Published in final edited form as:

Environ Mol Mutagen. 2012 December ; 53(9): 699–724. doi:10.1002/em.21735.

Modulation of mutagenesis in eukaryotes by DNA replication fork dynamics and quality of nucleotide pools

Irina S.-R. Waisertreiger¹, Victoria G. Liston¹, Miriam R. Menezes^{1,*}, Hyun-Min Kim^{2,**}, Kirill S. Lobachev², Elena I. Stepchenkova^{1,3,4}, Tahir H. Tahirov¹, Igor B. Rogozin^{5,6}, and Youri. I. Pavlov^{1,4,†}

¹Eppley Institute for Research in Cancer and Allied Diseases, ESH 7009, 986805 Nebraska Medical Center, Omaha, NE 68198-6805, U.S.A

²School of Biology and Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA 30332, U.S.A

³Saint Petersburg Branch of Vavilov Institute of General Genetics, Universitetskaya emb. 7/9, St Petersburg, 199034, Russia

⁴Department of Genetics, Saint Petersburg University, Universitetskaya emb. 7/9, St Petersburg, 199034, Russia

⁵National Center for Biotechnology Information NLM, National Institutes of Health, Bethesda, MD 20894, U.S.A

⁶Institute of Cytology and Genetics, 630090 Novosibirsk, Russia

Abstract

The rate of mutations in eukaryotes depends on a plethora of factors and is not immediately derived from the fidelity of DNA polymerases (Pols). Replication of chromosomes containing the anti-parallel strands of duplex DNA occurs through the copying of leading and lagging strand templates by a trio of Pols α , δ and ϵ , with the assistance of Pol ζ and Y-family Pols at difficult DNA template structures or sites of DNA damage. The parameters of the synthesis at a given location are dictated by the quality and quantity of nucleotides in the pools, replication fork architecture, transcription status, regulation of Pol switches, and structure of chromatin. The result of these transactions is a subject of survey and editing by DNA repair.

Keywords

DNA polymerases; nucleotide pools; mutagenesis; Okazaki fragments

Accuracy of DNA replication and mutagenesis: Overview

In multi-cellular organisms, programmed variation of mutation rates and changes in transcription lead to switches of developmental programs and adaptive responses; their aberrant regulation in pathology causes cancer and other diseases [Loeb et al., 1974; Jackson

[†]Address correspondence and requests for materials to: Youri Pavlov, ypavlov@unmc.edu.

^{*}Current address: Departments of Therapeutic Radiology and Genetics, Yale University School of Medicine, New Haven, CT 06520, U.S.A.

^{**}Current address: Department of Genetics, Harvard Medical School, Boston, Massachusetts, U.S.A.

Author contribution. YP, THT, ES and IBR wrote the manuscript. IW and VL performed experiments presented in Figures 5 and 6 and participated in writing. MM participated in writing and performed experiments related to Fig. 2. HMK and KL performed the experiments described in Fig. 6A and in writing. IBR performed the statistical analysis of the mutation spectra.

and Loeb, 1998]. The proper function of the eukaryotic genome is contingent upon the accurate replication and repair of genetic material, which relies on coordinated DNA synthesis reactions by multiple DNA polymerases (Pols). The fidelity of the individual Pols determines the accuracy of synthesis of specific DNA patches by an assigned enzyme. With and without DNA damage, hereditary changes happen only when DNA adducts or replication errors are converted into new information in both strands of duplex DNA. In this sense, all mutagenesis is mediated by DNA Pols and even highly accurate enzymes take responsibility for the mutation load, participating in mutation fixation. The problem of mutagenesis, however, is bigger than a mere sum of transactions by Pols. Here we highlight the recent novel discoveries in the literature and provide original data from our laboratory illustrating how the replication environment affects the function of DNA polymerases. Due to page limitations, we will not discuss DNA Pols of Y and X families and redirect the reader to excellent reviews [Sweasy, 2003; Sweasy et al., 2006; Yang and Woodgate, 2007; Sale et al., 2012]. We will only briefly mention the enormous field of genetic instability which is caused by a massive creation of base analogs in DNA by editing deaminases [Conticello et al., 2007; Liu and Schatz, 2009].

The nucleic acid metabolism includes transcription, replication, repair and recombination (Fig. 1). All of these transactions influence mutagenesis. Transcription affects DNA structure and leads to an elevation of mutagenesis by a variety of ways [Kim and Jinks-Robertson, 2012]. Replication errors accumulate during copying of undamaged templates, due to the intrinsic limits of accuracy of DNA Pols [Kunkel, 2004; Pavlov et al., 2006b; McCulloch and Kunkel, 2008], elevated or imbalanced levels of precursors in the dNTP pools [Chabes et al., 2003; Mathews, 2006; Kumar et al., 2011], the presence of base analog nucleotides [Maki and Sekiguchi, 1992; Negishi et al., 1994; Kozmin et al., 1998; Kamiya, 2010], or the combination of these factors. For example, decreased DNA Pol fidelity may lead to elevated mutagenesis due to errors of polymerization of normal nucleotides plus elevated levels of incorporation of non-canonical precursors [Shcherbakova and Pavlov, 1996; Nick McElhinny et al., 2010; Williams et al., 2012]. DNA Pols can occasionally incorporate rNTPs from the relatively larger pool of RNA precursors (Fig. 1). This contributes to genome instability [Nick McElhinny et al., 2010; Kim et al., 2011]. Ribonucleotide incorporation by primase for the synthesis of RNA primers is a regular process during replication that happens with every initiation of Okazaki fragment (Fig. 1, insert), but this patch is thought to be completely removed during the maturation [Kao and Bambara, 2003]. Yeast mutants with a partially defective primase, however, are mutators [Longhese et al., 1993], suggesting that, under certain conditions, the change in parameters of regular rNTP incorporation could be a source of genetic instability.

Intact DNA molecules are replicated with high fidelity (up to 10^{-11} per base replicated) due to three sequential fidelity control steps: base selection by DNA polymerases, exonucleolytic proofreading and DNA mismatch repair (MMR) [Morrison et al., 1993; Schaaper, 1993; Kunkel and Bebenek, 2000]. Reduced base selectivity causes a mutator effect [Niimi et al., 2004; Li et al., 2005; Venkatesan et al., 2006; Pursell et al., 2007b; Tanaka et al., 2010]. Proofreading elevates the fidelity of DNA synthesis, and mutants with defective proofreading are mutators [Morrison et al., 1991; Morrison et al., 1993]. The DNA polymerase errors that escaped proofreading are corrected by post-replicative MMR [Modrich and Lahue, 1996; Harfe and Jinks-Robertson, 2000; Kunkel and Erie, 2005]. The failure of both proofreading and MMR leads to a synergistic increase in mutation rate, often called an “error catastrophe,” and death in haploid yeast [Morrison et al., 1993]. This underscores the importance of the pathways of error removal that act in series. The MMR repair system recognizes base-base and insertion/deletion mismatches in double-stranded DNA and corrects them through a precisely regulated process, involving mismatch excision followed by correct DNA synthesis [Kadyrov et al., 2006]. The DNA synthesis step in

MMR is accomplished by PCNA-dependent DNA polymerase, mostly Pol δ , but the involvement of Pol ϵ is also possible [Longley et al., 1997; Gu et al., 1998; Tran et al., 1999; Zhang et al., 2005]. The multiplicative increase in the mutation rates of a given mutant when combined with the MMR defect indicates that the cause of the mutator effect were replication errors.

DNA molecules with errors arising during replication or damaged directly (Fig. 1, right upper corner) are the subject of DNA repair, or tolerance by so called “error free bypass” or by translesion DNA synthesis. In most cases, repair requires an undamaged template and, when it utilizes recombination, a homologous DNA [Friedberg, 2006]. The synthesis opposite a lesion during bypass reaction is accomplished by specialized DNA Pols with a relaxed active site, often tailored for specific lesions [Yang and Woodgate, 2007; Biertumpfel et al., 2010]. This only partially solves the problem of bypass of persisting damage, because the extension from the aberrant primer termini is not a trivial task. The final bypass often associated with mutation fixation is achieved by a switch to a specialized “extender” DNA Pols and then, most likely, to further synthesis by major replicative DNA Pols. The most prominent extender is Pol ζ , which belongs to the same the B-family as major replicative Pols. The whole chain of reactions leading to damage bypass uses specialized machinery and, therefore, is a form of DNA repair.

The crucial role of extender Pols is illustrated by the fact that the frequency of induced mutagenesis declines to negligible values when Pol ζ is inactivated [Lawrence et al., 2000; Gan et al., 2008]. The dependence of mutagenesis on Pol ζ allows for robust and unambiguous classification of mutagens into misreplication versus misrepair classes (Fig. 2). Classical “misrepair” mutagen, UV light, does not induce mutations in yeast with deletion of the *REV3*. Most mutagens act via this pathway. Quite contrary, mutagenesis by base analogs (Fig. 2), or mutagenesis caused by DNA Pol errors, such as those developed in proofreading exonuclease-deficient background, is independent of Pol ζ [Shcherbakova et al., 1996; Datta et al., 2000].

All of these transactions occur in the eukaryotic cell in the chromatin environment that has an impact on every DNA metabolism transaction [Ransom et al., 2010]. Chromatin structure is one of the important, but understudied, factors modulating mutation rates and shaping the eukaryotic genome [Sasaki et al., 2009].

