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REVIEW

Structure of Human DNA Polymerase Iota and the Mechanism of DNA Synthesis

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Abstract—Cellular DNA polymerases belong to several families and carry out different functions. Highly accurate replicative DNA polymerases play the major role in cell genome replication. A number of new specialized DNA polymerases were discovered at the turn of XX-XXI centuries and have been intensively studied during the last decade. Due to the special structure of the active site, these enzymes efficiently perform synthesis on damaged DNA but are characterized by low fidelity. Human DNA polymerase iota (Pol ι) belongs to the Y-family of specialized DNA polymerases and is one of the most error-prone enzymes involved in DNA synthesis. In contrast to other DNA polymerases, Pol ι is able to use noncanonical Hoogsteen interactions for nucleotide base pairing. This allows it to incorporate nucleotides opposite various lesions in the DNA template that impair Watson–Crick interactions. Based on the data of X-ray structural analysis of Pol ι in complexes with various DNA templates and dNTP substrates, we consider the structural peculiarities of the Pol ι active site and discuss possible mechanisms that ensure the unique behavior of the enzyme on damaged and undamaged DNA.

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Synthesis of complementary DNA copies during the process of replication in all living cells is performed by enzymes called DNA polymerases (DNAPs). Based on the analysis of sequences and structural features of DNAPs from different organisms, six different families of DNAPs have been identified: A, B, C, D, X, and Y. Eukaryotic DNAPs belong to four families: A, B, X, and Y (DNAPs from C and D families are present only in bacteria and archaea, respectively) and are characterized by high diversity. They vary in their biochemical properties, accuracy of DNA synthesis, and functions. In humans, there are at least 14 different DNAPs: α, β, γ, δ, ε, ζ, η, θ, ι, κ, λ, μ, ν, and Rev1 [1-5].

High accuracy of genetic information replication is crucial for normal functioning of cells and the whole organism. Replicative eukaryotic DNAPs of the B-family (Pol ε and Pol δ) carry out DNA synthesis with very high fidelity. The error rate of Pol ε and Pol δ replication is 10^{-6}-10^{-7} [6, 7]. The accuracy of replication is ensured by high selectivity of the DNAP active site during dNTP incorporation and the 3′-5′-correction exonuclease activity, which is localized in a distinct active site of the enzyme. In each catalytic cycle of replicative DNAPs, binding of incoming dNTP is accompanied by conformational rearrangements of the enzyme that "close" dNTP within the active site. The efficient catalysis is only possible in the case of Watson–Crick interactions and the correct geometry of the nucleotide pair in the active site. The incorporation of an incorrect nucleotide at the 3′-terminus of DNA primer results in a dramatic decrease in the efficiency of DNA synthesis and stimulates exonuclease activity of replicative DNAPs [6-9].

DNA of living organisms is constantly exposed to damage under the influence of various endogenous and exogenous factors of chemical and physical nature. For example, more than 10,000 apurinic/apyrimidinic sites (AP sites) are formed each day in each mammalian cell, while the total number of AP sites simultaneously present in the genome may reach 50,000-200,000 [10-12]. Not all DNA lesions can be quickly removed from the genome by repair enzymes. DNA damage makes a significant impact on the

Abbreviations: AAF, 2-acetylaminofluorene; AP site, apurinic/apyrimidinic site; BPDE, benzo(α)pyren-diol-epoxide; ε-dA, 1,N^6-ethenodeoxyadenosine; dBrU, 5-bromodeoxyuridine; DNAP, DNA polymerase; HG, Hoogsteen interactions; O^6-Me-dG, O^6-methyldeoxyguanosine; N^2-Et-dG, N^2-ethyldeoxyguanosine; 8-oxo-dG, 7,8-dihydro-8-oxodeoxyguanosine; WC, Watson–Crick interactions.

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functioning of accurate replicative DNAPs. Usually, the rigid closed active site does not allow these DNAPs to carry out efficient and accurate synthesis on damaged DNA. This causes a replication stop and can lead to cell death [13, 14].

An important mechanism for overcoming replication blocks and reducing the negative impact of DNA lesions to the organism is a recruitment of specialized DNAPs, participating in replication and repair of damaged DNA, to the replicative fork. Specialized DNAPs usually have a catalytic center that is more tolerant to the template structure and is not always guided by Watson–Crick interactions when pairing nucleotides [13, 15-17].

Most of the specialized DNAPs have been isolated and characterized relatively recently. The history of the discovery of these DNAPs begins in the 1970s, when genetic studies demonstrated that DNA damage-induced mutagenesis in a cell is not a passive process but requires the participation of specialized proteins. Thus, mutations in genes *umuC* (UV-induced mutability) in *Escherichia coli* and *REV1* (UV reversion) in *Saccharomyces cerevisiae* dramatically reduced the level of mutagenesis in cells treated with DNA-damaging agents [18-20]. Later, homology with UmuC and Rev1 was found in proteins of many organisms from various taxonomic groups: DinB (*E. coli*) [21], Dbh (Archaea) [22], RAD30 (*S. cerevisiae*) [23], leading to the conclusion that a whole family of proteins involved in mutagenesis existed in many organisms. In 1999, it was demonstrated that DinB, UmuC, and RAD30 are DNA polymerases: Pol IV and Pol V in *E. coli* and Pol η in *S. cerevisiae*, respectively [24-26]. Finally, the Human Genome Project revealed homologs of new DNAPs in humans. Most of them have been isolated and studied by biochemical and structural methods [15-17].

The majority of specialized DNAPs belong to families X and Y, and Y-DNAPs have the most unusual properties. There are five subfamilies of the Y-family of DNAPs: Pol V, Pol IV/Pol κ, Rev1, Pol η, and Pol ι-like DNAPs [27]. Two Y-polymerases were found in bacteria: Pol IV (DinB) and Pol V (UmuCD). The cells of *S. cerevisiae* also contain two Y-polymerases: Pol η and Rev1. In humans, four Y-family DNAPs, Pol κ, Rev1, Pol η, and Pol ι, have been identified.

DNAPs from the Y-family play an important role in the maintenance of genome stability. It is assumed that various Y-family DNAPs specialize in the synthesis of DNA opposite different types of lesions (DNA translesion synthesis, TLS) and use different strategies for incorporation of dNTPs opposite the damaged bases [15, 16]. For example, Pol η efficiently and accurately conducts DNA synthesis opposite thymine–thymine cyclobutane dimers (as well as opposite numerous other lesions), protecting the organism from ultraviolet (UV) radiation [24, 28, 29]. Mutations in the human *Pol η* gene lead to the development of a specific form of xeroderma pigmentosum syndrome (XP-V phenotype) that is characterized by cell sensitivity to UV, increased rate of mutagenesis, and high risk of skin cancer [30, 31]. Pol κ is able to incorporate nucleotides opposite dG residues (guanine deoxyribonucleotides) containing various chemical groups at N^2 position of the guanine (e.g. 2-acetylaminofluorene, AAF; benzo(α)pyren-diol-epoxide, BPDE; interstrand cross-links) (see, e.g. [32-34]), as well as to extend the unpaired 3′-ends of DNA primers (e.g. [35, 36]). Rev1 protein incorporates dCTP opposite various damaged dG residues and AP sites (see review [16]).

As a result of active site tolerance in interactions with the template and nucleotides, specialized DNAPs are characterized by lower accuracy of DNA synthesis compared with replicative DNAPs. The nucleotide pairing in the active site of Y-family DNAPs depends on the formation of hydrogen bonds between nitrogen bases rather than on their size and geometry [13]. The Y-DNAPs also lack a 3′-5′-exonuclease domain, which results in an increase of the DNA synthesis error rate to 10^{-1}-10^{-4} [4, 5, 16]. Therefore, uncontrolled activity of these DNAPs can lead to a variety of replication malfunctions, reduction of cell viability, and cancer. For example, high mutagenesis rate in human cells with XP-V phenotype, caused by Pol η mutations, results from activity of other DNAPs, including Pol ζ, Pol κ, and Pol ι, which incorporate incorrect nucleotides opposite thymine dimers [31, 37, 38].

