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A False Note of DNA Polymerase Iota in the Choir of Genome Caretakers in Mammals

L. V. Gening[1], A. V. Makarova[1], A. M. Malashenko[2], and V. Z. Tarantul[1]*

[1]*Institute of Molecular Genetics, Russian Academy of Sciences, pl. Kurchatova 2, 123182 Moscow, Russia; fax: (7-495) 196-0221; E-mail: tarantul@img.ras.ru*
[2]*Research Laboratory for Experimental Biological Models, Russian Academy of Medical Sciences, 143440 Svetlye Gory, Moscow Region, Russia*

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Abstract—DNA polymerase iota (Polι) of mammals is a member of the Y family of DNA polymerases. Among many other genome caretakers, these enzymes are responsible for maintaining genome stability. The members of the Y-family DNA polymerases take part in translesion DNA synthesis, bypassing some DNA lesions, and are characterized by low fidelity of DNA synthesis. A unique ability of Polι to predominantly incorporate G opposite T allowed us to identify the product of this enzyme among those synthesized by other DNA polymerases. This product can be called a "false note" of Polι. We measured the enzyme activity of Polι in crude extracts of cells from different organs of five inbred strains of mice (C3H/Sn, 101/H, C57BL/6, BALB/c, 129/J) that differed in a number of parameters. The "false note" of Polι was clearly sounding only in the extracts of testis and brain cells from four analyzed strains: C3H/Sn, 101/H, C57BL/6, BALB/c. In mice of 129/J strain that had a nonsense mutation in the second exon of the *polι* gene, the Polι activity was reliably detectable only in the extracts of brain. The data show that the active enzyme can be formed in some cell types even if they carry a nonsense mutation in the *polι* gene. This supports tissue-specific regulation of *polι* gene expression through alternative splicing. A semiquantitative determination of Polι activity in mice strains different in their radiosensitivity suggests a reciprocal correlation between the enzyme activity of Polι in testis and the resistance of mice to radiation.

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Key words: DNA polymerase iota, genome caretakers, mouse strains, brain, testis, sensitivity to radiation

The replicative DNA polymerases catalyzing error-free DNA replication play the key role in maintenance of genome stability. However, active sites of these enzymes are poorly suitable for DNA synthesis on damaged template; this results in replication arrest followed by subsequent cell death. In such cases, special DNA polymerases sometimes called genome caretakers may help. More than 15 such enzymes forming a whole assembly are known to date; each of them (separately or various combinations with others) is responsible for repair of various types of DNA damages in the cell. DNA polymerases of Y-family (Polη, Polι, Polκ, and Rev1) are examples of these caretakers [1]. Using undamaged DNA as template, such DNA polymerases make many mistakes and due to incorrect DNA synthesis these enzymes are often defined as error-prone DNA polymerases [1-3].

It is accepted that Polι is characterized by the highest error rate of DNA synthesis due to special arrangement of its catalytic site and non-Watson–Crick base pairing. This enzyme exhibits high dependence of correctness of DNA synthesis on nucleotides in the template strand. The most correct synthesis by Polι requires purine-containing templates. Acting as the genome caretaker, Polι cannot only replicate guanine DNA templates rather correctly, but also inserts the correct cytidine nucleotide opposite guanines modified with various chemical groups [4-6]. Usually this is sufficient for continuation of DNA synthesis at the damaged site, which further involves more processive DNA polymerases (e.g., Polκ) carrying out more correct DNA synthesis [7]. Copying cytidines, Polι follows Watson–Crick base pairing, whereas in the case of thymidines non-Watson–Crick base pairing occurs. Only mammalian Polι was shown to have the unique ability to insert guanine

* To whom correspondence should be addressed.

nucleotide opposite the thymidine template even in the presence of deoxyadenosine triphosphate [8]. Using this characteristic feature, it is possible to identify the product formed by Polι among all products formed by other DNA polymerases. We have named such product as the "false note of Polι".

