ISSN 0006-2979, Biochemistry (Moscow), 2008, Vol. 73, No. 3, pp. 346-352. © Pleiades Publishing, Ltd., 2008.
Original Russian Text © A. V. Makarova, V. Z. Tarantul, L. V. Gening, 2008, published in Biokhimiya, 2008, Vol. 73, No. 3, pp. 426-433.

Evolution of DNA Polymerase ι Structure and Function in Eukaryotes

A. V. Makarova*, V. Z. Tarantul, and L. V. Gening

Institute of Molecular Genetics, Russian Academy of Sciences, pl. Kurchatova 2, 123182 Moscow, Russia; fax: (499) 196-0221; E-mail: amakarova-img@yandex.ru

Received July 6, 2007
Revision received September 18, 2007

Abstract—Analysis of DNA polymerase ι (Pol ι) enzymic activity in different classes of eukaryotes has shown that error-prone activity of this enzyme can be found only in mammals, and that it is completely absent from organisms that are at lower stages of development. It was supposed that the emergence of the error-prone Pol ι activity in mammals is caused by structural alteration of the active center. Possible functions of error-prone Pol ι in higher eukaryotes are discussed.

DOI: 10.1134/S0006297908030176

Key words: DNA polymerase ι, error-prone activity, hypermutagenesis

In archaea, prokaryotes, and eukaryotes, along with DNA polymerases providing for error-free DNA replication, there are also about twenty DNA polymerases the main function of which is supposed to be the involvement in replication during passing over damaged sites. During efficient copying the damaged DNA, these enzymes are incorporate bases not always obeying the Watson–Crick rule. As a result, the frequency of errors on undamaged DNA increases up to 10^{-1}-10^{-3} [1-5]. These enzymes are also able to catalyze the incorporation of nucleotides to aberrant primer termini (unpaired, containing errors, and damaged DNA sites). Owing to this distinction from other DNA polymerases participating in replication and repair, these enzymes were called "error-prone" DNA polymerases. Recently more and more data have appeared showing that in the norm error-prone DNA polymerases may be not only involved in intracellular replication of damaged DNA, but they also can carry out some other functions providing for selective advantage of organisms.

DNA polymerase ι (Pol ι) [1, 5, 6], discovered in 2000 and found only in higher eukaryotes beginning from insects [7], occupies a particular place among these unusual enzymes. The Pol ι sequence is similar to that of another error-prone DNA polymerase, Pol η [6]. It is supposed that Pol ι arose as a genetic duplication of Pol η millions of years ago, not long before the emergence of insects [7].

Owing to a special structure of the catalytic center and the use of Hoogsteen rather than Watson–Crick's interaction, human Pol ι exhibits a set of unusual properties. Thus, Pol ι is able to carry out some functions for genome stabilization. For example, human Pol ι is able to remove phosphate of deoxyribose (5′-deoxyribonuclease activity) which is necessary for correct repair of the damaged DNA site upon excision base repair [8]. Human Pol ι is also able to incorporate guanine opposite uracil, which can be used by a cell to prevent transitions caused by cytosine deamination [9, 10]. Finally, this enzyme is able to carry out, with different efficiency and fidelity, syntheses opposite a number of DNA damages (AP-sites, various purine base adducts, etc.).

At the same time, human Pol ι exhibits the lowest fidelity of undamaged DNA synthesis among all known DNA polymerases [11]. In this case, incorporation of new nucleotides opposite four DNA bases happens with different accuracy and efficiency. The enzyme incorporates dGTP opposite thymidine (by the mechanism used for incorporation opposite uracil) several times more efficiently than canonical dATP [6]. Pol ι carries out more precise and efficient synthesis opposite template purines than opposite pyrimidines [2, 5, 6, 12].

Attention should be paid to the fact that Pol ι seems to be an exception among all DNA polymerases detected in members of different classes of organisms. It is known that in drosophila this enzyme differs little by its proper-

* To whom correspondence should be addressed.

ties from its homolog Pol η and does not show such high tendency to errors. Pol ι of the fruit fly drosophila by its properties more resembles human or yeast Pol η than human Pol ι. Thus, Pol ι of drosophila, like eukaryotic Pol η, is accurate and efficient for *cis-syn*-thymine dimers, whereas human Pol ι in this case makes errors and is inefficient [7, 13].

