ISSN 0006-2979, Biochemistry (Moscow), 2011, Vol. 76, No. 1, pp. 61-68. © Pleiades Publishing, Ltd., 2011.
Original Russian Text © L. V. Gening, 2011, published in Biokhimiya, 2011, Vol. 76, No. 1, pp. 76-85.

REVIEW

DNA Polymerase ι of Mammals as a Participant in Translesion Synthesis of DNA

L. V. Gening

Institute of Molecular Genetics, Russian Academy of Sciences, pl. Kurchatova 2, 123182 Moscow, Russia; fax: (499) 196-022; E-mail: geni@img.ras.ru

Received August 2, 2010
Revision received October 20, 2010

Abstract—This review describes the properties of some specialized DNA polymerases participating in translesion synthesis of DNA. Special attention is given to these properties *in vivo*. DNA polymerase iota (Polι) of mammals has very unusual features and is extremely error-prone. Based on available data, a hypothesis is proposed explaining how mammalian cells can explore the unusual features of DNA Polι to bypass DNA damages and to simultaneously prevent its mutagenic potential.

DOI: 10.1134/S0006297911010081

Key words: DNA translesion synthesis, error-prone DNA polymerase, DNA polymerase ι

DNA TRANSLESION SYNTHESIS IN EUKARYOTIC CELLS

The structural organization of a living cell and also physical and biochemical properties of its elements cannot completely protect DNA within cells against numerous damaging agents from the environment, such as ionizing radiation, carcinogens, and UV-radiation of sunlight, or from endogenous agents like reactive oxygen species. Due to some special molecular mechanisms, cells can eliminate damage to DNA, but some damage is retained and can block replication. To abolish the block and continue synthesis of the growing DNA chain, the cell also has some molecular mechanisms that provide for the translesion synthesis of DNA, but these mechanisms are not known in detail. Studies on these mechanisms are very important for biology and medicine, and in recent years there has been significant progress in studies on the replication of damaged DNA and DNA polymerases involved in the translesion synthesis of DNA.

DNA replication is a complex process resulting in effective and very accurate replication of maternal chains of DNA. Replicative DNA polymerases are enzymes fully corresponding to this process, and their active sites are adapted for replicating undamaged DNA. Therefore, many types of DNA damage are insurmountable obstacles for these DNA polymerases, and this can terminate replication and cause cell death. Regulatory systems of the cell resolve this problem depending on the type of damage and on difficulties of bypassing it. Thus, to bypass interchain cross-linking in DNA, the eukaryotic cells use three different approaches: nucleotide excision repair, DNA translesion synthesis, and homological recombination. Disorders in coordination of these approaches caused by mutation in a regulatory gene often results in severe genetic diseases, e.g. Fanconi anemia. This disease is characterized by a disturbance in genetic stability of cells. In addition to anemia, other specific symptoms appear in these patients, and cancer develops at an early age [1].

Interchain cross-links in DNA are a rather complex and rare kind of damage that must be bypassed during replication. They affect the two chains of DNA, prevent their untwisting, and thus inhibit both replication and transcription. Damage affecting one of DNA chains is more frequent. Such damage can often be bypassed only

Abbreviations: APE1, apurinic/apyrimidinic endonuclease 1; cisPt-GG, cisplatinum adduct; *cs*TT, *cis-syn*-thymine–thymine dimer; HIF1, hypoxia-inducible factor 1; HRE, hypoxia response element; PARP1, poly(ADP-ribose) polymerase 1; PCNA, proliferating cell nuclear antigen; PIP1(PIP2), PCNA interaction protein box; PNK, polynucleotide kinase; Polι, Polη, Polζ, Polκ, RevI, Polβ, Polλ, Polα, Polδ, and Polε are DNA polymerases ι, η, ζ, κ, RevI, β, λ, α, δ, and ε, respectively; RFC, replication factor C; RPA, replication protein A; XP-V, *xeroderma pigmentosum* variant V; XRCC1, X-ray repair complementing defective repair in Chinese hamster cells 1.

by the translesion synthesis of DNA, which is catalyzed by one or several specialized DNA polymerases. Due to a special structure of their catalytic sites, these enzymes can synthesize DNA using templates with damaged regions, but they are unable to accurately synthesize on undamaged template and frequently make errors during its replication. Therefore, these enzymes are sometimes termed "error-prone DNA polymerases" [2]. However, some of these DNA polymerases are able to bypass replication-blocking damage and insert nucleotides recovering the information that was present in the template previous to its being damaged. Consequently, the term "error-prone DNA polymerases" is not always just. Obviously, the function of these enzymes is not to create errors but to maintain genome stability. In fact, in the living cell a complex regulatory system is responsible for minimization of the number of errors generated by "error-prone DNA polymerases", and the ability to infallibly bypass DNA damage is used as one of major approaches for supporting genome stability.