High mutagenic potential of specialized DNAPs is controlled in the cell by a variety of regulatory mechanisms that coordinate their expression, activity, and recruitment to damaged DNA. There is a multi-level system of interactions between various DNAPs and protein factors that ensure switching from replicative DNAPs to specialized DNAPs during replication of damaged DNA. In particular, processivity factor PCNA and accessory proteins RFC and RPA play important roles in the switching of DNAPs [39-41].

Y-family DNAP Pol ι was discovered in 1999. Human Pol ι is encoded by the *RAD30B* gene (or *POLI*). Pol ι orthologs were found in different taxonomic groups of eukaryotes: mammals, amphibians, fish, insects, and some fungi [27, 42]. It was suggested that Pol ι arose from Pol η by a gene duplication [27, 43]. However, human Pol ι demonstrates biochemical properties very different from those of Pol η or other Y-family DNAPs. Pol ι has very low accuracy of DNA synthesis and, in contrast to most other known DNAPs, is able to use noncanonical interactions in nucleotide base-pairing. Analysis of biochemical data and the structure of human Pol ι suggests the mechanisms of DNA synthesis by Pol ι on damaged and undamaged templates. In this review, we briefly discuss the main structural features and possible functional mechanisms of the active center of Pol ι.

BIOCHEMICAL PROPERTIES OF Pol ι

Biochemical properties of human Pol ι have been examined in many studies using purified recombinant

preparations of the enzyme and DNA templates of different structure. The efficiency and accuracy of Pol ι on undamaged templates strongly depend on the DNA sequence context[1]. The incorporation of dNTP opposite purine nucleotides (dA and dG) in the DNA template occurs with higher efficiency than opposite pyrimidines (dC and dT). The enzyme inserts nucleotides most accurately opposite dA residues, with error rate of only 10^{-4}-10^{-5}. The error frequency opposite dG and dC is about 10^{-1}-10^{-3}. In the case of dT residues in the template, Pol ι demonstrates surprising behavior and inserts dGTP several times more effectively than complementary dATP. Thus, the accuracy of the enzyme during replication of undamaged DNA may vary up to 10^5 times depending on the sequence-context of the template [44-46].

Pol ι with varying efficiency and fidelity performs *in vitro* synthesis opposite a number of DNA lesions blocking replicative DNAP, including AP sites, products of oxidation and alkylation of nitrogen DNA bases, N^2-guanine adducts, N^6-adenine adducts (including such large adducts as AAF and BPDE), pyrimidine dimers (cyclobutane, (6-4) T-T, and T-U photoproducts), etc. [45, 47-56]. Similarly to other Y-DNAPs, Pol ι is characterized by a certain spectrum of DNA lesions that are replicated with the greatest efficiency, suggesting the unique functions of Pol ι in the bypass of these lesions *in vivo* (see below).

DNA synthesis carried out by Pol ι is distributive and is usually limited to the incorporation of just a few nucleotides in experiments *in vitro* (e.g. see [44, 45]). PCNA (together with accessory proteins) increases the efficiency of DNA synthesis by reducing K_M values of incoming dNTP, but it does not affect the processivity of Pol ι [57, 58]. The presence of incorrect (non-complementary) nucleotides at the 3′-end of the DNA primer significantly reduces the efficiency of DNA synthesis by Pol ι, by 2-4 orders of magnitude [59]. Different studies have also shown that DNA synthesis by Pol ι stops before dT residues in the DNA template; this phenomenon was named "T-stop" [45]. A likely explanation for such a stop is that opposite dT residues Pol ι preferentially incorporates incorrect dGTP nucleotides, further elongation of which is inefficient. Pol ι also extends with a very low efficiency the 3′-primer ends located opposite the damaged bases in DNA. Therefore, further DNA synthesis after the incorporation of the nucleotide opposite the lesion by Pol ι must be performed by another DNAP, for example, by Pol κ or Pol ζ [47, 50, 52, 60].

Recently it was suggested that Mn^{2+}, but not Mg^{2+} ions may serve as cofactors of DNA polymerization by Pol ι *in vivo*. Although most *in vitro* experiments with Pol ι were performed in the presence of relatively high concentrations of Mg^{2+} (5 mM), it was found that the maximum activity of Pol ι is achieved at low concentrations of Mg^{2+} (0.1-0.5 mM) [46, 61]. The use of low concentrations of Mn^{2+} (0.05-0.25 mM) in reaction further increases the activity of Pol ι by more than one order of magnitude. Interestingly, this is accompanied by a significant decrease in the difference in the efficiency and accuracy of nucleotide incorporation opposite purine (dA) and pyrimidine (dT) bases [46]. The efficiency of Pol ι synthesis on DNA templates with lesions also increases in the presence of Mn^{2+} [46, 61]. It should be noted that the concentration of Mn^{2+} in the cytoplasm of mammalian cells *in vivo* is probably much less than in experiments *in vitro* (see references in [46]). It is possible that the cellular concentration of Mn^{2+} can dramatically increase under certain conditions (see, e.g. [62]), but the role of manganese ions in the regulation of Pol ι *in vivo* remains questionable.

Pol ι also has a 5′-deoxyribose phosphate-lyase (dRP-lyase) activity [63, 64]. It should be noted that no dRP-lyase activity of Pol ι was detected in one of the published studies [65]. The removal of the dRP-residue from the 5′-terminus of a DNA gap is an important step in DNA base excision repair (BER). Therefore, it was suggested that Pol ι may participate in BER (in particular, under oxidative stress) and remove the dRP-group, followed by the filling of the single-strand gap in double-stranded DNA [63, 66]. Indeed, Pol ι is active on double-stranded DNA substrates containing short (1 to 3 nt in length) single-stranded regions [63]. The structural elements and amino acid residues involved in the cleavage reaction of the 5′-dRP-group are not yet identified. Presumably, the center of the dRP-lyase activity of Pol ι may be located in the catalytic "core" of the DNAP, in a "helix–hairpin–helix" motif of the thumb domain (see below), which is weakly homologous to the dRP-lyase center of human Pol β [63, 64].

STRUCTURE OF HUMAN Pol ι AND THE GENERAL MECHANISM OF CATALYSIS

Significant progress in the understanding of Pol ι functioning was reached after solving the 3D structures of the catalytic core of the enzyme in complex with different DNA templates, including those containing various lesions, and incoming dNTP substrates (table). The main structural elements and amino acid residues (a.a.) that are presumably involved in catalysis of DNA synthesis by Pol ι and provide unique biochemical properties of the enzyme were identified.

Pol ι is a single-subunit enzyme with molecular weight of ~80 kDa (715 a.a.) and a quite simple structure.

[1] DNA synthesis efficiency is characterized by k_{cat}/K_M (or V_{max}/K_M) ratio for the reaction of nucleotide incorporation to the 3′-end of DNA primer, and accuracy by the ratio of incorporation efficiencies of correct and incorrect dNTPs.

The solved structures of Pol ι with different DNA substrates and incoming nucleotides