Replicative DNA Pols

The four Pols, α , ϵ , δ and ζ , that are critical for replication and successful cell proliferation [Lange et al., 2012], belong to the B-family. In the last five years, there has been marked progress in the understanding of their structures and functions [Johansson and Macneill, 2010] [Tahirov, 2012](Fig. 3). Their catalytic subunits possess the same general arrangement of essential motifs on the primary amino acid sequence [Pavlov et al., 2006b; Gan et al., 2008; Tahirov et al., 2009]. In addition to the polymerase domain, all Pols have relatively conserved sequences for the 3'→5' exonuclease domains. Pol α and ζ , however, lack exonuclease activity, because the sequence of catalytic motifs in the Exo domains is altered and catalytic residues are wiped out. This suggests that ancestor enzymes possessed proofreading activity that was lost in evolution during specialization of Pol functions. The catalytic core of yeast Pol δ aligns with RB69 DNA Pol in the general plan of organization with unique structural features [Swan et al., 2009]. All Pols possess remnants of the uracil recognizing domain but do not sense uracil like their archael homologs [Wardle et al., 2008]. The C-terminal domain of Pols (CTD) has two cysteine-rich metal binding motifs (MBS1 and MBS2) critical for the assembly of the holoenzymes [Dua et al., 1998; Sanchez Garcia et al., 2004; Klinge et al., 2009; Tahirov et al., 2009]. Before 2012, both of these sites were thought to bind zinc. These two zinc atoms were indeed present in the structure of CTD of

the yeast Pol α [Klinge et al., 2009]. The understanding of Pols changed drastically when it was found that MBS2 at the CTD of the catalytic subunits of yeast B family Pols and human Pol δ and ζ Pols coordinates the iron-sulfur cluster [Netz et al., 2012b; Baranovskiy et al., 2012]. Mutations affecting MBS2 in yeast in either Pol δ or Pol ζ lead to the absence of induced mutagenesis [Giot et al., 1997; Baranovskiy et al., 2012; Johnson et al., 2012]. The possible role of iron will be discussed in the next section.

The size of the catalytic subunits widely varies due to the presence of additional and, sometimes very long, N-terminal (in yeast Rev3, human REV3L and yeast Pol1, human POLA) or C-terminal extensions (in yeast Pol2 and human POLE). The structure and roles of these extensions are mainly unknown. It was recently found that the anomalously large catalytic subunit of Pol ϵ is a fusion of two distinct, active and inactive, Pols of the B family (Fig. 3), [Tahirov et al., 2009].

Functional B-family Pols in eukaryotes are multi-subunit complexes (Fig. 3). The nomenclature for names of human and yeast homologs is described in the legend to this figure. Pol α is a four-subunit complex [Muzy-Falconi et al., 2003] (Fig. 3, top left). This complex is responsible for the initiation of Okazaki fragments by concerted action of primase and DNA Pol α . All four subunits are essential. The largest subunit, p180, is a catalytic polypeptide capable of relatively accurate and robust but low-processivity DNA synthesis [Takada-Takayama et al., 1990; Copeland and Wang, 1991; Mizuno et al., 1999]. The current model is partially based on the crystallography of yeast Pol α fragments, and partially based on low-resolution electron microscopy (EM) images. This suggests that one larger domain has all the structural elements required for the DNA polymerase reaction and is connected by a flexible linker to the C-terminal Zn-finger domain responsible for interactions with the other subunits [Klinge et al., 2009; Nunez-Ramirez et al., 2011]. The smallest polypeptide (p49) in the four-subunit complex is the catalytic primase subunit. It is tightly associated with the larger accessory primase subunit (p58) that, in turn, interacts with the CTD of the catalytic subunit of Pol α . The second largest primase subunit has a unique iron-sulphur domain, which is essential for the priming reaction in addition to the primase catalytic subunit and is also responsible for the association with the origin recognition complex [Uchiyama and Wang, 2004; Klinge et al., 2007; Vaithiyalingam et al., 2010].

Pol δ is a complex of four (three in budding yeast) polypeptides [MacNeill et al., 2001; McHenry, 2003; Garg and Burgers, 2005; Tahirov, 2012] (Fig. 3, top right). The largest catalytic subunit (p125) has DNA polymerase and 3'→5' exonuclease active sites, as well as sites for protein-protein interactions [Sanchez Garcia et al., 2004; Sanchez Garcia et al., 2009; Baranovskiy, 2012 #70], including the PCNA binding motif [Garg and Burgers, 2005]. One distinguishing characteristic of Pol δ is that its processivity and robust activity is contingent upon PCNA and accessory factors [Prelich et al., 1987]. The essential second subunit (p50) serves as a stabilizer for the catalytic subunit and as a matchmaker with the third subunit (p66). In yeast, mutations that abolish interactions between the second and third subunit phenocopy the deletion of the gene encoding for the third subunit [Baranovskiy et al., 2008]. The third subunit (often called C-subunit) plays several important roles. The N-terminal part stabilizes the second subunit. We hypothesized that this domain is actually an integral part of the second subunit (B-subunits) of Pol α and Pol ϵ [Baranovskiy et al., 2008]. The available NMR structure of 75 amino acid N-terminal fragments of the second subunit of Pol ϵ with a 24 amino acid tag sequence [Nuutinen et al., 2008] is difficult to interpret in the context of the Pol complex. The proof of our hypothesis awaits the determination of the corresponding full crystal structures.

The third subunit has a conserved PCNA-binding motif and a motif that mediates interaction with Pol α [Gomes and Burgers, 2000; Gray et al., 2004; Johansson et al., 2004]. However,

the corresponding gene, *POL32*, is dispensable for growth in budding yeast [Garg and Burgers, 2005]. Deletion of this gene leads to ultraviolet (UV) sensitivity and immutability, essentially the same phenotype as deletions of *REV3*, *REV7* and *REV1* genes encoding for components of translesion synthesis (TLS) machinery. This suggests a role of the yeast Pol32 protein in the regulation of error-prone TLS [Lawrence, 2002; Prakash et al., 2005; Pavlov et al., 2006b]. Pol32 interacts with Rev1 and can recruit Pol ζ via this interaction, which could explain the role of this subunit in induced mutagenesis [Acharya et al., 2009; Pustovalova et al., 2012]. A new finding of sharing subunits of Pol δ and Pol ζ allow for additional opportunities for the explanation of these effects (see the discussion in the next section). The role of the fourth subunit (p12) is less defined. The deletion does not result in noticeable phenotypes in fission yeast [Reynolds et al., 1998], while the experiments with human enzyme suggested that it plays a regulatory role in Pol δ response to DNA damage [Zhang et al., 2007].

Pol ϵ is a four-subunit complex [Pospiech and Syvaoja, 2003; Pursell and Kunkel, 2008] (Fig. 3 bottom left) with an available yeast low-resolution cryo-EM structure [Asturias et al., 2006]. The largest catalytic subunit (p261) has robust processive DNA polymerase and proofreading exonuclease activity alone, and even without a C-terminal half [Morrison et al., 1990; Maki et al., 1998; Bermudez et al., 2011]. The second subunit (p59) mediates protein-protein interactions within the holoenzyme and is essential. Mutations in the corresponding gene in yeast that weaken these interactions confer a mutator phenotype [Jaszczur et al., 2009], which is increased synergistically when either proofreading or mismatch repair are not operating [Jaszczur et al., 2008]. The exact mechanism of the generation of replication errors under these conditions has to be determined. The third (p17) and fourth (p12) subunits bind directly to the catalytic subunit [Bermudez et al., 2011] and are thought to be involved in the interaction with double-stranded DNA [Tsubota et al., 2003; Asturias et al., 2006], but in yeast are not required for growth. The absence of the third and fourth subunit in yeast results in an elevation of spontaneous mutagenesis [Araki et al., 1991]. This elevation is partially dependent on the *REV3* function and independent of mismatch repair [Northam et al., 2006; Aksenova et al., 2010]. The fourth subunit is involved in chromatin remodeling [Iida and Araki, 2004]. Pol ϵ is additionally regulated by multiple accessory factors involved in origin recognition [Pospiech and Syvaoja, 2003; Takayama et al., 2003; Pursell and Kunkel, 2008] and chromatin structure [Li et al., 2011].

Pol ζ has been isolated from yeast in the moderately active form as a two-subunit complex [Nelson et al., 1996; Johnson et al., 2000; Zhong et al., 2006]. A heterodimer of the Rev3 protein (p353 in humans) and the second subunit encoded by the *REV7* gene (p30 in humans) has DNA polymerase activity and is uniquely proficient in the extension of mismatched primer termini [Lawrence, 2002; Prakash et al., 2005]. The activity of this form of Pol ζ is enhanced by PCNA [Garg et al., 2005]. It is interesting that the interaction with PCNA of both Rev3 and translesion Pol of Y-family Rev1 occurs via a distinct site from other Pols [Northam et al., 2006; Sharma et al., 2011]. Our recent studies suggest that human Pol ζ is actually a four subunit complex (Fig. 3, bottom right), in which the C-terminal part of the catalytic subunit interacts with two subunits of Pol δ [Baranovskiy et al., 2012]. Similar conclusions were reached for yeast Pol ζ [Johnson et al., 2012]. If this is a major form of Pol ζ , the activity, fidelity and PCNA stimulation data obtained with the two-subunit complex should be revisited to accommodate for the possible effects of the new subunits.

The genetic data suggest that the role of Pol ζ as a key player in TLS is conserved between yeast and humans [Li et al., 2002; Gan et al., 2008]. In addition, the human homolog of the second subunit of the yeast enzyme may have an additional role in regulating cell cycle

progression. The human REV7 (p30) is similar to the spindle checkpoint protein MAD2 and was reported to interact with MAD2 *in vitro* [Murakumo et al., 2000].