An error-free translesion synthesis of DNA is exemplified by the bypass catalyzed by DNA polymerase Polη on *cs*TT replication. This enzyme can bypass this lesion in DNA with the same efficiency and accuracy as on the replication of an undamaged thymine-containing template [3]. Polη bypasses *cs*TT by insertion of nucleotides opposite the damage and then continues the synthesis beyond the inserted nucleotides. However, in many cases, for translesion synthesis of DNA consecutive actions of two or several DNA polymerases are required. One inserts the nucleotide opposite the lesion, whereas the other extends the synthesis beyond the inserted nucleotide. Some DNA polymerases have been shown to insert nucleotides opposite the lesion. Such DNA polymerases are termed inserters, whereas other DNA polymerases that extend synthesis of DNA are termed extenders.

Studies on *in vitro* translesion synthesis of DNA involving different specialized DNA polymerases have shown that the majority of such polymerases can function as inserters, whereas the extender function is mainly performed by DNA polymerase ζ [4]. This enzyme can efficiently elongate a primer that contains a noncomplementary base pair, a noncoding, or a destabilizing substitute on the 3′-terminus. However, elongation by Polζ of a primer with damage in the 3′-terminus caused by the polymerase itself occurs with very low efficiency. Based on this and some other properties of Polζ, it was supposed that it should act as an extender if the translesion synthesis of DNA is catalyzed by two DNA polymerases [4]. Moreover, in experiments on human cells with the expression of Polζ, Polη, and DNA polymerase κ suppressed by RNA interference, Polζ was shown to be an extender in translesion synthesis of DNA with involvement of three or even four DNA polymerases [4].

It should be noted that features of some DNA polymerases studied *in vitro* using homogenous preparations can be significantly different from the features displayed by these enzymes in a living cell. Thus, each DNA polymerase Polη and Polκ can catalyze *in vitro* translesion synthesis in the case of such DNA damage as cisPt-GG. But experiments with the suppressed expression of three DNA polymerases (Polζ, Polη, and Polκ) by means of RNA interference in human cell cultures have revealed that these three DNA polymerases are involved in DNA synthesis through this lesion [4]. Polη and Polκ are likely to act as inserters and be interchangeable, because suppression of expression of each of the two DNA polymerases insignificantly influences the efficiency of the translesion synthesis of DNA. But the simultaneous inhibition of expression of both DNA polymerases immediately results in a fivefold decrease in the efficiency of the translesion synthesis of DNA. It seems that human cells do not have a suitable equivalent of Polζ to bypass cisPt-GG, because inhibition of expression of the gene encoding this DNA polymerase decreases fivefold the efficiency of the translesion synthesis of DNA. It is still unclear why the cell uses three DNA polymerases for bypassing the damage, which can be bypassed *in vitro* by two of them. Perhaps when Polζ is used as an extender, which is more precise on synthesis and more accurate than Polη and Polκ in the elongation of unpaired 3′-termini of DNA, it prevents mutagenic effects of Polη and Polκ.

Studies on interactions of DNA polymerases with each other and with other proteins involved in translesion synthesis of DNA revealed a rather complicated organization of the system regulating activities of individual DNA polymerases. It is still unclear how cells can involve during replication one or several specialized DNA polymerases to most efficiently and accurately bypass damage. Moreover, it is necessary to remove the specialized DNA polymerases from the replication fork after the damage is bypassed and to create conditions for further synthesis of DNA by replicative DNA polymerases. The latter operation is necessary because all DNA polymerases involved in translesion synthesis of DNA have low efficiency and are very prone to errors on replicating undamaged template.

TWO HOMOLOGS (Polη AND Polι) IN MAMMALIAN CELLS

DNA polymerases of the Y family, which includes Polη and Polι, are different in their ability to bypass DNA regions with damaged nucleotides and are highly prone to errors on replicating an undamaged template. X-Ray crystallographic analysis reveals that these enzymes have a more open catalytic site that allows them to bind unpaired or damaged bases and thus to be less demanding to the structure of the DNA template. All DNA polymerases of the Y family contain five *N*-terminal conservative motifs, I-V. Three conservative motifs, I-III, con-

tribute to formation of the active site, whereas two conservative motifs form a characteristic structure consisting of helix-turn-helix, which interacts with DNA. The *C*-terminus of DNA polymerases of the Y family is less conservative, varies in size, and is involved in protein–protein interactions having a regulatory function.

Mammals have four members of the Y family of DNA polymerases: Polη, Polκ, Polι, and Rev1 [5, 6]. By now Polη of this family of DNA polymerases is the best characterized. The gene encoding Polη (*RAD30A*) belongs to the group of *RAD30* genes, which encode a subfamily of DNA polymerases found only in eukaryotes. Two members of this subfamily are described in mammals. The *RAD30A* gene product occurs in both yeast and humans [7, 8] and independently of the origin can efficiently bypass different lesions during synthesis of DNA. It has been already mentioned that Polη can efficiently and unerringly perform *in vitro* synthesis and bypass *cs*TT damage, which is responsible for ~80% of UV-induced mutations [9]. Obviously, Polη plays an important biological role protecting us against the deleterious effect of UV radiation of the Sun. Moreover, this DNA polymerase replicates an undamaged template with many errors, and thus contributes to somatic hypermutagenesis of genes encoding antibodies in mammals. This is confirmed by changes in the mutation spectrum in variable regions of immunoglobulin genes in patients having a mutant gene encoding Polη [10].