PDB, resolution	Nucleotide in the active center and its interactions				Me^{2+}	Conformation of the 5′-terminus of template DNA		Reference
	DNA	dNTP	WC/HG*	C1′–C1′ (Å)		+1 nt turn**	Y61***	
1T3N 2.3 Å	dA *syn*	dTTP *anti*	HG	8.9	1 Mg^{2+}	–	–	[69]
2ALZ 2.5 Å	dG *syn*	dCTP *anti*	HG	8.55	2 Mg^{2+}	no	o	[70]
2FLL 2.6 Å	dA *syn*	dTTP *anti*	HG	8.33	2 Mg^{2+}	yes	–	[71]
2FLN 2.5 Å	dA *anti*	no	–	–	no	yes	c	
2FLP 2.4 Å	dG *anti*	no	–	–	no	yes	–	
3GV5 2.0 Å	dT *anti*	ddADP *syn*	HG	8.32	1–2Ca^{2+}	yes	c	[73]
3GV7 2.2 Å	dT *anti*	dTTP	wobble	8.51	2 Mg^{2+}	yes	c	
3GV8 2.0 Å	dT *anti*	dGTP *anti*	wobble	8.96	2 Mg^{2+}	yes	c	
3H40 2.3 Å	dBrU *anti/syn* (0.5/0.5)	no	–	–	no	yes	c	[77]
3H4B 2.85 Å	dBrU *anti/syn* (0.9/0.1)	dATP *trans*	*trans*-WC	11.2	1 Mg^{2+}	yes	c	
3H4D 2.2 Å	dBrU *anti/syn* (0.7/0.3)	dGTP *anti*	WC	9.6	2 Mg^{2+}	yes	c	
3G6V 2.2 Å	AP site	dATP *anti*	–	8.57	2 Mg^{2+}	yes	c	[78]
3G6X 2.08 Å	AP site	dGTP *anti*	–	9.01	2 Mg^{2+}	–	c	
3G6Y 2.1 Å	AP site	dTTP *anti*	–	9.17	2 Mg^{2+}	–	c	
2DPI 2.3 Å	ε-dA *syn*	dCTP *anti*	HG	8.44	2 Mg^{2+}	no	o	[89]
2DPJ 2.3 Å	ε-dA *syn*	dTTP *anti*	HG	8.56	2 Mg^{2+}	no	o	
3EPG 2.5 Å	N^2-Et-dG *syn*	dCTP *anti*	HG	8.65	no	–	–	[61]
3EPI 2.9 Å	N^2-Et-dG *anti*	(dTTP)	–	–	no	–	c	
3NGD 2.8 Å	O^6-Me-dG *syn*	dCTP *anti*	HG	7.94	no	–	c	[88]
3OSN 1.9 Å	O^6-Me-dG *syn*	dTTP *anti*	HG	8.21	2 Mg^{2+}	–	o	
3Q8P 1.95 Å	8-oxo-dG *syn/anti* (0.8/0.2)	dCTP *anti*	HG	8.19	1 Mg^{2+}	–	c	[81]
3Q8Q 2.03 Å	8-oxo-dG *syn/anti* (0.8/0.2)	dATP *syn*	HG	7.39	1 Mg^{2+}	–	c	
3Q8R 2.45 Å	8-oxo-dG *syn/anti* (0.8/0.2)	dGTP *syn*	HG	8.88	1 Mg^{2+}	–	–	
3Q8S 2.09 Å	8-oxo-dG *syn/anti* (0.8/0.2)	dTTP *anti*	HG	8.41	1 Mg^{2+}	–	c	

* Interactions between nucleotides in the active center: WC, Watson–Crick; HG, Hoogsteen.

** The turn of the +1 nucleotide in the template DNA strand: “yes”, the turn of the DNA 5′-terminus towards the thumb domain is visible in the structure; “no”, the 5′-terminal nucleotide of DNA is directed towards the fingers domain; “–”, the +1 nucleotide position is not resolved.

*** Position of Y61: “o”, open, Y61 is rotated away from the active center; “c”, closed, Y61 is rotated towards the active center; “–”, position of the side chain is not resolved in the structure.

The catalytic core of Pol ι is located in the N-terminal part of the molecule (1-420 a.a.). The C-terminus of the enzyme is involved in protein–protein interactions and performs a regulatory function. In particular, the C-terminal part contains motifs responsible for interactions with PCNA and ubiquitin (which provides the interactions of Pol ι with ubiquitinated PCNA) [58, 66-68]. The spatial structure of the C-terminus of Pol ι remains unknown.

The general architecture of the catalytic core of Pol ι is similar to that of other DNAPs, including replicative ones, and resembles the human right hand (Fig. 1a; see color insert) [69-71]. The overall architecture of secondary structure elements and functional specialization of Pol ι domains show high similarity to Y-family DNAPs, primarily to Pol η (Fig. 1b; see color insert) [28, 29]. The catalytic core of Pol ι and of Y-family DNAPs consists of the palm domain (25-37 and 99-224 a.a.), the fingers domain (38-98 a.a.), the thumb domain (225-288 a.a.), and an additional, unique to this family of polymerases, the little finger domain (298-414 a.a.). The little finger is also known as the polymerase-associated domain (PAD) (Fig. 1).

The palm domain forms a "bottom" of the active site, and the fingers, thumb, and little finger domains form its "walls". Conservative residues of the palm and the fingers domains are involved in the binding of the dNTP-substrate and in the phosphodiester bond formation. The thumb and the little finger domains of Pol ι embrace DNA, the thumb makes a few contacts with the sugar-phosphate backbone of DNA in the minor groove, while the little finger forms multiple contacts with the major groove (Fig. 1). In addition, the little finger domain is involved in the positioning of the template nucleotide in the active site together with the fingers domain (Fig. 1) [69-71].

The little finger domain plays an important role in the interactions with DNA in all DNAPs of the Y-family. The appearance of this domain increases the total area of contacts of DNAP with the DNA template from approximately 600-700 Å^2 (in enzymes from A- and B-families) to 1000-1100 Å^2 in Y-polymerases [72]. Y-DNAPs differ in the structure of the little finger within the family, which presumably determines their substrate specificity to different types of lesions [15]. In comparison with other DNAPs, the little finger domain of Pol ι has greater mobility, which may be one of the reasons for the low processivity of DNA synthesis by this enzyme [70]. Increased mobility is caused by a smaller number of intramolecular contacts of the little finger with other domains, in particular with the finger domain (Fig. 1) [73].

The mechanism of dNTP incorporation by Pol ι is similar to other polymerases and involves nucleophilic substitution of the S_N2 type, which is catalyzed by two bivalent metal ions (A^{2+} and B^{2+}) [70]. Phosphodiester bond formation occurs as a result of nucleophilic attack of the 3′-terminal hydroxyl of the primer at the phosphorus atom of the α-phosphate group of dNTP. During the reaction the A^{2+} ion interacts with the attacking hydroxyl group of the primer and the α-phosphate group of nucleotide, and the B^{2+} ion coordinates the leaving pyrophosphate group. The binding of the metal ions involves conservative amino acid residues Asp and Glu of the palm domain from two consensus sequences of Y-DNAPs: (I/V)**D**(M/F/L) and (A/L)SI**DE**(V/A)(F/Y). Metal ions in human Pol ι are coordinated by D34, D126, and E127 residues located between the dNTP triphosphate and the 3′-OH group of the primer (they correspond to D13, D115 and E116 residues in human Pol η) (Fig. 1) [69, 70]. Substitutions of these residues completely inactivate Pol ι [44, 74]. The B^{2+} ion in Pol ι is coordinated by D34 and D126 residues, and their positions coincide in most published structures of Pol ι. At the same time, the A^{2+} ion, which should be coordinated by D34, D126, and E127 residues [70], is absent in some of the published structures (table; see also Fig. 3 below) or has a different position (e.g. in [73]), indicating its increased mobility. Thus, at least some published structures may correspond to an inactive or pre-catalytic state in which the reactive substrates occupy non-optimal positions. It should also be noted that all published three-dimensional structures of Pol ι were obtained in the complex with Mg^{2+} or Ca^{2+} ions but not Mn^{2+} ions.

THE STRUCTURE OF THE ACTIVE CENTER OF HUMAN Pol ι AND THE MECHANISM OF dNTP INCORPORATION ON UNDAMAGED DNA TEMPLATES

To date, the methods of X-ray analysis allowed to solve the structures of complexes of Pol ι with DNA substrates with template nucleotides dA, dG, and dT (and its analog 5-bromo-deoxyuridine, dBrU) in the absence or presence of various incoming nucleotides in the active site (table). In contrast to replicative DNAPs, the binding of dNTP in the active site of Pol ι and other Y-polymerases is not accompanied by conformational changes and closing of the active site (e.g. [71, 75]). This makes possible the formation of various base pairs, including those containing non-complementary nucleotides (Figs. 2 and 3) and damaged nitrogen bases (see below).