Although biochemical characteristics of purified Polι have been studied in detail, the putative role of this enzyme *in vivo* remains unclear. Results of *in vitro* studies suggest that Polι may both stabilize and modify genome structure. However, it remains unclear which activities of this enzyme are employed *in vivo* (and for what purpose). The available data are contradictory. Initially, use of Berkitt lymphoma cells with deletions of both *polι* alleles revealed Polι involvement in hypermutagenesis of the variable region of immunoglobulin genes [9]. Later studies on strain 129 mice mutant by *polι* [10] and mice containing mutations in genes encoding Polι and Polκ [11] revealed that these mutation do not influence hypermutagenesis of the variable region of these genes. A similar discrepancy was also found in studies of the putative role of Polι in the development of urethane-induced lung adenoma [12, 13]. In the earlier report [12], the authors considered tumorigenesis as the result of mutagenic effect of Polι, whereas results of the later study [13] suggested that tumors appear due to deficit of this enzyme determined by mutation in the corresponding gene.

Since information on Polι activity *in vivo* may be useful for evaluation of putative role(s) of this enzyme in the body, we have recently analyzed Polι activity in cell extracts of various murine organs and tissues. We have demonstrated that Polι-like activity was more pronounced in testicular and brain cell extracts of C57BL/6 mice [14]. There was a correlation between Polι catalytic activity in the mouse brain and aggressive behavior of these animals [15].

In this study, we have analyzed Polι in several murine strains differing in some parameters, particularly, by the presence of mutations in *polι* and also by sensitivity of the testes to radiation.

MATERIALS AND METHODS

Animals. DNA polymerase activities were determined in cell extracts from various organs of five inbred murine strains: C3H/Sn, 101/H, C57BL/6, BALB/c, and 129/J obtained from the Research Laboratory of Experimental Biological Models, Russian Academy of Medical Sciences.

Assay of DNA polymerase activity in cell extracts. The cell extracts were prepared from various murine tissues as described [14]. Two complementary oligodeoxyribonucleotides of 17 and 30 nucleotides [8] were used as substrate for the assay of DNA polymerase activity; after hybridization they form duplex with a protruding 5′-end:

5′-GGAAGAAGAAGTATGTT-3′

3′-CCTTCTTCTTCATACAATCTTACTTCTTCC-5′.

The shorter oligonucleotide (of 17 nucleotides) was labeled with ^{32}P at the 5′-end and prepared as described in our previous study [14]. Besides the oligonucleotide substrate, the reaction mixture also included dATP and dGTP. The reaction mixture (final volume 20 μl) contained 50 mM Tris-HCl buffer, pH 8.0, 5 mM $MgCl_2$, 1 mM dithiothreitol (DTT), 300 nM substrate, 1 μM dATP, 1 μM dGTP, and 5 μl of tissue extract. The reaction was carried out at 37°C for 15 min; in the case of extracts of testis, the reaction time was shortened to 4 min. The reaction was stopped by cooling in ice followed by subsequent adding of an equal amount of mixture containing 95% formamide, 50 mM EDTA, and 0.05% bromophenol blue. The reaction products were separated by electrophoresis in Tris-borate buffer in the presence of 7 M urea using 20% polyacrylamide gel. After electrophoretic separation, the gels were covered with a polypropylene film and radioautographed using a Storage Phosphor Screen (Molecular Dynamics, USA). The autographs were scanned using a Storm 840 phosphoimager (Amersham Biosciences, UK). Results were analyzed using Image Quant 5.2 software.

PCR gene typing of the 27th codon of *polι* gene. This procedure was carried out exactly as described [10] for analysis of putative mutations in the second exon of the *polι* gene. A DNA fragment of 88 bp from brain cell extracts was amplified and treated with *Taq*I restrictase.