Another member of the *RAD30* subfamily is Polι, which is encoded by the gene *RAD30B*. The amino acid sequence of Polη is reminiscent of a similar sequence of Polι, and Polι is supposed to have descended from Polη as a result of gene duplication shortly before the appearance of insects in evolution [11].

The biological functions of Polι are still unclear, and no human diseases are described that are directly associated with the gene encoding this enzyme. There have been attempts to obtain mice with knockout of this gene, but mice of the 129 strain, which are usually used as donors of embryonic stem cells for obtaining gene-knockout animals, were found to have a nonsense-mutation in the second exon of *RAD30B* [12]. In extracts from different organs of the 129 strain mice Polι was not detected by Western blotting. We also failed in detecting Polι activity in extracts from different organs of these mice [13].

Thus, Polι seems to be not necessary for survival of mice, at least under laboratory conditions. It was also shown that in mice of the 129 strain the mutation spectrum of immunoglobulin genes was the same as in mice without mutations in the gene encoding Polι [12]. This means that although Polι is extremely prone to incorrect synthesis of DNA, it is not involved in hypermutagenesis of variable regions of the immunoglobulin genes as discriminated from Polη. But mutation in the Polι-encoding gene was shown to increase in mice the frequency of urethane-induced lung cancer [14]. Therefore, it seems that a useful property of Polι is its direct involvement in processes preventing carcinogen effect in mammals. Moreover, experiments in inhibiting expression of the Polι-encoding gene on sensitivity of human fibroblasts to different agents damaging DNA [15] have revealed that Polι can be involved in the repair of genomic DNA under conditions of oxidative stress. Thus, the data accumulated so far indicate that Polι is not necessary for survival of mammals, but is important at least for their normal existence.

PROPERTIES OF Polι *in vitro*

The gene encoding human Polι was discovered 10 years ago [16]. The protein consists of 715 amino acid residues, and its properties are significantly different from those of other DNA polymerases described to date [17]. Orthologs of Polι are found in some fungi (except *Saccharomyces cerevisiae*), insects, fishes, and amphibian [18]. The enzymes of human, mouse, and drosophila have been studied [12, 17, 19, 20]. The exon–intron structure and biochemical properties of the human and mouse enzymes are very similar, whereas Polι of drosophila is sharply different and has features more like its precursor Polη. It is very likely that the properties of human and mouse Polι should be also specific for this enzyme from the majority of mammals.

The accuracy and efficiency of *in vitro* DNA replication by Polι significantly depend on the nucleotide composition of the template. Thus, opposite to the template adenine (A) the enzyme efficiently and accurately inserts thymine (T), and the error probability is 10^{-4}. On bypassing guanine (G) and cytosine (C) of the template the probability of Polι error is, respectively, 10^{-3} and 10^{-1}. On synthesizing DNA the enzyme inserts G opposite the template thymine instead of the complementary A 3-10 times more efficiently [6]. And Polι is a distributive enzyme that dissociates from the template after every inserted nucleotide. Some cases are described when on replicating T-containing template the enzyme stopped after the first incorrectly inserted G. This property described only for Polι is designated T-stop [17].

Recent studies have revealed another unusual property of Polι: it can catalyze synthesis of DNA in the presence of low concentrations of Mn^{2+}. Thus, Frank and Woodgate have shown that this enzyme prefers as a bivalent cation cofactor just Mn^{2+}, whereas the majority of DNA polymerases use Mg^{2+} [21].

Unusual properties of mammalian Polι are due to the specific structure of its active site. It is known that on incorporation of pyrimidines opposite the template purine the enzyme uses not Watson–Crick, but rather Hoogsteen interactions between nucleotides. X-Ray crystallographic analysis shows that the active site of Polι

contains large aliphatic amino acid residues (Gln59, Leu62), which prevent the template purine from adopting the anti-conformation necessary for the Watson–Crick interaction. This is associated with a purine turn by 180° and its taking the syn-conformation [22]. This is favorable for production of alternative hydrogen bonds between the template nucleotide and the incorporated one: this is the so-called Hoogsteen interaction.