Mouse Pol ι exhibits properties characteristic of the analogous human enzyme [14, 15]. However, properties of Pol ι in other vertebrates are still unclear, although sequences of this polymerase gene are already known for several organisms (*Drosophila melanogaster*, *Danio rerio*, *Ictalurus punctatus*, *Xenopus tropicalis*, *Bos taurus*, *Mus musculus*, *Rattus norvegicus*, *Homo sapiens*, *Pan troglodytes*). Functions of the human enzyme are also not completely clear because still no human diseases tightly associated with any mutation in Pol ι gene are described. In this situation, studying evolution of the enzyme structure and properties could be very helpful in improving its functions in man and mammals.

Previously we proposed a variant of the method of Pol ι biochemical activity determination in crude extracts of different animal tissues and organs [16, 17]. The unique ability of the enzyme for preferable dGTP incorporation opposite T template even under dATP excess in the reaction mixture allows one to estimate Pol ι activity in crude cell extracts. In our work, this approach was combined with analysis of the enzyme amino acid sequences in different species to estimate structural similarity and extent of accuracy of synthesis carried out by Pol ι in members of different vertebrate classes.

METHODS OF INVESTIGATION

Objects of investigation. The Pol ι error-prone activity was tested in crude organ and tissue extracts of sexually mature vertebrates of different sex and taxonomic groups:

– river loach (*Misgurnus fossilis*, bony fishes class, order Cypriniformes) was the courtesy of members of Department of Embryology, Lomonosov Moscow State University;

– crested newt (*Triturus cristatus*, class amphibia, order Caudata), an adult female was purchased in a zoological shop (Moscow);

– common frog (*Rana temporaria*, class amphibia, tail-less order), animals were purchased in a zoological shop (Moscow);

– Horsfield's terrapin (*Testudines agrionemys*, class reptile, order Chelonia), an adult female was purchased in a zoological shop (Moscow);

– green shrub snake (*Philothamnus punctatus*, class reptile, order snake); organs of an adult male for experiments were the courtesy of members of Department of Embryology, Lomonosov Moscow State University;

– domestic cock (*Gallus domesticus*, class birds, order Galliformes), an adult cock was purchased in a zoological shop (Moscow);

– quail (*Coturnix coturnix*, class birds, order Galliformes), adult males were the courtesy of members of the Institute of Medico-Biological Problems, Russian Academy of Sciences;

– brown rat (*Rattus norvegicus*, class mammals, order Muridae (rodents)), a male was obtained from the vivarium of the Institute of Molecular Genetics, Russian Academy of Sciences;

– domestic rabbit (*Oryktolagus cuniculus*, class mammals, suborder of double-toothed rodents) was obtained from the vivarium of the Institute of Molecular Genetics, Russian Academy of Sciences;

– domestic dog (*Canis familiaris*, class mammals, wolf family), organs of an adult male of Mastiff pedigree after castration procedure were the courtesy of a veterinary clinic "Micro Plus" (Moscow).

Determination of Pol ι activity in cell extracts. Preparation of cell extracts from different organs and tissues as well as DNA polymerase reaction were carried out as described previously [16, 17]. Semiquantitative estimation of Pol ι activity was achieved in two ways: by percentage of dGTP incorporation in position 18 opposite thymine of oligonucleotide substrate template ($G \times 100\%/(G + A)$) and by percentage of dGTP incorporated in position 18 among all nucleotides incorporated during this reaction ($G \times 100\%/(4A + 3B + 2C + D)$, where A, B, C, and D show the amount of reaction products in which 21-, 20-, 19-, and 18-membered products were formed, respectively). Calculations were done using the ImageQuant 5.2 program.

Analysis of amino acid substitutions in the active center of eukaryotic Pol ι and creation of a phylogenetic tree. Known base sequences of mRNA gene and amino acid sequences of Pol ι of animals from different phylogenetic taxons were used: *D. melanogaster* (NM_169207.1 and NM_141515.2), *D. rerio* (BC092781.1), *X. tropicalis* (M_001032308.1; BC121199; NM_001032308; DQ102380), *M. musculus* (AH013032 – strain 129/J, AY515316.1 – strain A/J, AF151691.1 – strain BALB/c, BC057575.1 – strain C57BL/6, AK154537.1 – strain NOD), *R. norvegicus* (XM_225844.3), *P. troglodytes* (XM_512140 and XM_512141), *B. taurus* (NM_001024692.1), and *H. sapiens* (NM_007195.1). The BLAST program [18] was used for their search.