The evolutionary history of the four polymerases is very complex [Koonin, 2006; Tahirov et al., 2009]. For example, in addition to the fusion of two Pols to generate Pol ϵ as discussed previously, the second subunits (so-called B-subunits) of Pol α , Pol δ and Pol ϵ share some similarity and have homologs in Archaea [Aravind and Koonin, 1998; Makiniemi et al., 1999]. In Archaea, these polypeptides have 3'->5' exonuclease motifs and are subunits of a so-called D family of DNA polymerases [Jokela et al., 2005]. Changes of critical catalytic residues in the phosphodiesterase motifs in the eukaryotic orthologs of B-subunits renders them inactive as nucleases. In addition, the B subunit of human Pol δ possesses an OB-fold DNA binding domain and a surface for interaction with the other subunits [Baranovskiy et al., 2008]. The evolution of metal binding sites of catalytic subunits of all four Pols involves duplication and the use of different ancestors for catalytic subunit of Pol ϵ and the other B-family Pols [Tahirov et al., 2009].

Dynamics of Pols at the replication fork

The asymmetric nature of the DNA duplex poses topological problems for the replication of the anti-parallel strands by a fork moving in one direction. So the replication of the two strands is inherently different [Kornberg and Baker, 1991]. In simple DNA replication systems, such as bacteriophage T4, one B-family DNA polymerase is sufficient for synthesis of leading and lagging strands [Trakselis et al., 2001]. A minimal set for eukaryotes is Pol α and Pol δ , and replication of the mammalian virus SV40 requires only these two Pols for synthesis of both strands [Waga and Stillman, 1994]. Yeast can also use only those two Pols to replicate their whole genome, but at the expense of a poor growth rate [Kesti et al., 1999] and genome instability [Ohya et al., 2002]. Under normal circumstances, robust chromosomal replication in eukaryotes requires three DNA polymerases, Pol α , Pol ϵ and Pol δ [Garg and Burgers, 2005; Pavlov et al., 2006b; Kunkel and Burgers, 2008]. Pol ζ assists major Pols when they experience difficulties with copying the template [Northam et al., 2006; Northam et al., 2010]. In addition, many specialized TLS polymerases assist replicative polymerases at certain conditions, e.g. DNA damage [Bebenek and Kunkel, 2004; Prakash et al., 2005; Zhong et al., 2006].

The process of replication of the eukaryotic genome is started at multiple sites by origin recognition by Orc1-6 followed by a series of steps, resulting in the assembly of the Cdc45-Mcm2-7-GINS complex, an active helicase [Araki, 2011]. Curiously, Pol ϵ and its accessory factors are obligatory components of the process and thus it is the first DNA Pol arriving at the emerging replication fork. Its Pol activity is useless at this time, however, because there are no primers available for the synthesis yet. Pol α -primase plays the main role in the actual start of DNA synthesis.

DNA polymerases cannot begin synthesis without primers, while RNA polymerases can. Therefore, specialized RNA polymerases called primases [Kuchta and Stengel, 2010] are indispensable for both the start of leading strand replication and the start of replication of each of the millions of Okazaki fragments on the lagging strands. Primase is a remarkable enzyme. It is able to count the length of RNA primers by itself and synthesizes *in vitro* 8-10 nucleotide-long unit-length RNA primers and multiples of this unit. It is inhibited by dNTPs and has a quite low fidelity of polymerizing of rNTPs [Sheaff and Kuchta, 1994]. The problem of switching from synthesis of RNA to synthesis of DNA is solved by a dedicated Pol α that works in tight complex with primase [Muzi-Falconi et al., 2003]. The reaction occurs intra-molecularly, without the dissociation of primase and Pol α after the switch inhibits primase [Copeland and Wang, 1993; Sheaff et al., 1994]. Pol α is not processive and lacks an intrinsic proofreading exonuclease. Pol α extends these RNA primers by

synthesizing short stretches of DNA. It is believed that the size of these patches is around 20-40 nucleotides [Bullock et al., 1991; Tsurimoto and Stillman, 1991b; Waga and Stillman, 1998; Liu et al., 2004]. A switch then occurs to start the processive synthesis of numerous Okazaki fragments by Pol δ or, rarely, to Pol ϵ , when it starts the leading strand. RNA primers as well as most of the DNA synthesized by Pol α are removed by nucleases: RNase H, Dna2, and flap endonuclease FEN1 [Kao and Bambara, 2003; Burgers, 2009; Zheng and Shen, 2011; Gloor et al., 2012; Reijns et al., 2012].

It was commonly accepted that Pol α synthesizes a small amount of DNA in comparison to other replicative DNA polymerases – 1/10 of Okazaki fragment, estimated to be from 165 to 250 base pairs, [Waga and Stillman, 1998] [Pearson et al., 2005; Smith and Whitehouse, 2012]. Thus, the *a priori* is not expected to contribute to the accuracy of genomic DNA replication. Moreover, these patches are removed with RNA primer during Okazaki fragment maturation, or errors by Pol α are the subject of correction by Pol δ [Pavlov et al., 2006a] and by MMR [Niimi et al., 2004; Pavlov et al., 2006a]. There is also a possibility that some of these errors are corrected by flap endonuclease [Rumbaugh et al., 1999]. However, mutations reducing base selectivity of Pol α (for example, *pol1-L868M* in yeast) can lead to very strong mutator phenotypes when combined with mismatch repair defects [Liu et al., 1999; Pavlov et al., 2001; Gutierrez and Wang, 2003; Ogawa et al., 2003; Niimi et al., 2004; Pavlov et al., 2004; Tanaka et al., 2010]. Thus, Pol α plays an important role in maintaining genome stability. The nucleotide selectivity of Pol α normally makes an important contribution to the overall mutation rate [Niimi et al., 2004; Tanaka et al., 2010].

After a short patch of synthesis of RNA-DNA primer a switch occurs to DNA synthesis by the more accurate proofreading proficient Pols. The switch to DNA Pol δ was investigated quite thoroughly in the SV40 system that utilized only two Pols [Tsurimoto et al., 1990; Tsurimoto and Stillman, 1991a; Tsurimoto and Stillman, 1991b], while the switch to Pol ϵ is understudied. Pol accessory factors RFC play the main role in the switch. RFC limits the extent of synthesis by Pol α [Tsurimoto and Stillman, 1991b; Mossi et al., 2000], and along with PCNA, assists in the transfer of the primer to the Pol δ active site [Maga et al., 2000]. Structural details of this reaction have not been studied.

Pol δ then continues the synthesis of the lagging strand until it bumps to the start of another Okazaki fragment [Garg et al., 2004]. Several solid genetic lines of evidence and direct biochemical experiments suggest that Pol δ is operating on the lagging strand *in vivo* [Garg et al., 2004; Garg and Burgers, 2005; Jin et al., 2005; Nick McElhinny et al., 2008]. This Pol is well suited for this, because, contrary to Pol ϵ , it can idle at the nick until it is ligated. The size of Okazaki fragments in eukaryotes is an order of magnitude smaller than in prokaryotes. Earlier estimates with the SV40 system were 40-290 nucleotides with an average of 135 [Anderson and DePamphilis, 1979]. It was immediately realized that it could be connected to chromatin structure and the size of nucleosomes in eukaryotes [Herman et al., 1979]. The contemporary estimate of the Okazaki fragment in yeast agrees with those earlier results and directly links the size of the fragments to the size of the nucleosomes [Smith and Whitehouse, 2012]. The measurements performed in ligase I deficient strains revealed that there is some heterogeneity of the sizes of Okazaki fragments, but a substantial fraction of them were around 165 base pairs, which parallels the size of the nucleosomal repeat. It is possible that nucleosome assembly by itself regulates the extent of synthesis by Pol δ . Notably, mutations in the genes of post-replicative nucleosome assembly factors (CAF-1) drastically altered the length distribution. Curiously, these mutations do not exert any strong deleterious effects in vegetative yeast cells.

We proposed that the leading DNA strand in eukaryotes is replicated both by Pol ϵ (near the origins) and Pol δ (most of the leading strand), while the lagging DNA strand is replicated

mostly by Pol δ [Pavlov and Shcherbakova, 2010]. The exact roles of these two Pols, which are equally processive with proper accessory factors [Chilkova et al., 2007; Bermudez et al., 2011], are not fully understood in biochemical terms (see reviews [Waga and Stillman, 1998; Pospiech and Syvaaja, 2003; Shcherbakova et al., 2003a; Garg and Burgers, 2005; Pavlov et al., 2006b]). One of the first models of the replication fork, that was proposed after the discovery of Pol ϵ , postulates that Pol ϵ is responsible for exclusively copying the leading strand DNA template, and Pol δ is responsible for the lagging strand replication [Morrison et al., 1990; Fukui et al., 2004; Garg and Burgers, 2005; Hiraga et al., 2005; Kunkel and Burgers, 2008]. The model is currently favored, however, there are no mechanistic insights into how the two Pols work together. The evidence is mainly based on genetic observations in yeast with the use of various Pol mutants and sensitized genetic backgrounds that may distort the replication parameters [Morrison and Sugino, 1994; Shcherbakova and Pavlov, 1996; Karthikeyan et al., 2000; Kunkel and Burgers, 2008; Miyabe et al., 2011]. We discussed the evidence for and against this broadly accepted model in-depth [Pavlov and Shcherbakova, 2010]. One of the main arguments against it is that the polymerase activity Pol ϵ is not absolutely required for replication and could be substituted by another Pol, which is based on the viability of mutants with deletions of the polymerization-proficient half of the catalytic subunit [Kesti et al., 1999].