In addition to the DNA polymerase activity, mammalian Polι can remove the deoxyribosophosphate group from the 5′-termini of DNA that is necessary for the correct repair of damaged regions of DNA during base excision repair [23]. However, the majority of the *in vitro* properties of this enzyme demonstrated its ability to participate in translesion synthesis of DNA. Thus, on replication of DNA containing modified nucleotides with a wrong nucleotide incorporated oppositely by other DNA polymerases, Polι can "by mistake" incorporate the due nucleotide and prevent mutations. Thus, experiments with replication of templates containing uracil and some of its derivatives have shown that Polι can incorporate G opposite these lesions and prevent C→T transitions caused by deamination of cytosine [24]. Moreover, the enzyme can synthesize DNA *in vitro* and bypass some lesions capable of arresting activities of replication DNA polymerases, aromatic adducts of deoxyadenosine, cyclobutane pyrimidine dimers, and apurine/apyrimidine sites [25-27]. However, the *in vitro* translesion synthesis of DNA by Polι depends on the presence of univalent cations and on the template context [28]. Results of experiments can also depend on the use of bivalent cations Mg^{2+} and Mn^{2+}. The optimal concentrations of these cations as cofactors of the DNA polymerase reaction catalyzed by Polι were determined only in 2007 [21], so the earlier data need to be revised.

REGULATION OF EXPRESSION OF THE GENE *RAD30B* IN MAMMALIAN CELLS AND PROTEIN–PROTEIN INTERACTIONS OF Polι

Different examples of *in vitro* translesion synthesis of DNA catalyzed by specialized polymerases reveal that in the living cell these enzymes are controlled by regulatory factors. Moreover, in some cases DNA polymerases involved in DNA synthesis also act themselves as regulatory factors, e.g. DNA polymerase Rev1. This protein capable of incorporating nucleotides in the growing DNA chain can physically interact with other DNA polymerases of the Y-family (Polι, Polη, and Polκ) and also with Rev7, which is a non-catalytic subunit of Polζ from the B-family of DNA polymerases [29, 30]. Note that Polκ and Rev7 are bound with the same region located on the *C*-terminus of Rev1. This suggests that Rev1 could play a key role in multienzymatic multistep translesion synthesis of DNA. Such a mechanism is sometimes designated as the Rev1-Polζ pathway of translesion synthesis of DNA [31].

Mechanisms regulating Polι expression and its availability to undamaged DNA in cells are similar to mechanisms regulating activities of other DNA polymerases of the Y-family. The expression of the gene Polι is controlled on both transcriptional and posttranscriptional levels. The level of Polι mRNA transcription sharply increases in human cells under conditions of hypoxia. In this case the transcription is activated as a result of interaction of hypoxia-inducible factor 1 (HIF1) with the consensus sequence located in the first intron of the gene encoding Polι [32]. The posttranscriptional regulation of expression of the Polι-encoding gene is realized at the level of splicing. Processing of mouse Polι mRNA can be associated with alternative splicing accompanied by removal of the second and fourth exons [33].

Polι interacts with some regulatory protein factors and also with other DNA polymerases. The interaction with protein factors involves the Polι *C*-terminus, which is responsible for regulatory functions and is less conservative than the *N*-terminus. Thus, Polι was shown to interact with Polη [34]. Both enzymes are accumulated in sites of replication upon arrest of the replication complex caused by UV-irradiation of cells. The existence of the two DNA polymerases in the nucleus is strictly coordinated: the accumulation kinetics of the two enzymes at the replication sites are similar, and the presence of Polι in the replicative complex depends on Polη. The key role in the interaction with Polη belongs to 224 *C*-terminal amino acid residues of Polι. Moreover, Polι interacts with Rev1 through the region located between amino acid residues 449 and 589 [35].

Because Polι is involved in translesion synthesis of DNA, the physical interaction of the enzyme with the proliferating cell nuclear antigen (PCNA) is important [36]. On the *C*-terminus of the human Polι molecule there are three consensus sequences capable of interacting with PCNA: PIP1 (amino acids from 420 to 427), PIP2 (amino acids from 540 to 542), and KA (amino acids from 412 to 424) [36]. But only the PIP1 sequence is involved in regulation of Polι activity. PCNA together with replication factor C (RFC) and replication protein A (RPA) stimulate DNA synthesis catalyzed by human Polι *in vitro*. Mutations in the PIP1 region suppress stimulation of the Polι activity and limit the accumulation of the enzyme in the region of replication [36].

Polι also interacts with monoubiquitinylated PCNA (UB-PCNA) through two *C*-terminal ubiquitin-binding motifs (UBM1 and UBM2), which can be an important mechanism for inclusion of the enzyme into the replicative fork at a DNA lesion [37].

The base excision repair factor XRCC1 is important for repair of oxidative damage. This factor interacts with many proteins, e.g. with PARP1, Polβ (DNA polymerase

of the X-family), PCNA, APE1, PNK, and ligase III [38]. Petta et al. have shown that Polι can also physically interact with XRCC1 *in vivo* [15]. Because Polι has 5′-deoxyribosophosphate activity [23], it is suggested that in addition to participation in translesion synthesis of DNA this enzyme should also be involved in base excision repair.

Because Polι can interact with different proteins, it seems to form transient complexes with them. Using approaches of gel filtration and centrifugation in glycerol gradient, Sabbioneda et al. recently showed that the molecular weight of Polι in extracts from MRC5 cells is 130 kDa, which is approximately 50 kDa higher than the weight calculated from the amino acid sequence [39]. None of the abovementioned protein factors interacting with Polι has such molecular weight. Most likely, the cells contain another protein with molecular weight of 50 kDa that forms a stable complex with Polι.