As mentioned above, the most part of the catalytic center is formed by the fingers and the palm domains of Pol ι that interact with the template nucleotide and the incoming dNTP-substrate. The deoxyribose of the incoming nucleotide interacts with the Y39 residue of the palm domain of Pol ι (corresponding to residue F18 in Pol η) (Figs. 4a and 4b; see color insert). This residue likely participates in the discrimination of ribo- and deoxyribonucleotides in the active site of Pol ι. Hydrogen bonds with the phosphate groups of the incoming dNTP

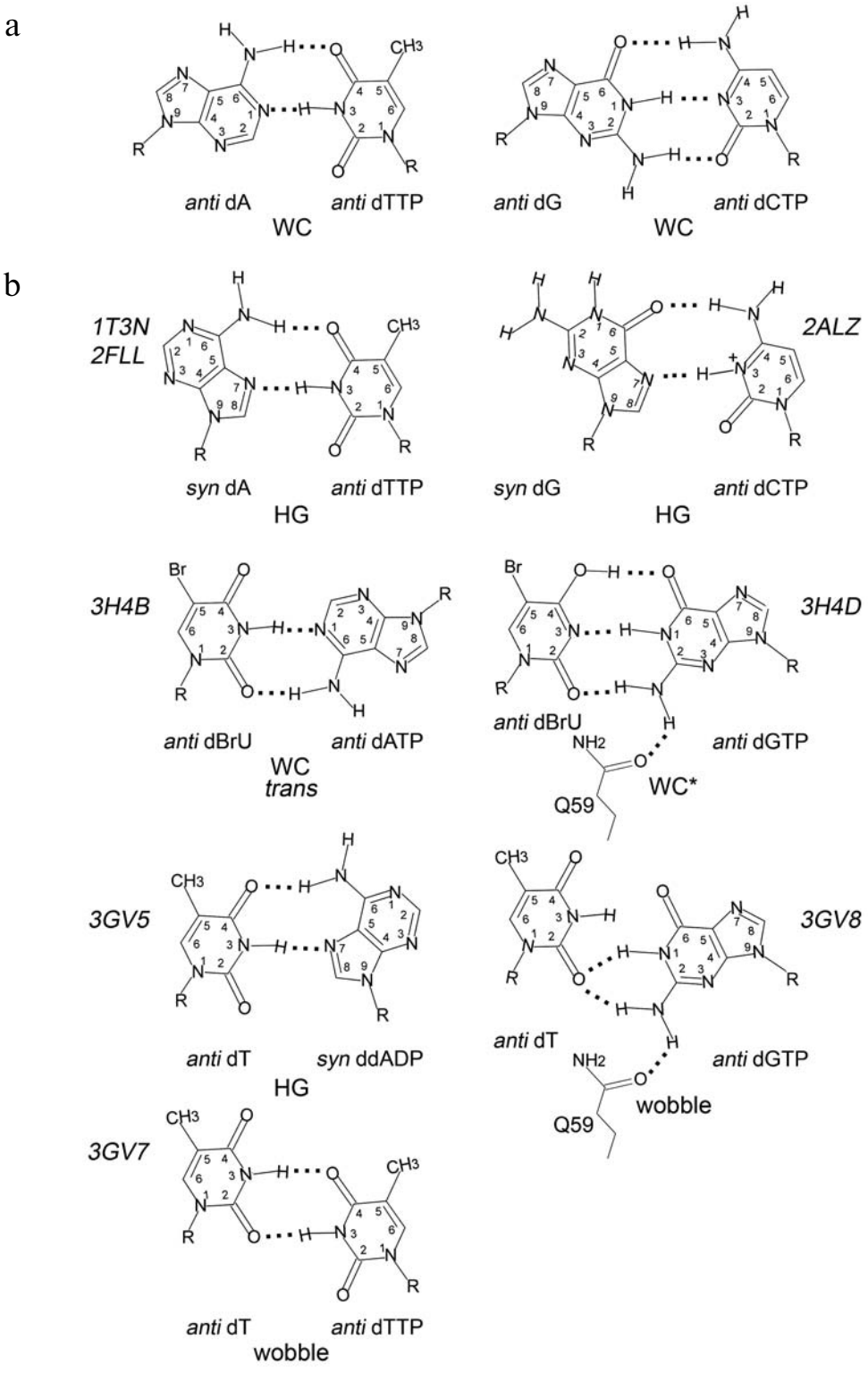

Fig. 2. Different variants of interactions between nucleotides in the active site of Pol ι. a) The scheme of Watson–Crick interactions formation between nucleotides; b) examples of different base pairs identified in the Pol ι three-dimensional structures. The database accession number is shown near each pair, and the conformation of each nucleotide and the type of interactions are indicated: WC, Watson–Crick; HG, Hoogsteen; WC *trans*, Watson–Crick interactions between dT and dATP in the *trans*-conformation; WC*, non-canonical Watson–Crick interaction between the dBrU residue (shown in the enol form) and dGTP.

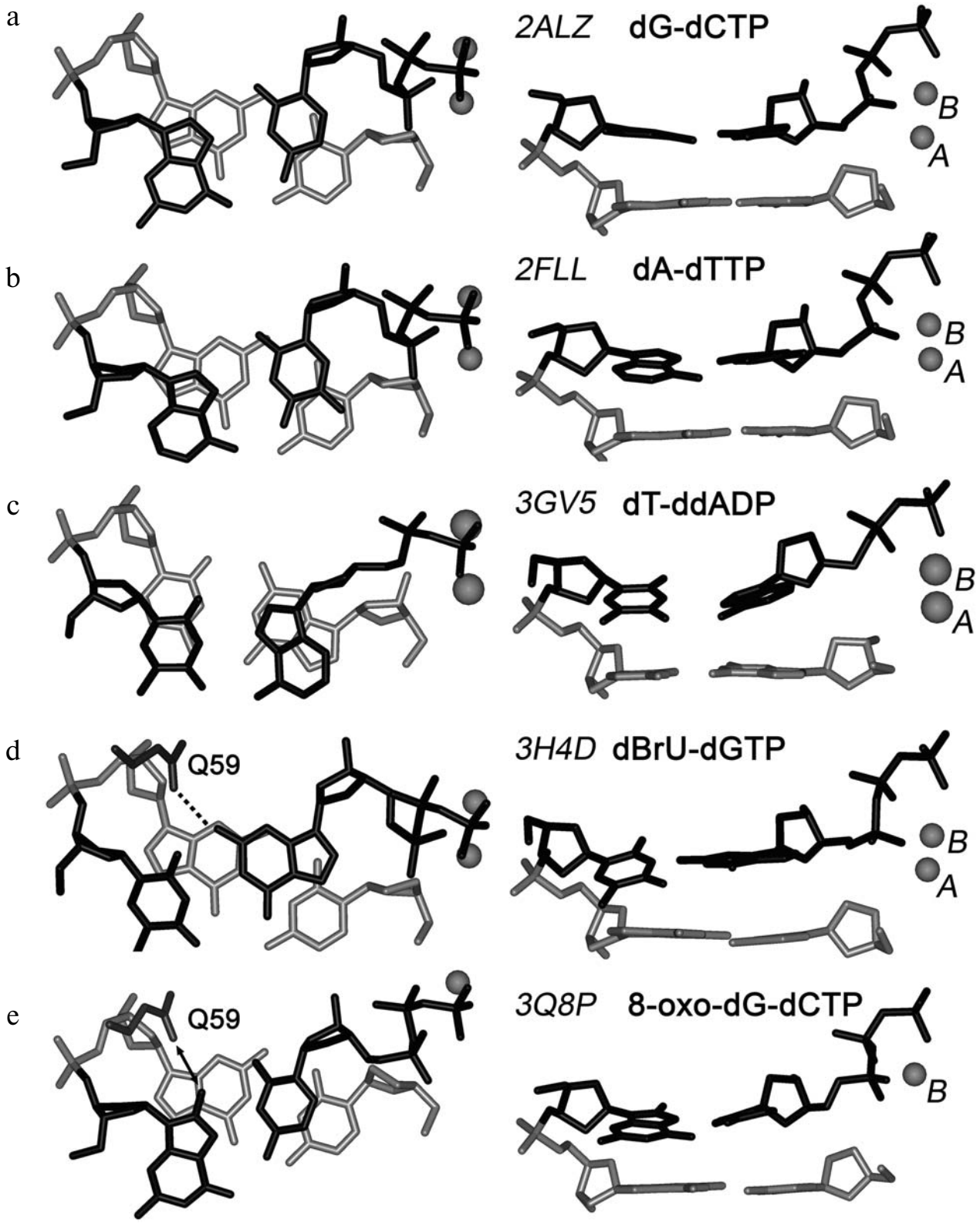

Fig. 3. Examples of nucleotide conformations in the active site of Pol ι. Each of the structures is shown in two orientations. Light gray color indicates the nucleotide pair in "–1" position of the active center. Two metal ions in the active site are indicated by letters A and B (Mg^{2+} in all structures except the structure shown at panel (c), which contains Ca^{2+} ions). Structures (d) and (e) show Q59 residue, which forms a hydrogen bond with the N^2-atom of dGTP (d), or repulses the O^8 atom of the 8-oxo-dG residue (e).

are formed by the fingers-domain residues Y68, R71, K77, as well as residue K214 of the palm domain (they correspond to Y52, R55, R61, K236 in human Pol η) (Fig. 4). These amino acids are conserved in all Y-DNAPs, and their mutations lead to a strong decrease of the catalytic activity of enzymes [76].