The Pol ι sequences were aligned using the CLUSTAL W Multiple Sequence Alignment Program. Parts of fragments that cannot be reliably aligned were excluded from the analysis. Phylogenetic analysis of primary mRNA sequences was carried out using program package MEGA 3.1 for phylogenetic analysis of nucleotide and polypeptide sequences. The phylogenetic tree was built by the neighbor joining method. Phylogenetic distance (p-distance) was calculated as

mean number of nucleotide substitutions per pair of homologous sites of base sequences under comparison.

RESULTS AND DISCUSSION

Previously cell extracts of different organs and tissues of several inbred strains of mice (C57/BL, C3H/Sn, 101/H, 129/J) [17] were used in investigation of Pol ι enzymic activity in mammals. In this case, we managed to detect a specific product of synthesis by Pol ι, a 18-membered oligonucleotide with guanine incorporated opposite template thymine at the 3′ end with violation of the Watson–Crick rule. The highest Pol ι activity was registered in extracts of testes of all strains of mice except 129/J. In these cases guanine was incorporated opposite thymine 2.6-2.7 times more frequently than canonical adenine, and mutagenic potential carried out by Pol ι was ~10^{-2}. The enzyme activity was also clearly detected in brain cell extracts (on average, it was 2-3 times lower than that in testes extracts) and only trace Pol ι activity was observed in cell extracts of liver and kidney. Significant error-prone activity of Pol ι was also high in extracts of mouse ovaries (unpublished data).

We continued analysis of error-prone Pol ι activity in other animals. The presence of this activity was determined as before by two radioactive bands corresponding to 18-membered oligonucleotides with adenine or guanine at the 3′ end, characterized by different electrophoretic mobility. In this case, the lower band with higher mobility is indicative of the amount of oligonucleotide with 3′-terminal dATP, whereas the upper band shows the amount of a less mobile oligonucleotide with dGTP incorporated in position 18.

Our experiments with organ extracts of other mammals have shown that Pol ι activity was clearly detected in testis and brain extracts of rat (*R. norvegicus*) (Fig. 1, lanes *1* and *5*) and rabbit (*O. cuniculus*) (Fig. 2, a and b),

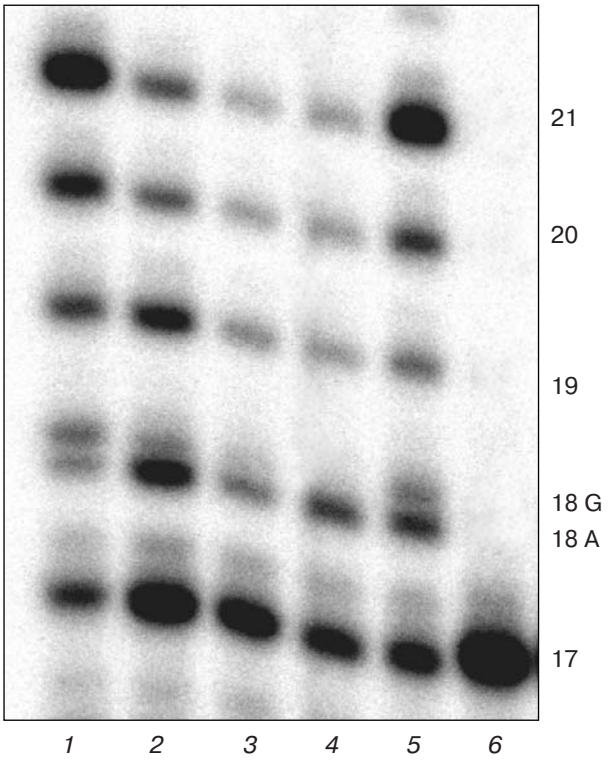

Fig. 1. DNA polymerase activity in cell extracts of rat tissues and organs: *1*) brain; *2*) liver; *3*) lungs; *4*) heart; *5*) testes; *6*) negative control (without extract).

as well as in extracts of dog (*C. familiaris*) testes (Fig. 3, lane *2*). Slight error-prone activity was also present in cell extract of rat liver (Fig. 1, lane *2*). The enzyme activity was detected in all parts of rabbit brain. However, error-prone Pol ι activity was very slight when cerebellum extracts were used as enzyme preparations (Fig. 2, a and b, lanes *7* and *8*). Thus, total character of Pol ι activity was similar in all studied animals.