RESULTS AND DISCUSSION

In the presence of the oligonucleotide duplex presented in the "Materials and Methods" section as substrate and dATP and dGTP, any known DNA polymerase (except Polι) will synthesize the AGAA tetranucleotide at the 3′-end of the primer. Since Polι preferentially incorporates G opposite T and all subsequent nucleotides with lower efficiency and is a distributive DNA polymerase, the presence of this enzyme in tissue extracts in general results in formation of the 18-membered oligonucleotide containing the primer and dGMP attached to its 3′-end.

Short DNA fragments of equal length but various nucleotide compositions are often characterized by different electrophoretic mobility. So it is really possible to separate the product of the Polι reaction from all other products of DNA synthesis formed under these conditions. In control experiments where only dGTP or dATP were present in the reaction mixture, we found that the band corresponding to the 18-membered oligonucleotide and exhibiting higher mobility represents the product of DNA-polymerase reaction containing canonic dAMP attached to the 3′-end of the primer (18 A). The 18-mem-

bered oligonucleotide of lower mobility was identified as the product formed in the reaction catalyzed by Polι. At the 3′-end, this product contains dGMP incorporated in non-Watson–Crick base pairing manner (18 G). We defined this product as the false note of Polι.

Figure 1 shows results of analysis of DNA polymerase activity of extracts of testis obtained from four inbred murine strains. In each mixture of reaction products, the 21-membered oligonucleotide representing the primer with the AGAA tetranucleotide synthesized at its 3′-end predominated. Other products of 20, 19, and 18 nucleotides in length were also detected. In the case of C3H/Sn, C57BL/6, and 101/H murine strains there were two bands corresponding to the 18-membered oligonucleotide. In the presence of extracts of testis from 129/J mice, the false note of Polι (18 G) was basically not detected. This may be attributed to the presence of a nonsense-mutation in the 27th codon of the *polι* gene encoding Polι in this murine strain [10].

But analysis of brain tissue extracts for DNA polymerase activity revealed that the false note of Polι is detected in mice of all four strains studied although with different intensity (Fig. 2). The activity of this enzyme varied in the mice of 129/J strain (but was reliably detected). In mice of three other strains, the activity of Polι was higher and less variable. Detection of Polι activity in brain extracts of 129/J mice was rather unexpected. Certain evidence exists that these mice are characterized by the presence of a nonsense-mutation in the second exon of the *polι* gene, and the activity of this enzyme was not found in testicular tissue extracts from these animals (Fig. 1).

For detection of such mutation in five animals used in our experiments, we carried out PCR gene typing of the 27th codon of the *polι* gene using the previously described test [10]. Indeed, in 129/J murine strain our analysis revealed the presence of the nonsense-mutation in the *polι* gene involving conversion of serine codon TCG into TAG stop-codon; this results in disappearance of the restriction site for *Taq*I restrictase (TCGA) in these mice (data not presented).

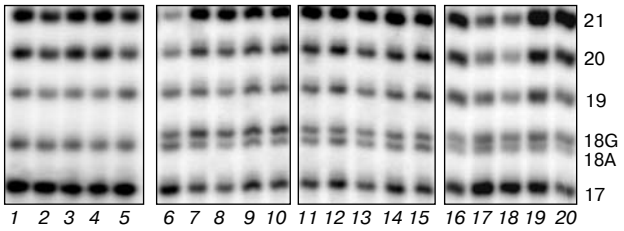

Fig. 1. Analysis of DNA-polymerase activity in extracts of testis of four murine strains. In the case of each strain, five animals were used. Lanes: *1-5*) 129/J; *6-10*) C3H/Sn; *11-15*) C57BL/10; *16-20*) 101/H.

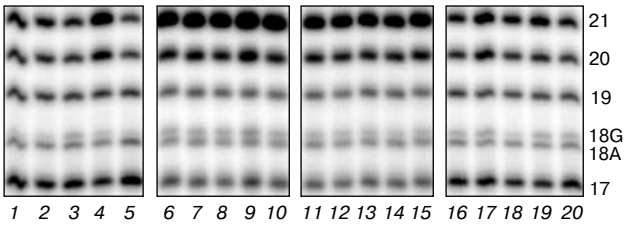

Fig. 2. Analysis of DNA-polymerase activity in brain extracts of four murine strains. In the case of each strain, five animals were used. Lanes: *1-5*) 129/J; *6-10*) C3H/Sn; *11-15*) C57BL/10; *16-20*) 101/H.