A distinctive feature of human Pol ι as compared to other Y-polymerases is a narrow active site, which stimulates the formation of noncanonical interactions between nucleotides. It was found that the distance between C1′-atoms of template and incoming nucleotides in the active site of Pol ι is reduced from 10.5 Å (the average value for most DNAPs) to 8.2-8.9 Å (table). As a result, the usual arrangement of nucleotides in which both nitrogen bases are in the *anti*-conformation and the formation of canonical Watson–Crick hydrogen bonds for purine–pyrimidine nucleotide pair is restricted.

Template purine nucleotides in the active site of Pol ι adopt the *anti*-conformation in the absence of dNTP and rotate to the *syn*-conformation upon binding of the incoming pyrimidine nucleotide, forming Hoogsteen hydrogen bonds with it (Figs. 2 and 3 (a and b)) [69-71]. In the case of the dA-dTTP pair, hydrogen bonds between the N^7 and N^6 atoms of adenine and the N^3 and O^4 atoms of thymine are formed, and in the case of the dG-dCTP pair two hydrogen bonds are formed between the N^7 and O^6 atoms of guanine and the N^3 and N^4 atoms of cytosine (Fig. 2). In the latter case, the cytosine residue must be protonated in the third position to form the second hydrogen bond, which may explain the decreased efficiency and accuracy of dCTP incorporation opposite dG in comparison with the incorporation of dTTP opposite dA [70].

The incorporation of nucleotides opposite pyrimidine bases occurs by a somewhat different mechanism. To date, the structures of Pol ι complexes with dT [73] and dBrU [77] residues in the DNA template, either in the absence of dNTP or in the presence of dATP, dGTP, and dTTP in the active site have been solved (table). It was shown that the dBrU residue adopts either *anti*- or *syn*-conformation in the absence of dNTP, but preferably rotates to the *anti*-conformation upon the binding of the incoming nucleotide (table) [77]. The incoming dGTP is also bound in the *anti*-conformation, forming either wobble interaction with formation of two hydrogen bonds (according to [73]) or noncanonical Watson–Crick interactions with formation of three bonds (according to [77]) with the template dT (Figs. 2 and 3d). It is assumed that dBrU ionization at the third position or its isomerization to the enol form should occur in the latter case. It should be noted that a bromouracil residue is more prone to such transitions than a thymine residue, which may explain the observed differences in nucleotide pairing in [77] and [73]. It should be noted that the C1′-C1′ distance between the nucleotides in the complex containing incoming dGTP (~9.6 Å; table) is close to the normal distance for the DNA B-form. However, due to the narrowed active site, the nucleotide pairing is accompanied by the rotation of bases from the plane of interactions (for example, the dT base is rotated by 15°) (Fig. 3d).

Additional stabilization of the dT/dBrU–dGTP interactions is provided by the formation of the unique hydrogen bond between the N^2 atom of dGTP and the OE1 atom of the Q59 amino acid residue of the fingers domain (Figs. 2b and 3d) [73, 77]. This may explain the preferential incorporation of dGTP opposite dT by Pol ι. In support of this, the replacement of dGTP in the reaction by inosine triphosphate lacking an amino group at the second position, reduced the synthesis efficiency 10-fold [77]. Interestingly, Pol ι prefers to incorporate dGTP opposite AP sites, likely due to the additional interactions between dGTP and the Q59 residue (see below, [78]). The stabilization of the dNTP substrate by the formation of hydrogen bonds directly with an amino acid residue of the active site was previously demonstrated for another Y-family DNAP – Rev1. In the active site of Rev1, the incorporation of dCTP opposite dG and a variety of its damaged analogs is stabilized by formation of hydrogen bonds between dCTP and the R324 residue [79, 80].

As shown in biochemical experiments, the incorporation of dATP opposite dT by Pol ι is inefficient (see above). Analysis of Pol ι complexes with dT/dBrU and the incoming adenine nucleotide in the active site explained it from a structural point of view. In one of the studies [73], the incoming ddATP adopts a *syn*-conformation accompanied by the formation of hydrogen bonds between the N^6, N^7 atoms of adenine and the O^4, N^3 atoms of thymine. However, in this case adenine is strongly tilted relative to the 3′-terminal nucleotide of the DNA primer, thus losing the stacking interactions that is energetically unfavorable (Fig. 3c). In another study [77], the binding of dATP occurs in a very unusual *trans*-conformation – the nucleotide is flipped "upside down" (Fig. 2), which makes impossible its incorporation into DNA.

The narrowing of the Pol ι active center in comparison with other Y-family DNAPs, which stimulates the formation of non-standard interactions between nucleotides, is mostly explained by the following features of the Pol ι structure (Fig. 4) [69, 71, 73, 77]:

– changes in the conformation and positions of the enzyme domains relative to each other and to DNA. Thus, as compared with Dpo4 DNAP (DNAP IV of *Sulfolobus solfataricus*) and human Pol η, the little finger domain of Pol ι lies closer to the fingers domain and is shifted towards the template DNA chain; the S307 and R347 residues of the little finger interact with the 5′- and 3′-phosphate groups of the nucleotide in the active site (Fig. 4a) [73]. In comparison with Dpo4, these changes are in part explained by shortening of the L23 loop between β2- and β3-layers of the fingers domain, which is located close to the +1 nucleotide of the DNA template

(Figs. 1 and 4). The L23 loop interacts directly with the little finger domain in Dpo4, which pushes this domain away from the DNA [73]. Compared to Pol η, Pol ι has shorter L56 loop between the β5- and β6-layers that connects the palm and the fingers domains and, in the case of Pol η, also contacts the little finger domain (Fig. 4b). The loss of these interactions in Pol ι causes a shift of the little finger towards the DNA template and the constriction of the active center;

– the presence of large aliphatic amino acid residues that contact the template nucleotide in the active site. The Q59, K60, Y61, and L62 residues form a hydrophobic pocket participating in the binding of deoxyribose residue of the DNA template nucleotide and forcing it to move towards the incoming nucleotide (Fig. 4a) [69, 73, 77]. The side chains of residues Q59, L62, V64, and L78 "hang" above the base of the template nucleotide and push it towards the major DNA groove; in some of the published structures this is accompanied by shifting of the nucleotide from the plane of stacking interactions (Figs. 3 and 4a) [71, 73, 77]. In other Y-family DNAPs, the Q59, K60, L62, V64, and L78 residues correspond, on average, to residues with smaller side chains: V32, G41, A42, A44, G58 in archaeal Dpo4 and Q38, Y39, G46, I48, S62 in human Pol η (Fig. 4b), respectively [28, 29, 73].

The role of some of the listed amino acid residues of the active center in DNA synthesis by Pol ι has been verified experimentally. Thus, the replacement of the entire fingers domain in DNAP Dpo4 with the corresponding domain of Pol ι shifts the spectrum of nucleotide incorporation towards Pol ι and, in particular, enhances the incorporation of dGTP opposite dT by the hybrid DNAP [73]. According to [81], the Q59A substitution increases the processivity of Pol ι, possibly by extending the active site cavity (see below). The L62A substitution leads to a significant decrease in the efficiency of incorporation of pyrimidine nucleotides opposite purine bases suggesting a role of this residue in positioning of the template nucleotide and, presumably, in stabilization of its *syn*-conformation required for the formation of Hoogsteen interactions [82]. At the same time, molecular modeling [83], as well as analysis of the available structures of Pol ι [77], demonstrates that the side chain of residue Leu62 can be rotated and moved away from the active site allowing for the formation of Watson–Crick interactions. However, the assumptions made about the role of individual residues of Pol ι in catalysis require further experimental verification.