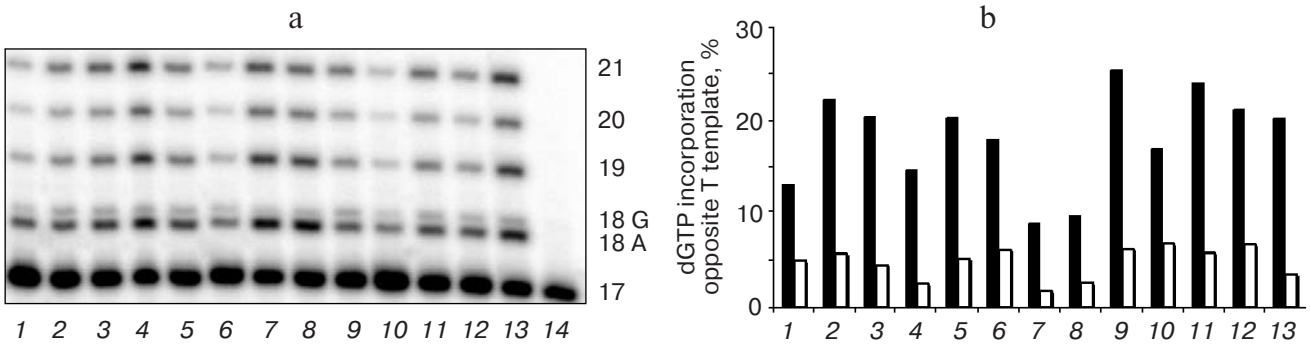

Fig. 2. DNA polymerase activity (a) and semiquantitative evaluation of Pol ι activity (b) in cell extracts of rabbit testes and different brain regions. Black columns, G × 100%(G + A); white columns, G × 100%(4A + 3B + 2C + D). *1*) Olfactory lobes; *2*) white matter of cerebral hemispheres; *3*) gray matter of cerebral hemispheres; *4*) quadrigeminal bodies; *5*) corpus callosum; *6*) hypothalamus; *7*) cerebellar hemispheres; *8*) vermis cerebelli; *9*) hippocampus; *10*) gray nuclei of subcortical region; *11*) medulla; *12*) pons Varolii; *13*) testes; *14*) negative control (extract-free).

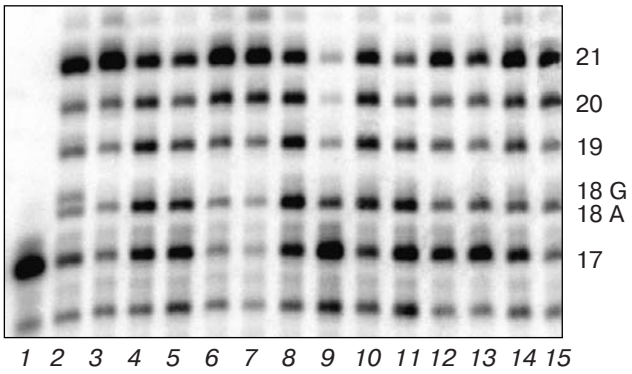

Fig. 3. DNA polymerase activity in cell extracts of tissues and organs of members of different classes of vertebrates: *1*) extract-free control; *2*) testes (dog); *3*) testes; *4*) brain (domestic cock); *5*, *8*) brain; *6*, *7*) testes (quail); *9*) liver; *10*) brain (common frog, female); *11*) brain; *12*) liver; *13*) eggs (crested newt); *14*) brain; *15*) testes (common frog, male).