Polι AS A SOURCE OF INCREASED MUTAGENESIS AND CARCINOGENESIS IN MAMMALIAN CELLS

In some cases Polι of mammals can act as a protector of the genome participating in translesion synthesis of DNA and, possibly, also in base excision repair. Being an extremely low-fidelity DNA polymerase, this enzyme is likely to be a source of an increased mutagenesis in cells of mammals and thus lead to development of malignancies.

Patients with variant pigment xeroderma (XP-V) are very prone to development of skin cancer induced by insolation. The initial defect of XP-V cells is a mutation in the Polη-encoding gene, which completely prevents functioning of this enzyme. In patients with XP-V the probability of appearance of UV-induced mutations is 25-fold higher than in persons with the normal genotype. This is associated with an unusual spectrum of mutations with the prevalence of G→T transversions [40, 41]. It has been already noted that *in vitro* Polη can efficiently and precisely bypass *cs*TT, which is a type of UV-generated damage in DNA on the translesion synthesis of DNA. The cell repair systems can efficiently correct *cs*TT [9], so the absence of functioning Polη is a threat for the survival of the cell due to arresting the replicative fork before the damage. In this situation another DNA polymerase is supposed to act in the role of Polη. Because the XP-V genotype is characterized by a high probability of mutagenesis and a specific spectrum of mutations, Polι is considered to be the main pretender for the role of alternative DNA polymerase. Studies *in vitro* have shown that this enzyme can incorporate G or T opposite the 3′-nucleotide in *cs*TT [26].

To determine whether Polι is really responsible for the increased mutagenesis in the cells of patients with XP-V, in work [42] an immortalized line of XP-V cells expressing two forms of Polι was used. The probability of appearance of mutations in DNA of the cells of this line was twofold higher than in DNA of the cells of the XP-V line expressing one form of Polι, but the spectrum of mutations did not change. These data confirm the hypothesis that, in the absence of Polη, Polι is responsible for the increased mutagenesis in the cells and for the specific spectrum of mutations. However, later experiments have shown that in the cells of patients with XP-V not only Polι, but also Polκ and Polζ are responsible for the increased mutagenesis and malignization of cells [43]. In other words, in the absence of Polη the *cs*TT is bypassed through the above-considered Rev1-Polζ pathway of translesion synthesis of DNA [31], but Polι and Polκ acting as inserters incorporate wrong nucleotides, whereas Polζ continues the synthesis fixing the mutations.

The examples under consideration unanimously indicate that disorders in the balance of expression of specialized DNA polymerases can induce mutations and malignization of cells. However, it is unclear whether this statement can be extended to different types of malignant tumors and if disorders in the balance of expression of specialized DNA polymerases can always result in cell malignization. In work [44] the levels of expression of specialized DNA polymerases Polβ, Polλ, Polι, and Polκ, and of replicative DNA polymerases Polα, Polδ, and Polε were determined in different types of normal and tumor tissues. The expression level of only one specialized DNA polymerase was increased at least twofold in 45% of tumors under study. In particular, in some cases the expression of Polι was increased [44].

The presented data show that cell malignization is associated, in particular, with an increase in Polι expression. But it is difficult to determine how the increased expression of this enzyme influences the increase in the number of mutations in cells because regulatory factors can prevent the availability of the enzyme to the replication of an undamaged DNA. A detailed study was performed by Yang et al. on cells of human mammary gland cancer [45]. They found increased levels of both mRNA and the Polι protein in different strains of human mammary gland cancer cells. The expression of *RAD30B* varied in different strains, and an increase in the expression level was accompanied by a decrease in the replication accuracy of the reporter plasmid introduced into the cells. Moreover, the expression of *RAD30B* additionally increased upon UV-irradiation of the cells and remained elevated during 30-120 min after the irradiation [45]. This indicated that the change in the expression of *RAD30B* was an early event in the response to UV-caused damage of DNA. For replication of the plasmid vector, the same authors also used nuclear extracts from cells of mammary gland cancer and from mammary gland benign tumor [45]. These experiments clearly indicated that the num-

ber of mutations correlated with cell malignization. Using anti-Polι-specific antibodies decreased the number of mutations, and in the mutation spectrum ~90% were point mutations. The number of C→T transitions was decreased and the number of C→A transversions was increased compared to observations in the absence of the antibodies [45].

Moreover, our studies on DNA synthesis in extracts of cells of some malignant tumors have shown that DNA synthesis can continue beyond the T-stop specific for Polι [13].

The presented data suggest that Polι can increase the total number of mutations and also mutations arising during replication as a result of translesion synthesis of DNA after UV-irradiation.