Another specific feature of Pol ι in comparison with other Y-DNAPs is the difference in the position of the 5′-region of the template DNA chain in front of the active site. In most structures of other DNAPs, DNA in front of the active site lies between the fingers and little finger domains and has more or less straight conformation (see, for example [28, 84]). As an example, Fig. 5c shows the structure of human Pol η. At the same time, in a number of Pol ι complexes the template DNA strand makes a sharp turn in the +1 position relative to the active center and is directed towards the thumb domain (table and Fig. 5a). Initially, such a turn of DNA was found in the structures of Pol ι with the template dT residue in the active site [73, 77]. However, careful analysis of available struc-

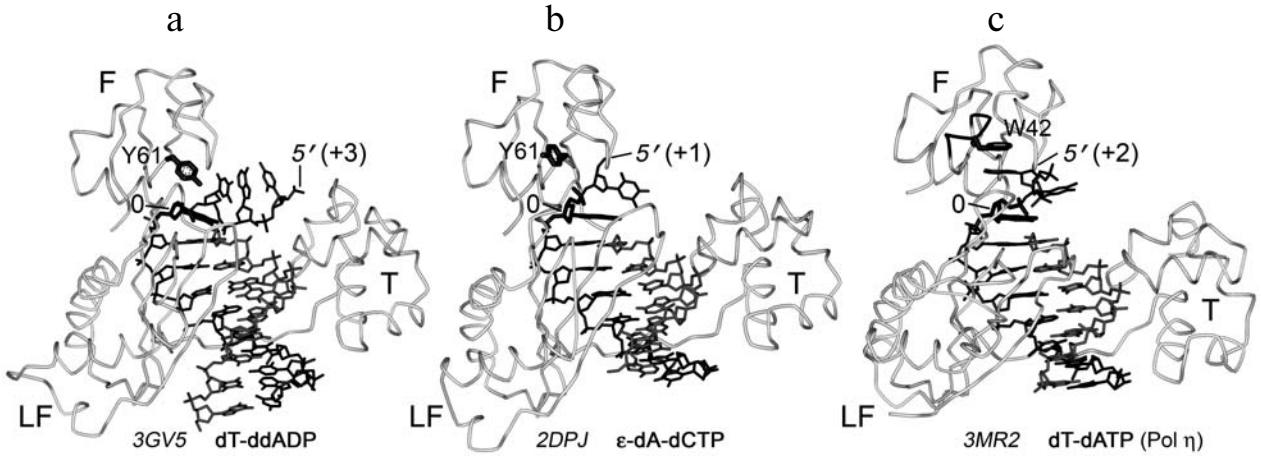

Fig. 5. The conformation of the 5′-terminus of the template DNA strand in different structures of Pol ι and Pol η. a) The structure of Pol ι with the dT–ddADP pair in the active site, a view from the side of the little finger domain; 3GV5 [73]). The palm domain and the incoming nucleotide are not shown on the figure. The template nucleotide in the "0" position of the active center is shown in black. The 5′-terminus of the DNA template is tilted towards the thumb domain; the 5′-terminal nucleotide in the position + 3 is marked. The Y61 residue is rotated towards the active center. b) The structure of Pol ι with the ε-dA–dCTP pair in the active site (2DPJ [89]). The 5′-terminal nucleotide in the +1 position of the template DNA strand is in the straight conformation. The Y61 residue is rotated away from the active center. c) The structure of Pol η with the dT–dATP pair in the active center (3MR2 [28]). The 5′-terminal nucleotide of the template DNA chain in the +2 position is marked. The nucleotide base forms stacking interactions with the W42 residue, which is located within the insertion in the L23 loop of the fingers domain.

tures shows that DNA also has a similar conformation in other cases, for example, in the presence of dA and dG residues (in the absence of incoming nucleotide), nucleotide pair dA-dTTP [71], and AP site [78] in the active site (table). It should be noted that DNA has a straight conformation in some of the structures (e.g. in the complex with the dG–dCTP pair in the active center; Fig. 5b). Comparison of the structures with different DNA conformations demonstrates that the DNA turn is accompanied by a change in the position of the Y61 residue in loop L23, which blocks the direct path between the finger and little finger domains for DNA (Fig. 5a). The observed turn of the template DNA in front of the active site may be considered as one of the reasons for the extremely low processivity of Pol ι.

EXPERIMENTAL EVIDENCE SUPPORTING FORMATION OF DIFFERENT TYPES OF NUCLEOTIDE INTERACTIONS IN THE Pol ι ACTIVE CENTER

After solving of the Pol ι structures, the possibility of noncanonical interactions between nucleotides in the active site of Pol ι was tested in biochemical experiments. One evidence of the formation of Hoogsteen interactions during the incorporation of pyrimidines opposite the purines of DNA template is a reduced efficiency of synthesis by Pol ι on DNA with 7-deaza-analogs of purine bases that prevent the formation of Hoogsteen interactions involving the N^7 atom of purine nitrogen bases (see Fig. 2b) [82]. Similar decrease in the efficiency of synthesis is observed when an 8-oxo-dG residue possessing an additional hydrogen atom in the N^7 position is present in the template (Fig. 6) [85]. The analysis of templates with a series of purine nucleotides analogs, which differ in their ability to form hydrogen bonds (including 7-deaza-purines, 2-aminopurine, purine, O^6-Me-dG), led to the general conclusion that the incorporation of dTTP opposite dA via Hoogsteen interactions requires only one hydrogen bond with the N^7 atom of dA, while the efficient and accurate incorporation of dCTP opposite dG requires the formation of two bonds with the N^7 and O^6 atoms (Fig. 2b) [82, 85].

Indirect biochemical evidence supporting formation of Hoogsteen interactions during incorporation of pyrimidines opposite template purines came from experiments that demonstrated efficient replication of DNA templates with purines containing major adducts that block the formation of Watson–Crick interactions (1,N^6-ethenoadenine, a variety of N^2-guanine adducts) (see below) [49-55].

It should be mentioned that Hoogsteen interactions are apparently not absolutely necessary for the incorporation of nucleotides opposite purine bases in the DNA template. According to the data of molecular dynamics simulation, Pol ι is able to sustain canonical Watson–Crick interactions with a purine nucleotide at the active site [83]. Increase of the C1′–C1′ distance between nucleotides, required for the formation of Watson–Crick interactions, is possible due to slight shifting of the DNA template from the little finger domain, as well as moving apart (by ~1 Å) of amino acid residues 59-62 in the pocket which binds the deoxyribose of the template nucleotide. Furthermore, Pol ι is able to incorporate nucleotides opposite DNA lesions that make the formation of Hoogsteen interactions with template purine nucleotide difficult or even impossible (e.g. N^3-methyl-dA [53], AAF-dG [83]). Finally, molecular modeling of the positions of damaged dG and dA bases in the active site showed that, depending on the type of the damage and the DNA sequence, Pol ι may preferably use either Hoogsteen or Watson–Crick interactions [83, 86].

Biochemical experiments studying the incorporation of nucleotides opposite pyrimidine bases in the DNA template using a wide range of modified purine dNTPs demonstrated that the pairing of an incoming nucleotide with template dC occurs through Watson–Crick interactions [87]. However, in the case of dT wobble interactions with incoming dGTP or dATP seem to be functionally important [87]. The formation of wobble interactions corresponds to the structural data obtained for the dT–dGTP pair, but contradicts the data for the dT–ddADP pair (in which ddADP adopts the *syn*-conformation, see above) [73]. Thus, the structural analysis of the Pol ι complex with the dT–ddADP pair probably registered an inactive conformation of the nucleotide pair in the active site.

It should be noted that the formation of hydrogen bonds between incoming purine nucleotides and template pyrimidine bases apparently does not make a large contribution to the binding energy of the incoming dNTP. In particular, this is supported by the observation that Pol ι demonstrates low accuracy of nucleotide incorporation opposite pyrimidines [44, 45, 47]. Furthermore, it was shown that the efficiency of incorporation of purine nucleotides opposite AP sites that does not contain a template base is comparable to or even exceeds the efficiency of synthesis on templates with pyrimidine nucleotides in the active site [56, 77, 78].