Based on data obtained on mammals, we have tested Pol ι activity in a number of other vertebrates. For this aim, cell extracts of testes, brain, liver, and kidneys of members of several animal classes were investigated: fishes (*M. fossilis*), amphibians (*R. temporaria*, *T. cristatus*), reptiles (*Ph. punctatus*, *T. agrionemys*), and birds (*C. coturnix*, *G. domesticus*). DNA polymerase reaction, using crude extracts of organs of different vertebrates as enzyme preparations, was carried out at various temperatures (25, 37, 40, and 42°C) to select optimal temperature conditions for DNA polymerase activity in different organisms. Temperature of 37°C appeared to be the most suitable for reaction using organ extracts of both cold-blooded animals (fishes, amphibians, reptiles) and mammals. Only for quail organ extracts optimal temperature was 42°C (data not shown).

Figures 3 and 4 show that in all cases using different organ extracts of all analyzed vertebrates, except mammals, an 18-membered product was synthesized, which consists of a single band corresponding to an oligonucleotide with canonical adenine at the 3′ terminus. Thus, Pol ι of fishes, amphibians, reptiles, and birds does not incorporate dGTP opposite template T, which is tested by our approach.

Our biochemical investigations show that crude cell extracts of some mammalian tissues (mainly testes and brain) exhibit stable ability to incorporate G opposite template T. We failed in detection of such activity for other vertebrate classes both in similar and changed (varied temperature of reaction) conditions.

Two versions can be proposed to explain the absence of Pol ι activity, similar to that in mammals, from other classes of animals that are at lower stages of development. The first version is based on the fact that Pol ι in the genome of these classes of animals is similar by its biochemical properties to the enzyme of mammals, but the enzyme gene expression in all cases is completely blocked. However, it is definitely known that Pol ι is expressed in *X. tropicalis*. Thus, mRNA of Pol ι of this organism was isolated from testes (BC121199) and gastrula cells (DQ102380). Besides, Pol ι of drosophila also does not exhibit significant error-prone activity compared to human Pol ι [13]. The other version suggests that Pol ι is not able to incorporate G opposite template T due to structural peculiarities. At the moment we consider the second version as more likely.

It is already known what functions are carried out by a certain site of human Pol ι protein and which amino acids are constituents of its active center [19]. We have carried out comparative analysis of known Pol ι amino acid sequences of different organisms (table) and tried to compare the results with data available in the literature on functional significance of amino acids of the enzyme active center. Data in the table on key amino acids of the human Pol ι active center and their functions are taken from [19]. It is seen that during evolution from insects to mammals, four substitutions of functionally significant amino acids occurred in Pol ι: three at the time of evolution from insects to vertebrates (positions 214, 307, and 343) and one (position 62) between mammals and other vertebrates and invertebrates. If error-prone activity of Pol ι emerges during evolution only in mammals, then it can be supposed that this enzyme property is due to the leucine substitution for isoleucine in position 62.

An essential argument in favor of this hypothesis is the data obtained during investigation of the enzyme active center by directed mutagenesis. Thus, in the course of investigation of the role of amino acid 62 of the human Pol ι active center in establishment of Hoogsteen hydro-

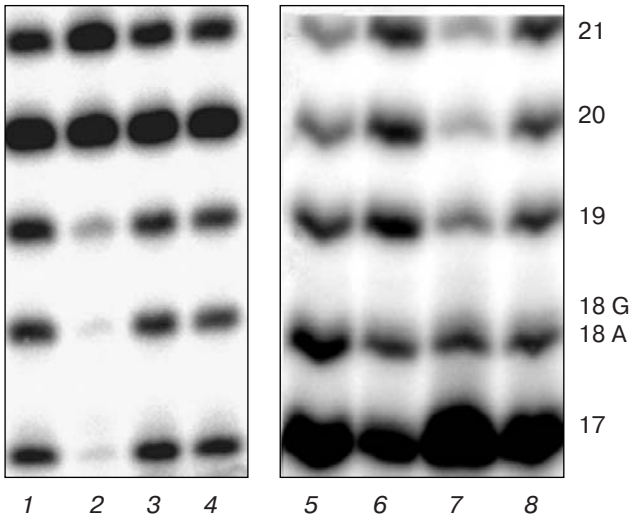

Fig. 4. DNA polymerase activity in cell extracts of tissues and organs of members of different classes of vertebrates: *1*) testes; *2*) brain; *3*) eggs; *4*) muscle (river loach); *5*) liver; *6*) brain (Horsfield's terrapin); *7*) brain; *8*) testes (shrub snake).