Similar to the currently accepted model, Pol α synthesizes short RNA-DNA fragments at the origins and on the lagging strand in our model. Pol δ extends these fragments on the lagging strand. The principal novel feature of the model is in the mechanism of leading strand synthesis. We postulate that Pol ϵ is responsible for the early steps of leading strand synthesis, as well as elongation of the leading strand in the vicinity of the origin. It dissociates from the primer terminus with an increasing probability as the distance from the origin increases, and Pol δ takes over the leading strand synthesis. As a result, the majority of the genome replication involves copying of both DNA strands by Pol δ . When transactions of Pol ϵ can be tracked genetically, errors attributable to this Pol are found on the leading DNA strand. These traces, however, fade with an increase in distance from replication origins [Larrea et al., 2010]. The extent and nature of the sites of participation of Pol ϵ in leading strand replication should be a subject of further biochemical and genetics experiments.

The fourth Pol at the replication fork is Pol ζ [Pavlov and Shcherbakova, 2010]. It is critical for replication at difficult template sites or when replicative DNA Pols are compromised [Northam et al., 2010] and during DNA damage bypass [Prakash et al., 2005; Pavlov et al., 2006b; Waters et al., 2009]. Induced mutagenesis associated with bulky lesions completely depends on Pol ζ , therefore, this Pol operates on damaged sites on both leading and lagging DNA strands.

DNA Pols ζ and δ in induced mutagenesis

One property of mutagenesis induced by the majority of DNA damaging agents (Fig. 2) was difficult to explain. Certain mutations affecting the function of Pol δ had the same “immortality” phenotype as the complete absence of Pol ζ . A severe decrease of UV and MMS-induced mutagenesis in yeast was described for mutations in the three genes for the subunits of Pol δ : for the point mutation partially destroying the iron cluster binding site in MBS2 of the catalytic subunit, *pol3-13* [Giot et al., 1997], for the mutations affecting the interaction of the second with the third subunit [Baranovskiy et al., 2008], and for the deletions of the whole gene or parts of the gene for the third subunit [Huang et al., 2000; Huang et al., 2002; Johansson et al., 2004]. What is the connection between the two Pols apparently playing different roles in replication?

The discovery that human Pol δ shares two accessory subunits with Pol ζ provides a simple explanation of these effects [Baranovskiy et al., 2012]. When Pol δ bumps in to the lesion, its catalytic subunit is exchanged in the complex to the heterodimer of the catalytic subunit of Pol ζ with Rev7 to accomplish the vital role of Pol ζ in mutagenesis. Bacteria are also known to exchange Pols at damaged sites. However, Pols are exchanged using a sliding clamp as a platform for docking/exchange [Furukohri et al., 2008]. In eukaryotes, the exchange appears to be more elaborate, because the docking/exchange platform is provided by the B-subunit of the Pol delta and the exchange is redox-dependent. The initiation of exchange depends on iron-sulfur clusters, which are present in both Pol δ and Pol ζ . Therefore, mutations affecting this site in catalytic subunits of either Pol lead to immutability.

How is this new idea accommodated in the existing models of DNA damage bypass [Prakash et al., 2005; Waters et al., 2009; Ulrich, 2011]? We envision the following. When Pol δ is stalled by a damaged site, PCNA is monoubiquitylated by Rad18/Rad6 [Hoege et al., 2002; Stelter and Ulrich, 2003], which signals to an effector protein to change the oxidation state of iron in the C-terminus of Pol δ . One candidate for the effector protein is Mgs1, which is known to modulate induced mutagenesis and to act downstream from PCNA modification [Saugar et al., 2012]. It is known that iron sulfur cluster function is required for the assembly of the catalytic subunit of Pol δ with accessory subunits [Netz et al., 2012b]. Then, the catalytic subunit of Pol ζ binds to the platform of p50/p66 abandoned by the catalytic subunit of Pol δ [Baranovskiy et al., 2012] and REV7 further strengthens this complex interaction with translesion Pol REV1 [Acharya et al., 2009; Pustovalova et al., 2012]. This complex extends the products of translesion DNA synthesis by Y-family DNA Pols. Subsequent oxidation of iron in the catalytic subunit of Pol ζ results in its dissociation and the binding of a reduced catalytic subunit of Pol δ back to the p50/p66 complex.

Recent biochemical evidence confirms that subunit sharing between Pol δ and Pol ζ is evolutionarily conserved and Pol31 and Pol32 subunits are part of the four-subunit Pol ζ complex in yeast [Johnson et al., 2012]. The full complex is more stable and more active.

It appears that Pol δ is central in the regulation of TLS. Mutations affecting the components of Pol δ responsible for polymerase switches abolish all induced mutagenesis, which means that this happens in both leading and lagging DNA strands. This suggests that either Pol δ is a main replicase for both DNA strands or TLS events on the leading strand should include the switch for Pol δ as an initiating event when this strand is replicated by Pol ϵ . The possibility of such a switch has never been investigated. If TLS is delayed and not coupled with fast replication [Daigaku et al., 2010; Diamant et al., 2012] the time should be sufficient for more complicated switches of Pols.

The studies of the exact roles and inter-dependence of the iron-sulfur clusters in DNA Pols δ and ζ and PCNA ubiquitylation in Pols switches have just begun, but it is already obvious that the cluster's role in genome stability is dramatic. The insertion of the iron-sulfur cluster into proteins requires elaborate machinery [Lill, 2009], therefore, it is likely that replication and DNA repair depend on the status of iron metabolism. One of the recent highlights is the finding that the classical "DNA repair" gene, *MMS19*, encodes for a protein responsible for the insertion of the iron-sulfur cluster into proteins of DNA metabolism, including DNA Pols [Gari et al., 2012; Stehling et al., 2012]. This has broad biological significance, because it opens a previously unrecognized new mechanism of regulation of DNA transactions by mitochondria and by cytosolic factors, where components of Fe/S clusters are synthesized and required for the proper assembly of iron-sulfur-containing proteins [Lill et al., 2012; Netz et al., 2012b].

As sometimes happens, the genetic proof of this concept was provided a decade ago without the realization of its significance, because many genes involved in iron-sulfur biogenesis were not described [Chanet and Heude, 2003]. The aforementioned allele *pol3-13* that encodes for MBS2-defective Pol δ and abolished UV mutagenesis was found to be synthetically lethal with mutations, which later has been found to affect the biogenesis of iron-sulfur cluster, e.g. *nbs35* [Netz et al., 2012a] and *tah18* [Netz et al., 2010]. It is noteworthy that mutations in the *RAD18* gene, which encodes for E3 ligase participating in PCNA monoubiquitylation, rescues lethal *mms19 pol3-13* double mutant combination [Chanet and Heude, 2003]. Because MMS19 is a cytosolic factor inserting an iron –sulfur cluster in Pol δ [Gari et al., 2012], this links the function of the iron cluster in DNA Pols to PCNA modification.

Quality of nucleotide pools and mutagenesis

It is well known that levels of nucleotides affect mutagenesis outcomes [Kunz, 1988; Mathews and Ji, 1992; Kumar et al., 2011]. Another problem is the overlap of NTP and dNTP pools (Fig. 1). As we discussed, ribonucleotides are a regular part of nascent DNA chains (Fig. 1). If the machinery of their removal fails, the remnants of these primers can be left in DNA [Reijns et al., 2012]. In addition, under certain conditions, DNA Pols can directly incorporate ribonucleotides into DNA, causing genetic instability [Nick McElhinny et al., 2010; Miyabe et al., 2011]. Here we will focus on mutagenesis by deoxynucleoside triphosphates of base analogs that deceive DNA polymerases and become drivers of replication errors (Fig. 4A).

Environmental factors and biochemical reactions during oxidative stress and inflammation damage the bases of natural nucleoside triphosphates [Simandan et al., 1998; Colussi et al., 2002; Hakim et al., 2003; Polidori et al., 2003; Dedon and Tannenbaum, 2004]. Such mutagenic contaminants in dNTP pools lead to elevated mutation rates, chromosome instability [Abolhassani et al., 2010], risk of cancer [Sekiguchi and Tsuzuki, 2002], and developmental problems [Behmanesh et al., 2009]. One of the best studied base analogs in the nucleotide pool is 8-oxo deoxyguanosine triphosphate [Maki and Sekiguchi, 1992]. An accumulation of oxyguanine in DNA leads to several diseases, including cancer and age-dependent trinucleotide expansions, leading to neurodegenerative diseases [Kovtun et al., 2007; D'Errico et al., 2008; De Luca et al., 2008].

Cleansing of the precursor dNTP pool from potentially mutagenic nucleotide analogs is an important prerequisite for high fidelity DNA replication [Hochhauser and Weiss, 1978; Ames and Gold, 1991; Maki and Sekiguchi, 1992; Michaels and Miller, 1992; Grollman and Moriya, 1993]. The significance of base analog mutagens is that, in most cases, they are produced within the cells intrinsically, as a result of normal biosynthetic pathways (Fig. 4B). In addition, they are widely exploited by nature to generate genetic instability on demand, such as in the immune system [Chelico et al., 2009], or change gene expression [Guo et al., 2011]. The use of such dangerous agents, capable of changing the genetic information in the genome, demands tight regulation of base analog mutagenesis to avoid genome destruction. In this paper, we discuss the effects of purine base analogs that are generated by normal cell metabolism; hypoxanthine and xanthine are generated by spontaneous or enzymatic deamination of adenine and guanine bases, respectively, and their mimic, hydroxylaminopurine (HAP, Fig. 4A).