ROLE OF MANGANESE IONS IN MANIFESTATION OF DNA-POLYMERIZING ACTIVITY OF Polι *in vitro* AND IN MAMMALIAN CELLS

Thus, Polι has been recently shown to act both as a "genome protector" and as an agent affecting its stability. However, many details of Polι functioning in mammals are still unclear. In particular, it is a mystery what bivalent cation, Mg^{2+} or Mn^{2+} is a cofactor of this enzyme in living cells. Of bivalent cations, Mg^{2+} is the most representative in living cell (its intracellular concentration is ~0.2 mM) [46, 47]. Concentration of Mn^{2+} in mammalian cells is much lower (~0.04 mM) [48].

DNA polymerases can catalyze DNA synthesis *in vitro* in the presence of Mn^{2+}. But the synthesis accuracy in this case is significantly lower. In the first studies on Polι, Mg^{2+} was used as a cofactor of the DNA polymerase reaction at the concentration of 5-8 mM. But recently the activity of Polι was studied in detail in the presence of different bivalent cations that can be present in the living cell [21]. The enzyme displayed the best accuracy on synthesis of DNA in the presence of Mn^{2+} as a cofactor; Polι was active over a wide range of Mn^{2+} concentrations with the optimum at ~0.075 mM. In the presence of Mg^{2+} in the reaction mixture the enzyme activity was tens times lower and the optimal concentration of Mg^{2+} for the DNA-polymerizing activity of Polι was ~0.25 mM [21]. This indicates that all data on biochemical properties of Polι described in the literature before 2007 were obtained not under optimal concentrations of Mg^{2+}. Because some of these data do not adequately present true properties of Polι, new experiments are required. Thus, low concentrations of Mg^{2+} and Mn^{2+} very significantly influence the ability of Polι to bypass *cs*TT. The efficiency of bypassing this damage was 2.5% in the presence of 5 mM Mg^{2+} and increased to 8% in the presence of 0.5 mM Mg^{2+}, whereas in the presence of 0.2 mM Mn^{2+} it was 60% [21]. Thus, under certain *in vitro* conditions Polι can very efficiently bypass *cs*TT. Possibly, conditions needed for the efficient synthesis of DNA by Polι bypassing these damages can also exist *in vivo*. This hypothesis is confirmed by some reports about the role of Polι in arising of UV-induced mutations in mice and humans [42, 49, 50]. Based on the available data, Frank and Woodgate supposed that Mn^{2+} should be the major cofactor of Polι in the living cell [21].

We have determined the Polι activity in extracts from different organs of some inbred mouse strains using an approach developed by us. These studies with Mg^{2+} as a cofactor of DNA polymerase reaction revealed Polι activity only in extracts from cells of testes and brain of the mice [51]. On using Mn^{2+} as a cofactor of the DNA polymerase reaction, activity of Polι was observed in extracts from cells of all mouse organs [13]. Moreover, using extracts from mouse testes resulted not only in the product of Polι synthesis, but synthesis of DNA continued beyond the incorrectly incorporated G. These experiments revealed that the synthesis was continued not by Polι but by yet another unidentified DNA polymerase [13].

Based on the above-described data, it seems reasonable to attempt to answer the question of why Polι, possessing great mutagenic potential that is in particular due to its ability to incorporate G opposite the template T, does not normally cause disorders in genome stability. Most likely this may be explained by the property of the enzyme to stop beyond the incorrectly incorporated G. In extracts from cells of normal mouse organs the characteristic T-stop occurred as a rule after G incorrectly incorporated by Polι. Only in extracts from the cells of mouse testes the active enzyme was found which could continue synthesis of DNA, but in the case when the concentration of Mn^{2+} in the reaction mixture was ~0.2 mM, which is much higher than the intracellular concentration. This can be the other cause of the absence of hypermutagenicity of Polι in living cells. It seems that in the presence of particular damages of DNA, some factors are activated that allow DNA polymerase to more efficiently interact as an extender with Polι and perform translesion synthesis of DNA.

During the decade after the discovery of Polι, many studies were performed in order to elucidate the role of this DNA polymerase in mammals. The properties of Polι as a DNA-synthesizing enzyme are significantly different from those of other DNA polymerases described to date. However, regulation of Polι activity in living cells has much in common with the regulation of activities of some specialized DNA polymerases. The expression of structurally homologous *RAD30A* and *RAD30B* is regulated most similarly.

The role of Polι in mammals is not clear in detail, although it is shown that a probable function of this enzyme is its involvement in translesion synthesis of DNA. However, some data suggest the potential involve-

ment of this protein in base excision repair. Thus, the function of Polι as a protector of the genome is clearly determined, but there are data that disorders in regulation of the gene *RAD30B* expression can increase the level of mutagenesis in cells and cause the initiation and development of cancer.

Some functions of Polι, which can be associated with specific features of mice of strain 129 with the mutant gene encoding this enzyme, are not explained. These features include a record resistance to radiation compared to mice of other strains [52], unstable epigenetic status [53], high incidence of teratocarcinomas [54], and unique pluripotency of embryonic stem cells [55]. Perhaps not all these properties are immediately associated with mutation in the Polι-encoding gene, but it is obvious that functions of this enzymes are not limited by its involvement in maintaining genome stability.