Comparative analysis of key amino acids of the Pol ι active center in different classes of eukaryotes

Amino acid number	Amino acid functions in the active center	*M. musculus, R. norvegicus, B. taurus, P. troglodytes, H. sapiens*	*X. tropicalis, D. rerio*	*D. melanogaster*
34	catalyzes transfer of a nucleotide to be incorporated; coordinates Me ion	Asp	Asp	Asp
39	selective function (interacts only with nucleotides carrying OH in 3′ position)	Tyr	Tyr	Tyr
59	responsible for acquiring *syn*-position by template nucleotide; stabilizes template nucleotide keeping it in *syn*-position	Gln	Gln	Gln
60	stabilizes template nucleotide by keeping it in *syn*-position by H-bonds	Lys	Lys	Lys
62	responsible for acquiring *syn*-position by template nucleotide	Leu	Ile	Ile
64	responsible for acquiring *syn*-position by template nucleotide	Val	Val	Val
68	stabilizes incorporating nucleotide by forming H-bonds with it	Tyr	Tyr	Tyr
71	stabilizes incorporating nucleotide by forming H-bonds with it	Arg	Arg	Arg
126	catalyzes transfer of a nucleotide to be incorporated; coordinates Me ion	Asp	Asp	Asp
127	catalyzes transfer of a nucleotide to be incorporated; catalyzes formation of a bond between primer and incorporating nucleotide	Glu	Glu	Glu
214	stabilizes incorporating nucleotide by forming H-bonds with it	Lys	Lys	Glu
307	stabilizes template nucleotide by keeping it in *syn*-position by H-bonds	Ser	Ser	Ala
343	tight contact with large DNA groove	Arg	Arg	Lys

gen bond authors of work [20] have shown that the substitution of alanine for leucine in position 62 strongly changes the efficiency and especially fidelity of nucleotide incorporations by the enzyme. Authors of this work emphasize that amino acids Leu62 and Lys60 are unique for Pol ι (they studied human Pol ι). These amino acid residues are absent from the enzyme precursor Pol η (both human and yeast) as well as from polymerase of Y family Dpo4 and are the key ones for establishment of Hoogsteen hydrogen bond. Owing to remarkable importance of Leu62, amino acid replacement in this position by another amino acid even with close biochemical properties and dimensions can significantly influence the characteristics of the enzyme.

Pol ι emerged at early stages of evolution, in particular, its ortholog was found even in the genome of drosophila. At the present time both base sequences of the enzyme gene and amino acid sequences of Pol ι protein are already known for many organisms including humans. Reptiles and birds are an exception. Phylogenetic analysis of Pol ι mRNA sequences in different vertebrata classes revealed (Fig. 5) that the longest phylogenetic distance which indicate the genetic range between human and drosophila base sequences reaches 27 ± 3.2%. The divergence between man and *P. troglodytes* makes up only 0.02 ± 0.001%, whereas the divergence between mouse and amphibian *X. tropicalis* makes up about 12 ± 0.5% nucleotide substitutions per pair of homologous sites.

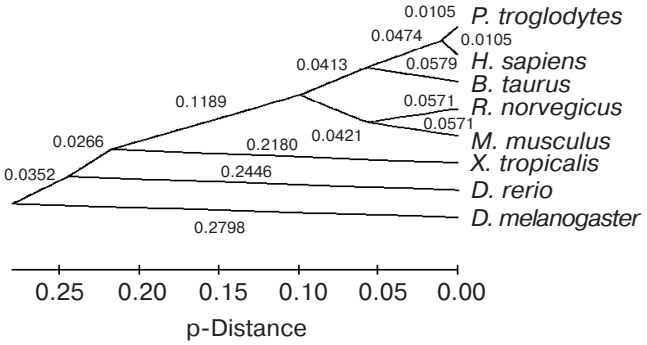

Fig. 5. Evolutionary tree of eukaryotic Pol ι. p-Distance was calculated as mean number of nucleotide substitutions per pair of homologous sites of base sequences under comparison.