IMP and XMP are central intermediates in purine metabolism (Fig. 4B). Inadvertent activation of these compounds into triphosphates (dotted red arrows in Fig. 4B) will result in their incorporation into DNA and lead to clastogenic effects [Budke and Kuzminov, 2009]. When the flow of biochemical reactions in purine biosynthesis in yeast is interrupted

genetically (by mutations in the genes regulating the downstream conversions of IMP - *ADE12*, or destruction of aberrant triphosphates containing base analogs - *HAM1*), there is a significant accumulation of base analogs in DNA [Pang et al., 2012] and an increase of HAP mutagenesis [Stepchenkova et al., 2009a]. Under certain conditions, the amount of ITP in the pool reaches 10% of the amount of adenine [Sakumi et al., 2010]. A related compound, HAP, can arise from hydroxylation of adenine and can be converted to adenine or hypoxanthine [Kozmin et al., 2008; Sakumi et al., 2010] (Fig. 4A). Deoxy- and ribonucleoside triphosphates XTP, ITP and HATP are destroyed by the same enzyme, inosine triphosphate pyrophosphatase (ITPA). HAP incorporations are easy to track because they are highly mutagenic. Many systems protect cells from the mutagenic and inhibitory effects of HAP [Pavlov Iu, 1986; Noskov et al., 1996; Kozmin et al., 1998; Kozmin et al., 2000; Stepchenkova et al., 2005]. This suggests that HAP might be a natural contaminant of dNTP pools [Kozmin et al., 2000]. That makes HAP a biologically relevant tool for the study of DNA replication and effects of the natural base analogs, hypoxanthine and xanthine.

Among multiple mechanisms protecting DNA from these base analogs [Kozmin et al., 1998; Bradshaw and Kuzminov, 2003; Burgis et al., 2003; Kozmin et al., 2008; Budke and Kuzminov, 2009], ITPA is one of the most important enzymes in eukaryotes (Fig. 4B) [Pavlov Iu, 1986; Noskov et al., 1996; Menezes et al., 2012]. ITPA orthologs from humans, yeast and bacteria control the levels of triphosphate forms of hypoxanthine, xanthine and HAP [Lin et al., 2001a; Burgis et al., 2003; Burgis and Cunningham, 2007]. ITPA is highly conserved among different species [Kozmin et al., 1998; Hwang et al., 1999; Lin et al., 2001b]. In *E. coli*, the *rdgB* mutation is synthetically lethal with the *recA* mutation that abolishes homologous recombination [Bradshaw and Kuzminov, 2003; Burgis et al., 2003]. The *rdgB* mutation sensitizes cells to the mutagenic and recombinogenic effects of HAP in the molybdenum-cofactor defective strain background (another system protecting from HAP [Kozmin et al., 2000; Kozmin and Schaaper, 2007; Kozmin et al., 2008]) because of a massive accumulation of breaks in DNA [Burgis et al., 2003; Lukas and Kuzminov, 2006]. DNA damage is caused by intermediates in the repair of base analogs in DNA by Endo V encoded by the *nfi* gene. This has been proven by the viability of triple *rdgB recA nfi* mutants [Burgis et al., 2003]. In most organisms, including yeast *S. pombe* and humans, there are orthologs of endonuclease V [Moe et al., 2003], [Dalhus et al., 2009], but there is no evidence that this enzyme can robustly repair hypoxanthine in DNA. It is known that human APNG can excise at least hypoxanthine [Saparbaev et al., 2000]. We propose that DNA fragmentation and genomic instability, due to the presence of base analogs in DNA in humans, are caused by the intermediates of DNA repair reactions, but the exact players in these reactions are unknown [Waisertreiger et al., 2010; Menezes et al., 2012].

It is worth mentioning that the *rdgB* mutation in *E. coli* is suppressed by an overexpression of the *purA* (involved in the conversion of IMP to AMP in *de novo* biosynthesis of adenine), thereby unequivocally pointing out that the main source of ITP and deoxy ITP in the pools is normal cellular metabolism [Clyman and Cunningham, 1987]. The relationship between ITPA, ADSS and ADSL enzymes of purine biosynthesis is indicated by a systems biology approach but it was not studied in human cells experimentally.

ITPases prevent contamination of the nucleotide pool, which could lead to the incorporation of abnormal nucleotides into DNA and RNA or dilution of the ATP/GTP needed by numerous proteins. Underscoring the importance of this process, when *Itpa* knockout mice were developed, it was discovered that more than half of the mice die before birth. Those that do survive display growth retardation, heart abnormalities, ataxia, and abnormal breathing and die within two weeks [Behmanesh et al., 2009]. Furthermore, cells from *Itpa* knockout mice exhibited elevated levels of inosine in the RNA and DNA and had higher

levels of chromosomal abnormalities than wild type cells until they are suppressed in later passages by the spontaneous expression of the NUDIX protein NUDT16 [Abolhassani et al., 2010]. This clearly demonstrates that if non-canonical nucleotides are allowed to accumulate, then they can interfere with normal cellular processes and can affect genome stability.

The precise cellular function of human ITPase, ITPA, is not clearly defined. ITPA is expressed in many human tissues [Lin et al., 2001b]. Several polymorphic variants have been identified in the *ITPA* gene, which result in varying degrees of ITPA deficiency. A 94C>A polymorphism, encoding for a protein with a proline to threonine change at amino acid 32 (P32T), is a clinically significant variant, because it causes ITPA deficiency, measured typically in erythrocytes [Cao and Hegele, 2002; Sumi et al., 2002]. Individuals heterozygous for this mutation have approximately 25% residual enzymatic activity, while homozygotes have essentially zero activity, which could be explained if heterodimeric ITPA/ITPA P32T leads to the same defect of ITPA as the ITPA P32T homodimer. The allelic frequency of this mutation ranges from 5–19% with the highest frequency found in the Asian population [Marsh et al., 2004]. Additional gene variants causing ITPA deficiency have been identified, but the P32T mutation leads to the most severe reduction in activity [Bierau et al., 2007].

Base analog HAP as a tool for studying replication

HAP (Fig. 4A) is a potent universal base analog mutagen with ambiguous base pairing capacity (see reviews [Pavlov et al., 1991; Kozmin et al., 1998]). In an amine state, it forms two hydrogen bonds with “T”, and in an imine state it makes two hydrogen bonds with “C.” Consistent with this, dHAPTP replaces both dATP and dGTP during *in vitro* DNA synthesis by prokaryotic and eukaryotic DNA polymerases [Abdul-Masih and Bessman, 1986]. The proof that the active mutagenic form of HAP is deoxyribonucleoside triphosphate comes from the fact that mutants deficient in a pyrophosphatase specific for abnormal purine triphosphates are hypersensitive to HAP [Noskov et al., 1996; Porta et al., 2006]. Both dCTP and dTTP can be incorporated opposite HAP *in vivo* in yeast [Noskov et al., 1994]. HAP induces both G-C to A-T and A-T to G-C transitions [Shcherbakova and Pavlov, 1993; Noskov et al., 1994; Kulikov et al., 2001]. Most likely, HAP is converted to dHAPTP inside the cell and is misincorporated into the DNA opposite template C. In the next round of DNA replication, T is incorporated opposite HAP with a high probability [Noskov et al., 1994]. Therefore, the C to T transition pathway is initiated when dHAP is misincorporated opposite the cytosine during replication. The C-containing strand defines the template for C-G to T-A mutagenic replication after HAP treatment. The initial HAP incorporation opposite T and consequent C misincorporation opposite HAP in the next replication cycle leads to an A-T to G-C transition [Shcherbakova and Pavlov, 1996]. This occurs less frequently.

HAP is a very useful tool for studying the mechanism of DNA replication fidelity because its mutagenic action does not depend on general DNA repair systems dealing with DNA damage [Pavlov et al., 1991]. HAP mutagenesis is also not dependent on mutations inactivating damage bypass/specialized DNA polymerase genes, *REV3* (Pol ζ) [Shcherbakova et al., 1996](Fig. 2), *RAD30* (Pol η) and *REV1* (Pol Rev1) (unpublished observations). Mutants defective in proofreading have elevated levels of HAP mutagenesis, suggesting that errors produced by HAP are subject to proofreading.

In the conclusion for this section we would like to emphasize the dependence of mutagenesis of DNA Pol fidelity and the quality of nucleotide pools. *In vivo*, DNA Pols utilize natural pools that may have many contaminants. Therefore, the cases of correlation between the fidelity of DNA pols and their inaccurate variants *in vitro*, when the Pols are highly controlled by experimenters, and mutational signatures of the same Pols *in vivo*

[Pavlov et al., 2002b; Shcherbakova et al., 2003b; Pursell et al., 2007a], could be regarded as fortunate exceptions rather than the rule.

Mutagenesis during the synthesis of leading and lagging DNA strands in the presence of base analogs

In the case of spontaneous base-pair substitutions, it is difficult to determine the DNA strand where the initial error occurred. For example, the change of a G-C base pair to an A-T base pair could be initiated by a G-dTTP mispair on one strand or a dATP-C mispair on the other strand. In this case, strand assignment relies on assumptions that the ratio of these reciprocal mispairs is different [Iwaki et al., 1996; Fijalkowska et al., 1998]. Assigning a DNA strand where a mutation occurs is more reliable with the use of specific means to target DNA changes to one strand. Possible solutions are strand-specific DNA damage [Veaute and Fuchs, 1993], the use of base analogs [Shcherbakova and Pavlov, 1996], nucleotide pools imbalances [Roberts et al., 1994] or a mutator DNA polymerase with strong asymmetry in rates of dPyTP/Pu versus dPuTP/Py errors [Nick McElhinny et al., 2007; Pursell et al., 2007a]. We are advocates of the base analog approach, because HAP is not as toxic as many mutagens or pool imbalances and it is mutagenic even in wild-type strains, so no specific mutational alteration of replication parameters are required [Kozmin et al., 1998].