Pol ι STRUCTURES WITH DAMAGED DNA TEMPLATES AND MECHANISMS OF NUCLEOTIDE INCORPORATION OPPOSITE DAMAGED BASES

As mentioned above, *in vitro* Pol ι is able to synthesize DNA on templates with different types of lesions. Based on the structural and biochemical data discussed above, it was suggested that the ability of Pol ι to form Hoogsteen interactions might play an important role in replication of damaged DNA template. These assumptions were con-

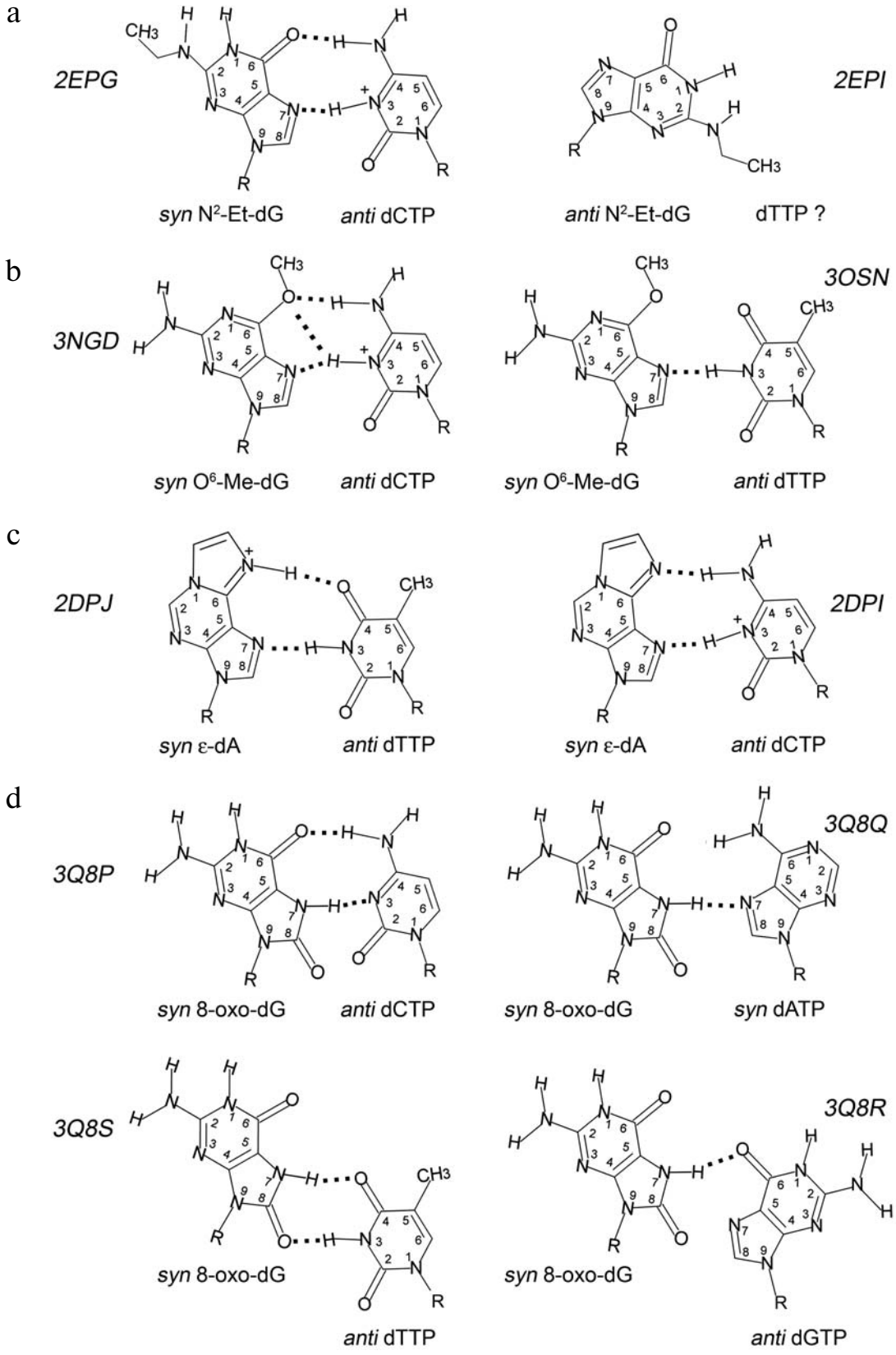

Fig. 6. Interactions between damaged bases and incoming dNTPs in the active center of Pol ι. Various interactions identified in solved Pol ι three-dimensional structures are shown.

firmed experimentally after determination of the three-dimensional structures of Pol ι complexes with damaged DNA templates containing N^2-ethylguanine (N^2-Et-dG) [61], 7,8-dihydro-8-oxoguanine (8-oxo-dG) [81], O^6-methylguanine (O^6-Me-dG) [88], 1,N^6-ethenoadenine (ε-dA) [89], as well as an AP site [78] (Fig. 6 and table). In addition, the results of molecular dynamics simulations for the complexes of Pol ι with two major adducts of dG and dA residues in the active center, 8-AAF-dG and N^6-BPDE-dA, were published [83, 86]. The analysis of these structures has shown that Pol ι does use Hoogsteen interactions for incorporation of nucleotides opposite most of the damaged bases mentioned above. The peculiarities of these complexes, as well as the mechanisms of incorporation of different nucleotides opposite the damaged bases, are briefly discussed below.

AP site. AP sites are the most frequent damage of genomic DNA [11, 12]. AP sites block replication, and most DNAPs with low efficiency preferentially incorporate dATP opposite AP sites [90]. Pol ι is able to perform DNA synthesis opposite AP sites, and it inserts dGTP with higher efficiency than other dNTP substrates (dGTP ≥ dTTP > dATP > dCTP, though with minor differences). However, as in the case of many other lesions, Pol ι cannot carry out a further DNA extension step [47, 56, 91]. In work [78] the structures of Pol ι complexes with dGTP, dATP, and dTTP opposite the AP site were solved (the tetrahydrofuran analog of the AP site was used for structure determination). In all three cases, the complexes have almost identical structure in which the incoming nucleotide adopts the *anti*-conformation (table). Preferential incorporation of dGTP opposite the AP site is due to the formation of a network of hydrogen bonds between amino acid residues Q59 and Y39 of the fingers domain, water molecules, and the N^2-atom of dGTP. The deoxyribose group of the AP site is located in the same hydrophobic pocket (formed by residues Q59, K60, Y61, L62 of the fingers domain) as in the case of undamaged nucleotides. Owing to the narrow active center, the base of the incoming nucleotide lies very close to the deoxyribose residue of the AP site, which is possible due to the absence of the template base. As a result, the efficiency of dGTP incorporation opposite the AP site is several times higher than that in the presence of template pyrimidine nucleotides (dC or dT) [77, 78, 87].

N^2-Ethylguanine. Pol ι is able to efficiently and accurately synthesize DNA on templates containing a variety of purine base adducts exposed in the minor groove of DNA and blocking the replication by many other DNAPs, such as N^2-guanine adducts, (e.g. [50-52, 55]) and 3-methyl-adenine [53, 54]. According to the X-ray diffraction analysis of complexes of Pol ι with one of these lesions, N^2-Et-dG, the damaged base in the active site is able to adopt the *syn*-conformation, which provides the correct dCTP incorporation with the formation of Hoogsteen hydrogen bonds, similarly to the dCTP incorporation on undamaged DNA (Fig. 6a) [61]. The damaged side of the nucleotide is exposed into the major groove of DNA. The Q59 amino acid residue of the active center of Pol ι is located in the immediate proximity of the N^3-atom of N^2-Et-dG and may play a role in stabilization of Hoogsteen interactions [61].

As in the case of undamaged dG, Pol ι is able to incorporate not only dCTP but also dTTP opposite N^2-Et-dG, and in certain conditions (in the presence of manganese ions) dTTP incorporation is even more efficient [61]. Unexpectedly, analysis of Pol ι structure containing N^2-Et-dG and dTTP in the active center revealed that N^2-Et-dG remains in the *anti*-conformation (Fig. 6a). It has been suggested that Watson–Crick interactions with dTTP may be formed in this case [61]. However, the structure of the incoming dTTP in this complex remains unresolved (only the position of the γ-phosphate group was determined). This may suggest that dTTP does not occupy the correct position in the active site in this complex.