Altogether phylogenetic sequence ratios in studied clusters mainly correspond to molecular-phylogenetic classification of eukaryotes, but at the same time there are also some peculiarities. This may be the result of uneven rate of Pol ι evolution as various taxons of higher eukaryotes were formed.

It is possible that error-prone activity of Pol ι, emerging during evolution, could provide for the participation of this enzyme in maturation of variable regions of immunoglobulin genes. Evolutionarily the humoral immunity function is relatively young and reaches its full development only in mammals. It is known that the heterogeneity of antibodies produced in fishes, amphibian, reptiles, and birds in response to antigenic stimulation is much restricted compared to that in mammals. Some immunoglobulin species are also found only in mammals [21-24]. However, data in the literature concerning the participation of Pol ι in this process are very contradictory. The first experiments allowed us to consider Pol ι as a candidate for the mutator of immunoglobulin genes. Cultivation of Burkitt's lymphoma cells with antigen resulted in 5-10-fold increase in mutations, mainly in RGYW sequences [25], and was accompanied by fourfold increase in Pol ι mRNA expression [26]. Experiments with the Pol ι gene deletion also showed a strong decrease in the level of immunoglobulin gene mutagenesis [27].

At the same time, following experiments with 129 strain of mice carrying a nonsense mutation in the second exon of the Pol ι gene have shown that the level and spectrum of mutations in immunoglobulin genes of these mice do not differ from the norm [14, 28, 29]. These data make doubtful the role of Pol ι in hypermutagenesis. However, studying the Pol ι activity in mice 129/J by our method has shown that in this case error-prone activity of the enzyme is partially retained at least in brain [17]. This fact suggested that in certain cell types, even in the presence of nonsense mutations in the gene, synthesis of active enzyme may take place due to alternative splicing at the second exon or to nonsense codon suppression by certain transcription factors [30]. This means that the problem of Pol ι involvement in hypermutagenesis of immunoglobulin genes still cannot be considered as completely resolved.

Another hypothesis concerning the role of error-prone Pol ι activity in higher organisms is the possible participation of the enzyme in specific repair functions in mammals, in particular, in gametes and brain. The ability of Pol ι to use Hoogsteen interactions for prevention of some transitions and to carry out DNA synthesis in certain damaged sites that cannot be used by other polymerases is especially important for tissues with increased inclination for creation of damage in DNA.

It is possible that in different types of tissue and/or upon involvement of different types of biochemical activity regulation, different enzyme variants (full-sized, truncated, or alternatively spliced) can have various biological functions, retaining the primary one (repair) and carrying out a new function specialized for mammals.

In order to obtain a final answer to the question, which of two proposed versions concerning the absence of Pol ι from lower vertebrates is correct, it is necessary to carry out large-scale molecular-genetic investigations including both sequencing genes encoding Pol ι in reptiles and birds, not yet done, and studying regulation of expression of these genes in different classes. Besides, it is necessary to study biochemical properties of homogeneous preparations of this enzyme in members of lower classes. This work points to the importance of such investigations.

The authors are grateful for help in this investigation to members of the Department of Embryology, Lomonosov Moscow State University, members of the Institute of Medico-Biological Problems, Russian Academy of Sciences, to members of Veterinary Clinic "Micro Plus" (Moscow), and to Ya. V. Agniullin.

This work was supported by the Presidium of the Russian Academy of Sciences (program No. 10 on Molecular and Cell Biology).

REFERENCES

1. Haracska, L., Prakash, L., and Prakash, S. (2002) *J. Biol. Chem.*, **277**, 15546-15551.
2. Johnson, R. E., Washington, M. T., Haracska, L., Prakash, S., and Prakash, L. (2000) *Nature*, **406**, 1015-1019.
3. Matsuda, T., Bebenek, K., Masutani, C., Hanaoka, F., and Kunkel, T. A. (2000) *Nature*, **404**, 1011-1013.
4. Ohashi, E., Bebenek, K., Matsuda, T., Feaver, W. J., Gerlach, V. L., Friedberg, E. C., Ohmori, H., and Kunkel, T. A. (2000) *Biol. Chem.*, **275**, 39678-39684.
5. Tissier, A., McDonald, J. P., Frank, E. G., and Woodgate, R. (2000) *Genes Dev.*, **14**, 1642-1650.
6. Zhang, Y., Yuan, F., Wu, X., and Wang, Z. (2000) *Mol. Cell. Biol.*, **20**, 7099-7108.