This work was supported by the Russian Foundation for Basic Research (project No. 10-04-01434-a).

REFERENCES

1. Moldovan, G.-L., and D'Andrea, A. D. (2009) *Annu. Rev. Genet.*, **43**, 223-249.
2. Rattray, A. J., and Strathern, J. N. (2003) *Annu. Rev. Genet.*, **37**, 31-66.
3. Yoon, J. H., Prakash, S., and Prakash, L. (2009) *Proc. Natl. Acad. Sci. USA*, **106**, 18219-18224.
4. Livneh, Z., Ziv, O., and Shachar, S. (2010) *Cell Cycle*, **9**, 729-735.
5. Goodman, M. F. (2002) *Annu. Rev. Biochem.*, **71**, 17-50.
6. Prakash, S., Johnson, R. E., and Prakash, L. (2005) *Annu. Rev. Biochem.*, **74**, 317-353.
7. Johnson, R. E., Prakash, S., and Prakash, L. (1999) *Science*, **283**, 1001-1004.
8. Masutani, C., Kusumoto, R., Iwai, S., and Hanaoka, F. (2000) *EMBO J.*, **19**, 3100-3109.
9. You, Y.-H., Lee, D.-H., Yoon, J.-H., Nakajima, S., Yasui, A., and Pfeifer, G. P. (2001) *J. Biol. Chem.*, **276**, 44688-44694.
10. Zeng, X., Winter, D. B., Kasmer, C., Kraemer, K. H., Lehmann, A. R., and Gearhart, P. J. (2001) *Nat. Immunol.*, **2**, 537-541.
11. Vaisman, A., Frank, E. G., McDonald, J. P., Tissier, A., and Woodgate, R. (2002) *Mutat. Res.*, **510**, 9-22.
12. 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.
13. Kazakov, A. A., Grishina, E. E., Tarantul, V. Z., and Gening, L. V. (2010) *Biochemistry (Moscow)*, **75**, 905-911.
14. Lee, G.-H., and Matsushita, H. (2005) *Cancer Sci.*, **96**, 256-259.
15. Petta, T. B., Nakajima, S., Zlatanou, A., Despras, E., Couve-Privat, S., Ishchenko, A., Sarasin, A., Yasui, A., and Kannouche, P. (2008) *EMBO J.*, **27**, 2883-2895.
16. McDonald, J. P., Rapic-Otrin, V., Epstain, J. A., Broughton, B. C., Wang, X., Lechmann, A. R., Wolgemuth, D. J., and Woodgate, R. (1999) *Genomics*, **60**, 20-30.
17. Tissier, A., McDonald, J. P., Frank, E. G., and Woodgate, R. (2000) *Genes Dev.*, **14**, 1642-1650.
18. Waters, L. S., Minesinger, B. K., Wiltrout, M. E., D'Souza, S., Woodruff, R. V., and Walker, G. C. (2009) *Microbiol. Mol. Biol. Rev.*, **73**, 134-154.
19. Zhang, Y., Yuan, F., Wu, X., and Wang, Z. (2000) *Mol. Cell Biol.*, **20**, 7099-7108.
20. Ishikawa, T., Uematsu, N., Mizukoshi, T., Iwai, S., Iwasaki, H., Masutani, C., Hanaoka, F., Ueda R., Ohmori, H., and Todo, T. J. (2001) *J. Biol. Chem.*, **276**, 15155-15163.
21. Frank, E. G., and Woodgate, R. (2007) *J. Biol. Chem.*, **282**, 24689-24696.
22. Nair, D. T., Johnson, R. E., Prakash, S., Prakash, L., and Aggarwal, A. K. (2004) *Nature*, **430**, 377-380.
23. Bebenek, K., Tissier, A., Frank, E. G., McDonald, J. P., Prasad, R., Wilson, S. H., Woodgate, R., and Kunkel, T. A. (2001) *Science*, **291**, 2156-2159.
24. Vaisman, A., and Woodgate, R. (2001) *EMBO J.*, **20**, 6520-6529.
25. Frank, E. G., Sayer, J. M., Kroth, H., Ohashi, E., Ohmori, H., Jerina, D. M., and Woodgate, R. (2002) *Nucleic Acids Res.*, **30**, 5284-5292.
26. Vaisman, A., Takasawa, K., Iwai, S., and Woodgate, R. (2006) *DNA Repair*, **5**, 210-218.
27. Zhang, Y., Yuan, F., Wu, X., Taylor, J. S., and Wang, Z. (2001) *Nucleic Acids Res.*, **29**, 928-935.
28. Vaisman, A., Frank, E. G., Iwai, S., Ohashi, E., Ohmori, H., Hanaoka, F., and Woodgate, R. (2003) *DNA Repair*, **2**, 991-1006.
29. Ohashi, E., Muracumo, Y., Kanjo, N., Akagi, J.-I., Masutani, C., Hanaoka, F., and Ohmori, H. (2004) *Genes Cells*, **9**, 523-531.
30. Hara, K., Hashimoto, H., Murakumo, Y., Kobayashi, S., Kogame, T., Unzai, S., Akashi, S., Takeda, S., Shimizu, T., and Sato, M. (2010) *J. Biol. Chem.*, **285**, 12299-12307.
31. Zhou, Y., Wang, J., Zhang, Y., and Wang, Z. (2010) *Nucleic Acids Res.*, **38**, 5036-5046.
32. Ito, A., Koshikawa, N., Mochizuki, S., Omura, K., and Takenaga, K. (2006) *Biochem. Biophys. Res. Commun.*, **351**, 306-311.
33. Lee, G. H., Nishimori, H., Sasaki, Y., Matsushita, H., Kitagawa, T., and Tokino, T. (2003) *Oncogene*, **22**, 2374-2382.
34. Kannouche, P., Fernandez de Henestrosa, A. R., Coull, B., Vidal, A., Gray, C., Zicha, D., and Woodgate, R. (2002) *EMBO J.*, **21**, 6246-6256.
35. Ohashi, E., Muracumo, Y., Kanjo, N., Akagi, J.-I., Masutani, C., Hanaoka, F., and Ohmori, H. (2004) *Genes Cells*, **9**, 523-531.
36. Haracska, L., Acharya, N., Unk, I., Johnson, R. E., Hurwitz, J., Prakash, L., and Prakash, S. (2005) *Mol. Cell Biol.*, **25**, 1183-1190.
37. Plosky, B. S., Vidal, A., Fernan dez de Henestrosa, A. R., McLenigan, McDonald, J. P., Mead, S., and Woodgate, R. (2006) *EMBO J.*, **25**, 2847-2855.
38. Caldecott, K. W. (2003) *Biochem. Soc. Trans.*, **31**, 247-251.
39. Sabbioneda, S., Gourdin, A. M., Green, C. M., Zotter, A., Giglia-Mari, G., Houtsmuller, A., Vemeulen, W., and Lehmann, A. R. (2008) *Mol. Biol. Cell*, **19**, 5193-5202.