Recently alternative splicing of Polι mRNA has been described [12]. In lungs of several murine strains including the 129/J strain, besides full length mRNA containing the second exon, the product of mRNA alternative splicing lacking the second exon was found. This shortened mRNA encoded a protein exhibiting Polι activity [12]. Thus, mice of the 129/J strain may synthesize the shortened form of Polι, and this is the most plausible explanation for detection of the false note of Polι in brain cell extracts of these mice. Comparing data of that report with results of our study, we can suggest that various murine organs are characterized by preferential expression of different forms of Polι encoded by different products of mRNA splicing. If this suggestion is right, lack of Polι activity (as well as the enzyme protein) might be attributed to the presence of defective mRNA containing the mutant second exon in the testicular tissue of 129/J mice.

Our data question conclusion of some authors indicating that Polι is not involved into hypermutagenesis of the variable region of immunoglobulin genes [10, 11]. This conclusion is based on the fact that murine cells used for experiments contained the *polι* allele originating from the 129/J mouse strain and totally inactive Polι. Results of our study suggest that in this murine strain only one route of Polι expression is blocked.

The same argument can be addressed to the authors of [13] suggesting that Polι deficit increases the probability of the development of urethane-induced lung adenoma in mice. Since these authors have used the *polι* allele originating from the 129/J mouse strain, it is possible that the actual reason consists in lack of only one form of this enzyme.

Catalytic activity of this enzyme was not detected not only in testes but also in all other organs of 129/J mice (Fig. 3). However, in BALB/c mice it was rather high both in testicular and brain extracts; this activity was somewhat lower than in C3H/Sn mice (data not shown). In four murine strains (C3H/Sn, 101/H, C57BL/6, and BALB/c) small quantities of Polι activity were detected in kidney, liver and lung extracts (but not in lymphoid and cardiac tissues). In lung and kidney extracts of BALB/c mice, traces of Polι activity were clearly detected com-

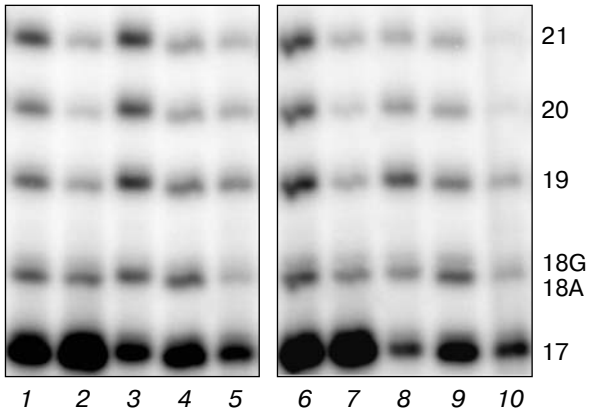

Fig. 3. Analysis of DNA polymerase activity in cell extracts from various organs of 129/J (*1-5*) and BALB/c mice (*6-10*): lungs (*1*, *6*), heart (*2*, *7*), liver (*3*, *8*), kidneys (*4*, *9*), and lymph nodes (*5*, *10*).

pared with corresponding extracts prepared from animals of other strains (see, for example, Fig. 3).

Our results do not correspond to the literature data indicating that Polι mRNA is ubiquitously distributed in organisms with quantitative predominance in testicular, cardiac, and pancreatic cells [16]. This suggests the importance of posttranscriptional regulation of *polι* gene expression.