7. Vaisman, A., Frank, E. G., McDonald, J. P., Tissier, A., and Woodgate, R. (2002) *Mutat. Res.*, **510**, 9-22.
8. Bebenek, K., Tissier, A. K., Frank, E. G., McDonald, J. P., Prasad, S., Wilson, S. H., Woodgate, R., and Kunkel, T. A. (2001) *Science*, **291**, 2156-2159.
9. Vaisman, A., Takasawa, K., Iwai, S., and Woodgate, R. (2006) *DNA Repair (Amst.)*, **5**, 210-218.
10. Vaisman, A., and Woodgate, R. (2001) *EMBO J.*, **20**, 6520-6529.
11. McDonald, J. P., Tissier, A., Frank, E. G., Iwai, S., Hanaoka, F., and Woodgate, R. (2001) *Philos. Trans. Roy. Soc. (Lond. B Biol. Sci.)*, **356**, 53-60.
12. Washington, M. T., Johnson, R. E., Prakash, L., and Prakash, S. (2004) *Mol. Cell. Biol.*, **24**, 936-943.
13. Ishikava, T., Uematsu, N., Mizukoshi, T., Iwai, S., Masutani, C., Hanaoka, F., Ueda, R., Ohmori, H., and Todo, H. (2001) *J. Biol. Chem.*, **276**, 15155-15163.
14. 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.
15. Wang, M., Devereux, T. R., Vikis, H. G., McCulloch, S. D., Holliday, W., Anna, C., Wang, Y., Bebenek, K., Kunkel, T. A., Guan, K., and You, M. (2004) *Cancer Res.*, **64**, 1924-1931.
16. Gening, L. V., Petrochenkov, A. N., Reshetnyak, A. B., Andreeva, L. E., and Tarantul, V. Z. (2004) *Biochemistry (Moscow)*, **69**, 435-440.
17. Gening, L. V., Makarova, A. V., Malashenko, A. M., and Tarantul, V. Z. (2006) *Biochemistry (Moscow)*, **71**, 155-159.
18. Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D. J. (1997) *Nucleic Acids Res.*, **25**, 3389-3402.
19. Nair, D. T., Johnson, R. E., Prakash, L., and Aggarwal, A. K. (2004) *Nature*, **430**, 377-380.
20. Johnson, R. E., Prakash, S., and Prakash, L. (2005) *Proc. Natl. Acad. Sci. USA*, **102**, 10466-10471.
21. Galaktionov, V. G. (2005) *Evolutionary Immunology* [in Russian], Akademkniga, Moscow.
22. Du Pasquier, L. (1982) *Nature*, **296**, 311-313.
23. Du Pasquier, L., and Flajnik, M. (1997) *Fundamental Immunology* (Paul, W., ed.), LW&W.
24. Hsu, E. (1998) *Immunol. Rev.*, **162**, 25-36.
25. Denepoux, S., Razanajaona, D., Blanchard, D., Meffre, G., and Capra, J. D. (1997) *Immunity*, **6**, 35-46.
26. Poltoratsky, V., Woo, C. J., Tippin, B., Martin, A., Goodman, M. F., and Scharff, M. F. (2001) *Proc. Natl. Acad. Sci. USA*, **98**, 7976-7981.
27. Faili, A., Aoufouchi, S., Flatter, E., Gueranger, Q., Reunaud, C. A., and Weill, J. C. (2002) *Nature*, **419**, 944-947.
28. Marmoto, S. A., Yang, W. W., Vaisman, A., Maas, A., Yokoi, M., Hoeijmakers, J. H., Hanaoka, F., Woodgate, R., and Gearhart, P. J. (2006) *DNA Repair (Amst.)*, **5**, 392-398.
29. Shimizu, T., Azuma, T., Ishiguro, M., Kanjo, N., Yamada, S., and Ohmori, H. (2005) *Immunol. Lett.*, **98**, 259-264.
30. Gening, L. V., and Tarantul, V. Z. (2006) *Immunol. Lett.*, **106**, 198-199.