In earlier studies, HAP-induced errors occurred preferentially in one DNA strand [Shcherbakova and Pavlov, 1993; Shcherbakova and Pavlov, 1996; Kulikov et al., 2001] and HAP-induced errors were proofread by Pol δ and by Pol ϵ on opposite DNA strands [Shcherbakova and Pavlov, 1996]. In order to study this phenomenon in more detail, we placed a reversion reporter gene within chromosome III, in two orientations, and at multiple locations relative to early replication origins [Pavlov et al., 2002a]. The rate of G-C to A-T transitions, resulting from HAP misincorporation, differed by three- to tenfold, depending on the reporter orientation and its distance from the flanking replication origins. Those results demonstrated that active origins establish a mutational strand bias *in vivo* that is maintained over at least 70,000 base pairs, and reflects lower mutagenesis associated with replication of the lagging strand DNA template. The mechanism of the bias is unknown. It could be connected to the intrinsic property of lagging strand replication, to the identity of Pols replicating leading and lagging DNA strands or the efficiency of MMR on different strands. We have shown that MMR is the most important contributor to strand bias for another base analog, 8-oxoguanine [Pavlov et al., 2003]. We decided to investigate this phenomenon in more depth using a forward mutation system.

Mutational system of the *URA3* gene

A system for the selection of the *ura3* mutants with 5-fluoroorotic acid was devised in 1984 [Boeke et al., 1984]. The *URA3* is 804 bp-long, not too large for sequencing, yet not too small to limit a variety of sequence contexts where mutations could be found. The first spectrum of induced mutations in the *URA3* gene was published in 1986 [von Borstel and Lee, 1986]. Later, the *URA3* system was used for studies of induced and spontaneous mutagenesis in wild-type strains [Lee et al., 1988]. Mutation spectra were also determined in different polymerase mutants [von Borstel et al., 1993; Morrison and Sugino, 1994; Pursell et al., 2007a; Nick McElhinny et al., 2008]. Rates of mutations in the *URA3* vary about six-fold when the gene is placed artificially at different chromosomal locations. This may be connected to replication timing [Lang and Murray, 2011].

Several studies have utilized the *URA3* gene mutational system to study the effects of carcinogens, 2-acetylaminofluorene [Roy and Fuchs, 1994] and benzopyrene diol epoxide [Xie et al., 2003]. It was found that, under certain conditions, this forward mutation system

provides biased estimates of error specificity due to the presence of “jackpot” mutation sites. We have found several hotspots for HAP-induced errors in a spectrum with a limited number of mutations [Shcherbakova and Pavlov, 1993]. In extreme cases, in strains with a variant of Pol ϵ with low base selectivity, 60% of mutations in the *URA3* target occur in one spot, which severely reduces the power of this forward mutational system [Pursell et al., 2007a].

We placed the *URA3* gene to the right of a strong replication origin ARS306 using PCR fragments of the gene region flanked by the tails homologous to the selected site as described [Pavlov et al., 2002a] in yeast strain 9A-YPOM206 [Baranovskiy et al., 2008] (Fig. 5A). One location is close to the origin, another is 2 kb away and the last one is 8 kb away from the origin. For the two locations closest to the origin, we also inserted the gene in reverse orientation. Treatment by HAP caused a 50-fold increase of mutation rates which were similar in all variants. Most of the mutations were transitions in GC pairs. Pie diagrams near the arrows representing the *URA3* gene in Fig. 5A show types of mutational changes represented on the non-transcribed strand of the *URA3*. This sequence corresponds to the sequence of mRNA. Therefore, G to A transitions in forward orientation occur when HAPTP is incorporated opposite C into the leading DNA strand (Fig. 5B). In the reverse orientation, G to A transitions are caused by the incorporation of dHAPTP into the lagging DNA strand.

Mutation spectra of five variants were generally dissimilar: the change of location and the inversion of the reporter resulted in a statistically different distribution of mutations (Fig. 5C). Contrary to our expectations based on the data with the reversion system (see above), the proportion of G to A mutations was independent from the orientation of the gene, but partially dependent on the location (Fig. 5A). It appeared that initial HAP incorporations were predominant when replicating the transcribed DNA strand, irrespective of the direction of replication. The effect could be due to the dependence on transcription or a unique collection of HAP mutable sites in the transcribed strand. To consolidate the data with the previous results, we propose that there are two components of HAP-induced mutagenesis, comprising the final mutation spectra. One, minor component, which we were lucky to reveal in reversion system [Shcherbakova and Pavlov, 1996; Pavlov et al., 2002a], depends on replication direction. The major component depends on transcription or the identity of the transcribed DNA strand of the *URA3* gene, which is clearly visible in the forward spectra. Because of this mixture, the spectra in forward and reverse orientations were statistically different. It is known that HAP mutagenesis is dependent on replicative DNA Pols, therefore, the results will stimulate new experiments to reveal the additional properties of replication of the two strands of the *URA3* gene by these enzymes. The possible mechanism of transcription dependence of HAP mutagenesis is not known.

Okazaki fragments and mutagenesis hotspots

The mechanisms of coordination of activities of all DNA Pols at the fork are largely unknown. The best defined, as we discussed previously, is the role of Pol α . The patches synthesized by Pol α are at the beginning of each Okazaki fragment. Therefore, hotspots of mutations attributed to inaccurate Pol α are informative for the size of Okazaki fragments *in vivo*. In the current study we have chosen the *pol1-L868M* allele that encodes for very inaccurate Pol α [Niimi et al., 2004]. To prevent the correction of errors made by inaccurate Pol α , we inhibited MMR by cadmium (Cd^{2+}) [Jin et al., 2003], which we have shown synergistically increases mutation rates in the *pol1-L868M* mutant [Pavlov et al., 2006a].

The probability of detecting the same sites of Okazaki fragments in the genome among different cells is undoubtedly low. Therefore, it is generally impossible to find mutations

that happen in the beginning of the Okazaki fragments. There is one exception. The start sites of the Okazaki fragments will be at the same site in the reporter gene very close to the origin. Such a site could be used to map mutations associated with short patches synthesized by Pol α at the 5' ends of Okazaki fragments. Therefore, we inserted the *URA3* cassette in strain (-2)I-7B-YUNI300 (nicknamed $\Delta 1$) that we used previously to monitor strand-specific replication errors [Pavlov et al., 2002a]. We performed the analysis of the timing of the *ARS306* firing in the selected region of chromosome III by the two-dimensional gel analysis of chromosomal replication intermediates [Poloumienko et al., 2001]. The results for the wild-type strain and the strain with insertion very close to the right side of *ARS306* (R306) are presented in Fig. 6A.

We observed that replication intermediates started to appear 15 minutes after release from the cell cycle arrest and reached the maximum at 30 minutes. The placement of our reporter cassette very close to the replication origin does not affect *ARS306* firing. Control experiments reveal that the *pol1-L868M* mutation or the addition of HAP to growth medium also did not change the timing of *ARS306* firing (Fig. 6A). Next, we collected FOA^r mutants induced by Cd²⁺ in the *pol1-L868M* strain with the insertion of the *URA3* cassette in the R306 location and compared this distribution with the distribution of mutations under UV or in the strain with the *RAD27* deletion (Fig. 6B).

During the first crude analysis we divided the whole gene into 100 bp intervals and found that the distribution of mutations in the strains with the *pol1-L868M* mutation is similar to the distribution in the *rad27* strain (correlation coefficient 0.76, $P=0.02$) but different from the UV spectrum (left panels versus top right panel). It is known that a defect on the maturation of Okazaki fragments on the lagging DNA strand in *rad27* strains results in an accumulation of duplications [Tishkoff et al., 1997; Jin et al., 2001]. The mechanism of duplication involves the ligation of unrepaired 5' flaps. Therefore, the 5' end of duplications in the *rad27* strains marks sites where Okazaki fragments were initiated. The similarity of the two spectra, when boundaries of Okazaki fragments are affected by different mechanisms, indicates that the approach we have designed could be legitimate. It is noteworthy that the distribution of a detectable position in the *URA3* gene is quite uniform (Fig. 6B, bottom right panel), so all the observed differences reflect the peculiarities of the mutation process itself.

Next, in order to get further proof that the hotspots might be connected with patches at the 5' ends of Okazaki fragments, we moved the reporter by small increments away from the origin. The rationale for this experiment is outlined in a hypothetical scheme in Fig. 6 C. We expected that the hotspots of mutations would exhibit periodicity, which would depend on the position of the reporter in respect to the origin, and the positions of the peaks of mutations inside the gene would shift, but the distance of the peaks from the origin would be maintained (Fig. 6C). To implement this idea, we inserted the *URA3* gene in two orientations at positions differing by 75 bp in respect to replication origin and sequenced *ura3* in the *pol1-L868M* strains induced by Cd²⁺ in all locations (Fig 6D). The results show remarkable patterns of mutation distribution changes when the reporter is moved or inverted (Fig. 6D). First, the distribution of hotspots changed with the inversion of the reporter (compare three forward locations, upper half, with three reverse locations, lower half), suggesting that the mutation distribution depends on what strand the synthesis by inaccurate Pol α happens. Second, some hotspot regions are independent of the distance from the origin and are fixed for each orientation (red dotted vertical lines). It is interesting that all of these hotspots, corresponding for both orientations, were detected among 78 sequenced mutations when the reporter is located in forward orientation far from the origin (between *ARS305* and *ARS306*, where either strand could be replicated as a leading or lagging location in the *AUT4* gene [Pavlov et al., 2002a]). These mutation hotspots may be related to starts of

sequence-specific Okazaki fragments or reflect an unknown pathway of mutagenesis in strains with inaccurate Pol α . Third, we detected another class of hotspots, “moving hotspots” (black dotted vertical lines in Fig. 6D). When the reporter moved, they also moved, but their location is constant in respect to the distance from ARS. They were not detected in the location *URA3::AUT4* mentioned above due to the sample size limitation and likely the asynchronous starts of Okazaki fragments at this location.