O^6-Methylguanine. The use of Hoogsteen interactions by Pol ι was also demonstrated for incorporation of dNTP opposite the O^6-Me-dG lesion that is exposed in the major DNA groove [88]. In case of such a lesion, Pol ι conducts highly error-prone synthesis, incorporating dTTP 6-10 times more effectively than complementary dCTP [85, 88]. According to X-ray analysis, O^6-Me-dG is in the *syn*-conformation, and the incorporation of both pyrimidine nucleotides occurs with the formation of hydrogen bond between the N^7-atom of O^6-Me-dG and the N^3-atom of dTTP and dCTP (Fig. 6b). In the case of incoming dCTP, formation of this bond requires protonation of dCTP at the N^3 position that decreases the efficiency of interaction. In addition, dCTP in this complex occupies a slightly different position in comparison with the dG–dCTP pair, and the hydrogen bond with the N^3 atom of dCTP is shared between the N^7 and O^6 atoms of the O^6-Me-dG residue, which decreases its energy [88]. In the case of dTTP, the relative position of the O^2 atom of dTTP and the N^7 atom of O^6-Me-dG residue is optimal for the formation of a hydrogen bond, which ensures its preferential incorporation.

1,N^6-Ethenoadenine. This lesion is formed in DNA as a result of exposure to some chemical mutagens, as well as natural cellular metabolites. The 1,N^6-etheno group disrupts Watson–Crick interactions and blocks the replication by most DNAPs, including some Y-polymerases [92]. However, Pol ι was found to be able to incorporate nucleotides opposite ε-dA [89]. Compared to dA, the presence of ethenoadenine in the template reduces the efficiency of dTTP insertion by approximately one order of magnitude and increases the efficiency of dCTP insertion by more than two orders of magnitude. It results in a dramatic decrease in the fidelity of synthesis, with dCTP being incorporated only several times less efficiently than dTTP [89]. The analysis of the structures of Pol ι complexes with ε-dA and incoming dTTP and dCTP in the active site provides an explanation for these effects.

Similarly to intact dA, ε-dA adopts the *syn*-conformation in the active site (Fig. 6c). The presence of the etheno group allows for the formation of an additional hydrogen bond between the N^6-atom of adenine and the N^4-atom of cytosine and stimulates incorporation of dCTP. At the same time, formation of the ε-dA–dTTP pair requires protonation of the N^6-adenine atom, which is less favorable (Fig. 6c) [89].

7,8-Dihydro-8-oxoguanine is one of the most common lesions caused by reactive oxygen species [93]. Most replicative DNAPs and other Y-family DNAPs preferably incorporate dATP opposite 8-oxo-dG due to the formation of Hoogsteen bonds between 8-oxo-dG in the *syn*-conformation and dATP in the *anti*-conformation [94, 95]. Pol ι is one of a few DNAPs that insert complementary dCTP opposite 8-oxo-dG more efficiently than dATP (though still with less specificity than in the case of intact dG residue) [81, 85]. It should be noted that the presence of an 8-oxo-dG residue in the DNA decreases the efficiency of replication in comparison with the intact template [81, 85, 91].

The structures of Pol ι complexes with 8-oxo-dG in the active site with each of the four incoming nucleotides were solved in [81] (Fig. 6d). 8-Oxo-dG was found to be in a mixture of *syn*- and *anti*-conformations (in the ratio of 0.8 : 0.2) and to have a high B-factor, indicative of its mobility [81]. Notably, binding of the incoming nucleotides is apparently possible only in the *syn*-conformation of 8-oxo-dG. The differences in the specificity of nucleotide incorporation opposite 8-oxo-dG between Pol ι and replicative DNAPs are due to the narrowed active site that forces the incoming dATP (and dGTP) to adopt the *syn*-conformation, which is unfavorable and is stabilized by only one hydrogen bond with the N^7-atom of 8-oxo-dG [81]. At the same time, the incoming dTTP and dCTP nucleotides adopt the *anti*-conformation and form two hydrogen bonds; in the case of dTTP the geometry of these bonds is not optimal, which results in the preferential incorporation of dCTP (Fig. 6d).

It is assumed that the Q59 residue in the active site of the enzyme may play an important role in nucleotide incorporation opposite 8-oxo-dG. The repulsion between the O^8 atom of the 8-oxo-dG residue and the OE1 atom of the Q59 residue results in a tilting of the 8-oxo-dG base by 30° towards the major groove of DNA. This destabilizes the *syn*-conformation of 8-oxo-dG as compared with intact dG residue (Fig. 3e). Indeed, the Q59A substitution in the active site of Pol ι increases the efficiency of nucleotide incorporation opposite 8-oxo-dG, but reduces the accuracy of Pol ι synthesis on damaged DNA, perhaps due to widening of the enzyme active site (which may promote the binding of dATP in the *anti*-conformation) [81].

This review of available structures of Pol ι in complex with various undamaged and damaged templates and incoming dNTP substrates reveals a wide spectrum of possible interactions between nucleotides in the active site of Pol ι. The ability to support such a variety of interactions between nitrogen bases is a unique feature of Pol ι that distinguishes this DNAP from most other polymerases. It appears that the main feature of Pol ι underlying this ability is a narrow but open active site. The narrowed active site in comparison with other DNAPs stimulates the formation of Hoogsteen hydrogen bonds between nucleotides and provides a mechanism for the efficient replication of DNA with damaged nitrogen bases blocking Watson–Crick interactions. As a result of rotation of the nucleotides to the *syn*-conformation, the damaged bases are removed from the active center and exposed to the major groove of DNA, where the lesion does not interfere with interactions of the template nucleotide with polymerase and incoming dNTP. At the same time, Pol ι can use not only Hoogsteen, but also Watson–Crick interactions depending on the structure of damaged bases [83, 86]. This further expands the range of lesions that can be bypassed by Pol ι *in vitro*. In addition, the efficiency of a particular type of nitrogen base pairing by Pol ι may also vary depending on the sequence context and structure of the DNA template. This demonstrates a high flexibility of Pol ι in replication of damaged DNA, which may be of great importance for successful DNA synthesis opposite different types of lesions *in vivo*. An important area for future research is the mutational analysis of various elements of the active site, which should help to determine the functional role of these elements in DNA synthesis.

The functions of Pol ι in human cells remain largely unknown. A brief analysis of the possible biological functions of Pol ι can be found in reviews [96, 97]. The biological significance of the highly error-prone synthesis by Pol ι on undamaged DNA in the cell is not clear. It was suggested that Pol ι may be involved in somatic hypermutagenesis in mammalian cells, particularly during the maturation of immunoglobulin genes, but no unambiguous confirmation has been found for these assumptions [98-100]. It is possible that the low accuracy of Pol ι DNA synthesis on undamaged template bears no biological function by itself, but is a side effect of the adaptation of this DNAP to the synthesis on damaged DNA.

The ability of Pol ι to synthesize DNA *in vitro* opposite a variety of lesions blocking the functioning of replicative DNAPs suggests its possible role in the replication of damaged DNA. However, there are only a few proofs of the participation of Pol ι in synthesis on damaged DNA *in vivo*. Experiments in cell cultures demonstrated that Pol ι is involved in cell protection from oxidative stress and reduces the mutagenic potential of 8-oxo-dG residues [66]. Other studies have shown that Pol ι is important for the survival of cells in the presence of methylating agents [53, 54]. Mouse fibroblasts cells lacking Pol ι are characterized by an increased frequency of mutations opposite N^2-dG adducts, suggesting the role of the enzyme in the correct replication of these lesions [101]. At the same time,

the role of Pol ι on the whole organism level remains unknown. Mice harboring a nonsense mutation in the gene encoding for Pol ι (line 129) do not possess distinct phenotypic characteristics except for an increased sensitivity to some mutagenic agents [102]. Therefore, the analysis of the functions of Pol ι and other Y-family DNAPs *in vivo* is one of the most important areas for future research.

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