The relative intensity of the Polι false note (18 G) in organs of various murine strains may be quantitatively analyzed using band intensity corresponding to the "correct note" or the 18-membered oligonucleotide fragment containing A at the 3′-end (18 A) as the internal control. Although this fragment is mainly formed by other DNA polymerases, some quantities of the oligonucleotide may be also formed by Polι. Table 1 shows results of such evaluation made for extracts of testis and brain by means of densitometry of the autoradiographs shown in Figs. 1 and 2. Calculating testicular Polι activity in 129/J mice, we took into consideration that insignificant background in the region corresponding to 18 G may be attributed to the product of this enzyme. Data of Table 1 show that in the lanes corresponding to products formed in the presence of extracts of testis the ratio of 18 G and 18 A band intensities calculated as 18 G/(18 G + 18 A)·100% varies from 0.9 to 70.7%. For brain extracts this parameter varies from 11.0 to 35.7%. As expected the most pronounced difference exists between Polι activity in extracts of testis and brain of 129/J mice compared with animals of the other strains investigated. However, the other strains also exhibit marked differences. For example, the highest Polι activity was found in testes of C3H/Sn and 101/H mice and in the brain of C3H/Sn mice.

Our results suggest that in most murine organs Polι is not a vitally important enzyme required for bypass of various types of DNA damage. It is possible that Polι activity is necessary for normal functioning only of brain cells. It should be noted that the inner cell mass of blastocyst of 129/J mice carrying mutation in *polι* gene is the most suitable material for preparation of embryonic stem cells compared with other murine strains [17]. For a long time this was the only murine strain employable for isolation of

Table 1. Relative catalytic activity of Polι in brain and testicular extracts of different murine strains

Murine strain	Organ	Relative Polι activity, % (18G/18G+18A) × 100
129/J	brain	11.0 ± 5.1
	testis	0.9 ± 0.4
C57BL/6	brain	18.5 ± 1.4
	testis	56 ± 2.2
C3H/Sn	brain	35.7 ± 3.1
	testis	70.0 ± 3.7
101/H	brain	22.4 ± 4.3
	testis	70.7 ± 4.7
BALB/c	brain	25.5 ± 4.1
	testis	58.2 ± 2.0

Table 2. Reversed correlation between relative Polι activity and radioresistance of testes

Murine strain	Radioresistance*		Relative Polι activity	
	whole organism	testes	brain	testes
C3H/Sn	moderate	decreased	+++	+++
C57BL/6	moderate	moderate	++	++
BALB/c	decreased	moderate	++	++
101/H	increased	decreased	++	+++
129/J	increased	increased	+	—

* Data from [19, 20].

embryonic stem cells suitable for manipulations in cell cultures and maintenance in non-differentiated state over many passages. It is reasonable to suggest that Polι may be involved in cell differentiation, and its lack in embryonic cells promotes maintenance of pluripotence of stem cells derived from the inner cell mass of the blastocyst.

Ability of Polι for incorrect DNA synthesis demonstrated *in vitro* experiments may be responsible for susceptibility of mice to mutagenesis and carcinogenesis. This is supported by results of experiments on human breast cancer cells demonstrating correlation between increased level of mutagenesis and increased content of Polι [18].

According to our data, the mutagenic potential of Polι in testicular cells of 129/J mice is two orders of magnitude less than in mice of other strains. So, it is reasonable to suggest that in testicular cells this enzyme is not ultimately required for animal viability. However, very high mutagenic potential of Polι in testicular extracts of animals from all strains investigated (except 129/J) suggests importance of this enzyme for DNA mutagenesis in spermatozoa. This might represent some adaptive mutagenesis increasing genetic diversity of offspring.

Comparison of radioresistance of testes of various murine cells with their Polι activity suggests another possibility. Table 2 shows the existence of reversed correlation between radioresistance and Polι activity. These results indicate that lack of Polι promotes testicular viability in the case of radiation-induced mutations.

Data obtained suggest the existence of certain mutagenic potential of Polι in mammalian cells. The increase in this potential may cause loss of control of genome stability by repair enzymes accompanied by susceptibility to malignant cell transformation.

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