We hypothesize that the moving hotspots represent mutations made by Pol α at the starts of Okazaki fragments. In a more likely mechanism, the location of the majority of Okazaki fragments is determined by the distance from the origin of replication. Of course, the hotspots are not in identical positions, but they are close. Some hotspots shown by dotted yellow lines (for example, at position 167 in the forward orientation, or 605 in the reverse orientation) belong to a mixed class (they are observed in a pair but not in all three locations), adding to the complexity of the analysis.

In attempt to correlate the mutation hotspots to the sizes of Okazaki fragments, we argue that the moving hotspots are the most plausible “witnesses” of transactions of Pol α at the beginning of the fragments. In Fig. 6D we used pink rectangles to mark the areas of the reporter that have the “moving” hotspots at the same distance from the *ARS306* in at least one (out of three) location of the *URA3* gene simultaneously with such a hotspot in at least one reverse orientation. Note that “moving” hotspots sometimes coincide with constant “red” hotspots or “yellow” hotspots (Fig. 6D). We have found three such regions, separated by 140–175 bp. This indicates that the sizes of the Okazaki fragments estimated by our method *in vivo* fall within the size range consistent with biochemical estimates of the size of the Okazaki fragment mentioned previously and a recent estimate of the size of the Okazaki fragment in yeast [Smith and Whitehouse, 2012].

The mechanisms of mutation generation in “constant” and “moving” hotspots in a mutant with inaccurate Pol α are not apparent. If all hotspots correlate with the start of Okazaki fragments it could be predicted that the start sites of some Okazaki fragments are fixed by the sequence of the reporter, and in some, depend only on the distance from the origin. We understand that the observed spectra are the result of many factors, such as the distribution of detectable positions and mutation hotspots. This may obscure the detection of some hotspots.

Our results suggest that base pair substitution mutability of the gene could be influenced by a very slight (75 bp) change of its location in the chromosome, because mutation hotspots are influenced by the positions of Okazaki fragments. Okazaki fragments in eukaryotes are linked to nucleosomes [Smith and Whitehouse, 2012], and therefore, it is likely that chromatin remodeling factors will affect the distribution of mutations in the genome. Taken together with the critical role of these factors in mismatch repair [Kadyrova et al., 2011; Schopf et al., 2012], in forthcoming years we expect to see more interpretations of mutagenic specificity that take chromatin structure into account. Mutagenesis is much more complicated than it was thought several years ago and mechanisms regulating the genome wide distribution of mutations are just beginning to emerge [Lang and Murray, 2011]. Sequencing of cancer genomes revealed that tumor genomes are highly enriched with mutations [Loeb, 2011] and the mutations are sometimes found in clusters, named mutation storms or kataegis [Nik-Zainal et al., 2012]. The exact nature of kataegis is not known and it is likely that multiple factors are involved, including the generation of base analogs in DNA by editing deaminases, patches of DNA synthesis by inaccurate translesion Pols, collisions with transcription and others.

Mutagenesis during specialized replication processes

In the cell there are several other processes beyond genome duplication requiring DNA synthesis. During the repair of DSB by homologous recombination, DNA synthesis is initiated by the invasion of the 3' end of the broken DNA-Rad51 nucleofilament into a donor DNA sequence. It was originally proposed that the classical replication fork, with leading and lagging strands, is formed and, hence all three replicative DNA polymerases, Pol α , Pol δ and Pol ϵ , were required for the completion of DSB-induced recombination [Holmes and Haber, 1999]. Later, the roles of polymerases were reevaluated and it was shown that DNA synthesis during recombinational repair involved the leading DNA strand and only redundant functions of Pol δ and Pol ϵ were required [Wang et al., 2004]. These replicative DNA polymerases are accurate, which is consistent with the notion that recombinational repair of DNA damage does not frequently generate mutations (hence, it is often referred to as “error-free” repair) [Friedberg, 2006]. However, the observation that the mutation rates are higher in meiosis than in mitosis led to a proposal that recombination can cause mutations [Magni and von Borstel, 1962]. It has been demonstrated that DNA synthesis associated with DSB repair in yeast is two orders of magnitude less accurate than during normal DNA replication, suggesting the involvement of a rather inaccurate DNA polymerase [Strathern et al., 1995]. The mutagenesis during recombination in their system was largely dependent on Pol ζ [Holbeck and Strathern, 1997; Rattray et al., 2002]. This suggests that Pol ζ can also be recruited to perform DNA synthesis on substrates that are generated during homologous recombination. Interestingly, the rate of recombination in the absence of Pol ζ was not changed. The unanswered questions are why and how “mutagenic” Pol ζ is allowed to participate in the reactions that could be performed by accurate DNA polymerases. One possibility is that Pol ζ could participate in the processing of damaged DNA recombination intermediates. Because the proportion of such abnormal intermediates is presumably small, the absence of Pol ζ would affect the frequency of mutation, but not the overall rate of recombination.

Recently, the interest for the phenomenon of mutagenesis during recombination was renewed. It was found that the synthesis of DNA during gene conversion is even more inaccurate than was thought originally and was dependent on replicative DNA Pols [Hicks et al., 2010]. Importantly, the specialized synthesis of DNA driven by double strand breaks was inaccurate over long distances (hundreds of kilobases) in break-induced replication [Deem et al., 2011]. Replication established under these conditions suffers from moderate but numerous limitations, such as elevated dNTP pools, somewhat reduced proofreading, and less effective mismatch repair. Multiplication of these mild effects results in a tremendous increase of mutation rates.

Another special condition of mutagenesis involves circumstances when single-stranded DNA persists longer than usual and results in localized mutagenesis and clustered mutations [Burch et al., 2011; Roberts et al., 2012]. The appearance of these mutations is proposed to contribute to cancer development [Nik-Zainal et al., 2012]. The hypothesis that persistent ssDNA is a substrate of editing deaminases [Roberts et al., 2012] has to be reconciled with the ability of replication protein A, that covers ssDNA, to inhibit activity and processivity of editing deaminases [Pham et al., 2008; Lada et al., 2011].

Human diseases caused by replication problems

Genome stability is compromised not only by DNA damage. Some DNA sequence contexts can impede DNA replication or repair. A classic example of genomic instability caused by problems in replicating an unusual DNA template is repeat expansions. These so-called “dynamic mutations” are the cause of more than 40 human disorders with a wide range of

manifestations, such as mental retardation, muscular atrophy, cranial dysplasia, and increased risk of prostate cancer [Pearson et al., 2005]. The current models of triplet instability predict that the maximal size of repeat expansion depends on the size of the Okazaki fragments on the lagging strand of DNA [Pearson et al., 2005; Shishkin et al., 2009]. This size may depend on the relative activity of primase and Pol α as well as on the efficiency of chromatin remodeling [Trakselis et al., 2001; Smith and Whitehouse, 2012]. The adverse effects of the repeated DNA on replication and repair are linked to the ability of these sequences to form aberrant DNA structures, such as intra-strand hairpins, and triple- and quadruple-stranded DNA [Kovtun and McMurray, 2001; Lahue and Slater, 2003; Cleary and Pearson, 2005; Mirkin, 2005; Mirkin, 2006], which are difficult to replicate by Pols δ and ϵ [Abdulovic et al., 2011; Korona et al., 2011]. Mutations under these conditions are most likely generated by various switches of template DNA and the forks discussed in excellent reviews [Maki, 2002; Cleary and Pearson, 2005; Chang and Cimprich, 2009].

The perturbation of DNA replication due to depletion or imbalance of DNA precursor pools or insufficient DNA polymerase activity, leads to chromosome instability localized in the regions that are difficult to replicate [Mathews and Ji, 1992; Glover et al., 2005; Lemoine et al., 2005; Nakamura et al., 2005; Admire et al., 2006]. In the case of replicative DNA polymerases, any significant changes in their activity are lethal, but variations in their activity or fidelity may have consequences for human disease. It is intriguing that a well known cancer cell line, DLD-1, possesses an allele of the *POLD1* gene encoding for the most inaccurate variant of Pol δ ever seen [Daee et al., 2010].

Defects in the proofreading activity of replicative Pol δ and Pol ϵ in mice result in an increased cancer incidence [Goldsby et al., 2001; Goldsby et al., 2002; Albertson et al., 2009]. The defect of proofreading of mitochondrial Pol γ in mice leads to an accumulation of mutations in mitochondria and to premature aging [Kujoth et al., 2005]. This suggests that defective proofreading in humans will likely result in cancer predisposition and accelerated aging as well. Several mutations in the polymerase and exonuclease domains of human Pol γ or in the gene encoding the accessory subunit of Pol γ have been associated with progressive external ophthalmoplegia (PEO), a rare disease characterized by the accumulation of point mutations and large deletions in mitochondrial DNA [Van Goethem et al., 2001; Lamantea et al., 2002]. Mutations in the *POLG* gene have also been associated with Parkinson's disease, male infertility and ataxia-neuropatia [Graziewicz et al., 2006]. Recent work demonstrated that mice heterozygous for active site mutation leading to compromised base selection of Pol δ accumulate tumors at a higher rate than control mice, while homozygosity for such a mutation is incompatible with mouse life [Venkatesan et al., 2006].

