated regulatory system. The TLS process resumes the replication fork movement in the damaged DNA region when cessation of replication threatens the cell with death. This means that regulation of this process should be associated with the cell cycle control systems, as has been shown by a number of works [12-15]. Any tumor disease is the result of disturbance in the work of these systems. The T-stop overcoming might be due to the metabolic peculiarities in tissues characterized by intensive cell division.

It should be noted in conclusion that the results were obtained due to our earlier developed method of Pol ι activity testing in cell extracts. Although this system is not devoid of shortcomings, it is a significantly more adequate reflection of intracellular processes compared to usually used analyses of mRNA or isolated enzyme contents. Only the study of Pol ι activity in cell extracts allowed us to detect its increase as well as T-stop overcoming in certain types of cancer. In addition, T-stop overcoming was also found in normal tissues where cell division is not blocked.

REFERENCES

1. Rattray, A. J., and Strathern, J. N. (2003) *Annu Rev. Genet.*, **37**, 31-66.
2. Zhang, Y., Yuan, F., Wu, X., and Wang, Z. (2000) *Mol. Cell Biol.*, **20**, 7099-7108.
3. Gening, L. V., Makarova, A. V., Malashenko, A. M., and Tarantul, V. Z. (2006) *Biochemistry (Moscow)*, **71**, 155-159.
4. Gening, L. V., Petrochenkov, A. N., Reshetnyak, A. B., Andreeva, L. E., and Tarantul, V. Z. (2004) *Biochemistry (Moscow)*, **69**, 435-440.
5. Kazakov, A. A., Gening, L. V., Grishina, E. E., Petrochenkov, A. N., and Tarantul, V. Z. (2008) *Med. Genet. (Moscow)*, **9**, 27-31.
6. Dominguez, O., Ruiz, J. F., Lain de Lera, T., Garcia-Diaz, M., Gonzalez, M. A., Kirchhoff, T., Martinez, A. C., Bernad, A., and Blanco, L. (2000) *EMBO J.*, **19**, 1731-1742.
7. Blanca, G., Shevelev, I., Ramadan, K., Villani, G., Spadari, S., Hubscher, U., and Maga, G. (2003) *Biochemistry*, **42**, 7467-7476.
8. Batra, V. K., Beard, W. A., Shock, D. D., Pedersen, L. C., and Wilson, S. H. (2008) *Mol. Cell.*, **30**, 315-324.
9. McDonald, J. P., Frank, E. G., Plosky, B. S., Rogozin, I. B., Masutani, C., Hanaoka, F., Woodgate, R., and Gearhart, P. J. (2003) *J. Exp. Med.*, **198**, 635-643.
10. Frank, E. G., and Woodgate, R. (2007) *J. Biol. Chem.*, **282**, 24689-24696.
11. Acharya, N., Johnson, R. E., Prakash, S., and Prakash, L. (2006) *Mol. Cell. Biol.*, **26**, 9555-9563.
12. Livneh, Z. (2006) *Cell Cycle*, **5**, 1918-1922.
13. Bi, X., Barkley, L. R., Slater, D. M., Tateishi, S., Yamaizumi, M., Ohmori, H., and Vaziri, C. (2006) *Mol. Cell Biol.*, **26**, 3527-3540.
14. Avkin, S., Sevilya, Z., Toube, L., Geacintov, N., Chaney, S. G., Oren, M., and Livneh, Z. (2006) *Mol. Cell.*, **22**, 407-413.
15. Soria, G., Podhajcer, O., Prives, C., and Gottifredi, V. (2006) *Oncogene*, **25**, 2829-2838.