40. Wang, Y. C., Maher, V. M., Mitchell, D. L., and McCormick, J. J. (1993) *Mol. Cell. Biol.*, **13**, 4276-4283.
41. Wang, Y. C., Maher, V. M., and McCormick, J. J. (1991) *Proc. Natl. Acad. Sci. USA*, **88**, 7810-7814.
42. Wang, Y., Woodgate, R., McManus, T. P., Mead, S., McCormick, J. J., and Maher, V. M. (2007) *Cancer Res.*, **67**, 3018-3026.
43. Ziv, O., Geacintov, N., Nakajima, S., Yasui, A., and Livneh, Z. (2009) *Proc. Natl. Acad. Sci. USA*, **106**, 11552-11557.
44. Albertella, M. R., Lau, A., and O'Connor, M. J. (2005) *DNA Repair*, **4**, 583-593.
45. Yang, J., Chen, Z., Liu, Y., Hickey, R. J., and Malkas, L. (2004) *Cancer Res.*, **64**, 5597-5607.
46. Gee, J. B., II, Corbett, R. J., Perlman, J. M., and Laptook, A. R. (2001) *Pediatr. Neurol.*, **25**, 304-308.
47. Wang, S., McDonnell, E. H., Sedor, F. A., and Toffaletti, J. G. (2002) *Arch. Pathol. Lab. Med.*, **126**, 947-955.
48. Ash, D., and Schramm, V. L. (1982) *J. Biol. Chem.*, **257**, 9261-9264.
49. Dumstorf, C. A., Clark, A. B., Lin, Q., Kissling, G. E., Yuan, T., Kucherlapati, R., McGregor, W. G., and Kunkel, T. A. (2006) *Proc. Natl. Acad. Sci. USA*, **103**, 18083-18088.
50. Ohkumo, T., Kondo, Y., Yokoi, M., Tsukamoto, T., Yamada, A., Sugimoto, T., Kanao, R., Higashi, Y., Kondoh, H., Tatematsu, M., Masutani, C., and Hanaoka, F. (2006) *Mol. Cell Biol.*, **26**, 7696-7706.
51. Gening, L. V., Makarova, A. V., Malashenko, A. M., and Tarantul, V. Z. (2006) *Biochemistry (Moscow)*, **71**, 155-159.
52. Roderick, T. H. (1963) *Radiat. Res.*, **20**, 631-639.
53. Simpson, E. M., Linder, C. C., Sargent, E. E., Davisson, M. T., Mobraaten, L. E., and Sharp, J. J. (1997) *Nat. Genet.*, **16**, 19-27.
54. Inoue, K., Ogonuki, N., Mochida, K., Yamamoto, Y., Takano, K., Kohda, T., Ishino, F., and Ogura, A. (2003) *Biol. Reprod.*, **69**, 1394-1400.
55. Kawase, E., Suemori, H., Takahashi, N., Okazaki, K., Hashimoto, K., and Nakatsuji, N. (1994) *Int. J. Dev. Biol.*, **38**, 385-390.