The activity of TLS polymerases modulates the rate of genotoxicant-induced mutations. The dysfunction of these enzymes is associated with disease, too. Humans carrying mutations in the XPV (*POLH/RAD30*) gene that inactivate the function of Pol η suffer from Xeroderma pigmentosum, exhibiting sensitivity to sunlight and skin cancer predisposition [Johnson et al., 1999; Masutani et al., 1999]. In XPV patients that lack Pol η , cyclobutane pyrimidine dimers are processed in a manner that generates the mutations that lead to skin cancer. It is also possible that the mutagenic processing of oxidative DNA damage produced by sunlight radiation is elevated when Pol η is defective [Kozmin et al., 2005]. Interestingly, the two seemingly opposite properties of Pol η are important for human health. Relevant to protection from UV-induced carcinogenesis is the ability of Pol η to contribute to the prevention of mutations during the bypass of damage. At the same time, the ability of Pol η to generate mutations at a high rate is important for the proper function of the immune system [Vaisman et al., 2004]. In the cells from XP-V patients lacking Pol η , related Pol ι is responsible for the high frequency of UV-induced mutagenesis, and ultimately malignant transformation [Lawrence et al., 2000]. Defects and polymorphism in the *POLI* are

associated with an increased risk of lung cancer [Lee and Matsushita, 2005; Sakiyama et al., 2005] and 129/J mice, devoid of Pol ι , are prone to an elevated occurrence of UV-induced skin tumors [Dumstorf et al., 2006; Ohkumo et al., 2006].

While DNA polymerases can contribute to genome stability and cancer incidence, they are also critical for the propagation of tumors. A better understanding of their roles is important for the identification of new prognostic markers, as well as for the design of anti-cancer drugs [Wang, 2001; Madhusudan and Middleton, 2005].

Single nucleotide polymorphisms (SNPs) in the human *REV1* gene were also found to be associated with increased cancer risk [Sakiyama et al., 2005]. In addition, changes in the expression of genes encoding Pol ι and Pol κ have been found in human tumors [O-Wang et al., 2001; Yang et al., 2004; Albertella et al., 2005; Bavoux et al., 2005]. While DNA polymerases can contribute to genome stability and cancer incidence, they are also critical for the propagation of tumors. A better understanding of their roles is important for the identification of new prognostic markers, as well as for the design of anti-cancer drugs [Wang, 2001; Madhusudan and Middleton, 2005].

ITPA deficiency causing alterations of RNA and DNA precursor pools has been found to play a role in the response to several drugs. Marinaki et al. were the first to discover an association between ITPA deficiency and increased levels of adverse reactions from the drug azathioprine used in the treatment of inflammatory bowel disease [Marinaki et al., 2004]. Azathioprine and other mercaptopurine drugs are also used in the treatment of leukemia and auto immune disorders. Stocco and coworkers have shown that ITPA deficiency is associated with febrile neutropenia in acute lymphoblastic leukemia patients treated with mercaptopurines [Stocco et al., 2009]. On the other hand, ITPA deficiency was observed to be associated with a better response to low-dose azathioprine in the treatment of systemic lupus erythematosus [Okada et al., 2009]. ITPA deficiency was found to protect against hemolytic anemia in hepatitis C patients treated with ribavirin [Fellay et al., 2010]. The ITP which accumulates in erythrocytes compensates for ribavirin-induced decreases in ATP and GTP by substituting for GTP in the adenylosuccinate synthase reaction, which leads to ATP production [Hitomi et al., 2011]. This is additional evidence for the connection of the pathways of base analogs and purine biosynthesis (Fig. 4).

In the crystal structure, the location of P32 is away from the active site [Porta et al., 2006; Stenmark et al., 2007]. Consistent with that, ITPA P32T is almost as active as wild-type ITPA but it is temperature labile [Stepchenkova et al., 2009b; Herting et al., 2010]. The level of ITPA protein in human dermal fibroblasts with endogenous ITPA-P32T is approximately nine-fold lower than by a corresponding cell line with wild-type ITPA, thus alluding to the possibility that this mutation triggers degradation of ITPA [Stepchenkova et al., 2009b]. Taken together, these two observations help to understand why the severity of the defect of ITPA P32T is much less than anticipated by extrapolation from mouse knockout data. ITPA P32T can provide enough ITPase activity in some tissues but not enough in the other. It is known that the activity and levels of ITPA are very different in different tissues [Verhoef et al., 1980]. Although ITPA deficiency in humans is currently considered benign, it is possible that the presence of ITPA P32T creates a predisposition to cancer, in general, and a drug-induced secondary cancer, in particular.

We have found that the P32T variant of ITPA aggravates base analog induced killing and instability of genetic material [Waisertreiger et al., 2010]. In cell lines, inhibition of the *ITPA* by shRNA leads to elevated levels of DNA breaks, mutations and apoptosis [Menezes et al., 2012]. Therefore, the investigation of ITPA goes beyond the study of the adverse reactions to purine analogs in patients with ITPA polymorphism [Maeda et al., 2005;

Zelinkova et al., 2006]. Given the high allele frequency of this variant in the human population, it is clear that it represents potential risk factors for cancer and other diseases affecting a substantial proportion of the human population.

Acknowledgments

We are grateful to Dr. Polina Shcherbakova for critical reading of the manuscript and Dr. Peter Burgers and Dr. Helle Ulrich for valuable discussions related to Pol ζ. We thank Kristi Berger for expert editing. The work was supported by UNMC Eppley Cancer Center seed grants and, in part, by NIGMS grant R01GM082923 to THT, NCI grant CA129925 to YIP, Smoking Disease Research Program DHHS grant 2011–27, and by the Russian federal program “Innovation scientific personnel,” State contract 14.740.11.0916 to YIP. IBR was supported by the Intramural Research Program of the National Library of Medicine at the National Institutes of Health/DHHS. Undergraduate summer student Ms. Marie Rose Donnelly assisted in genetic experiments. Her participation was made possible by grants from the National Center for Research Resources (5P20RR016469) and the National Institute for General Medical Science (NIGMS) (8P20GM103427).

References

- Abdul-Masih MT, Bessman MJ. Biochemical studies on the mutagen, 6-N-hydroxylaminopurine. Synthesis of the deoxynucleoside triphosphate and its incorporation into DNA *in vitro*. J Biol Chem. 1986; 261(5):2020–2026. [PubMed: 3944125]
- Abdulovic AL, Hile SE, Kunkel TA, Eckert KA. The *in vitro* fidelity of yeast DNA polymerase delta and polymerase epsilon holoenzymes during dinucleotide microsatellite DNA synthesis. DNA Repair (Amst). 2011; 10(5):497–505. [PubMed: 21429821]
- Abolhassani N, Iyama T, Tsuchimoto D, Sakumi K, Ohno M, Behmanesh M, Nakabeppu Y. NUDT16 and ITPA play a dual protective role in maintaining chromosome stability and cell growth by eliminating dIDP/IDP and dITP/ITP from nucleotide pools in mammals. Nucleic Acids Research. 2010; 38(9):2891–2903. [PubMed: 20081199]
- Acharya N, Johnson RE, Pages V, Prakash L, Prakash S. Yeast Rev1 protein promotes complex formation of DNA polymerase zeta with Pol32 subunit of DNA polymerase delta. Proc Natl Acad Sci U S A. 2009; 106(24):9631–9636. [PubMed: 19487673]
- Admire A, Shanks L, Danzl N, Wang M, Weier U, Stevens W, Hunt E, Weinert T. Cycles of chromosome instability are associated with a fragile site and are increased by defects in DNA replication and checkpoint controls in yeast. Genes Dev. 2006; 20(2):159–173. [PubMed: 16384935]
- Aksenova A, Volkov K, Maceluch J, Pursell ZF, Rogozin IB, Kunkel TA, Pavlov YI, Johansson E. Mismatch repair-independent increase in spontaneous mutagenesis in yeast lacking non-essential subunits of DNA polymerase epsilon. PLoS Genet. 2010; 6(11):e1001209. [PubMed: 21124948]
- Albertella MR, Lau A, O'Connor MJ. The overexpression of specialized DNA polymerases in cancer. DNA Repair (Amst). 2005; 4(5):583–593. [PubMed: 15811630]
- Albertson TM, Ogawa M, Bugni JM, Hays LE, Chen Y, Wang Y, Treuting PM, Heddle JA, Goldsby RE, Preston BD. DNA polymerase epsilon and delta proofreading suppress discrete mutator and cancer phenotypes in mice. Proc Natl Acad Sci U S A. 2009; 106(40):17101–17104. [PubMed: 19805137]
- Ames BN, Gold LS. Endogenous mutagens and the causes of aging and cancer. Mutation Research. 1991; 250(1–2):3–16. [PubMed: 1944345]
- Anderson S, DePamphilis ML. Metabolism of Okazaki fragments during simian virus 40 DNA replication. J Biol Chem. 1979; 254(22):11495–11504. [PubMed: 227871]
- Araki H, Hamatake RK, Morrison A, Johnson AL, Johnston LH, Sugino A. Cloning *DPB3*, the gene encoding the third subunit of DNA polymerase II of *Saccharomyces cerevisiae*. Nucleic Acids Res. 1991; 19(18):4867–4872. [PubMed: 1923754]
- Araki H. Initiation of chromosomal DNA replication in eukaryotic cells; contribution of yeast genetics to the elucidation. Genes Genet Syst. 2011; 86(3):141–149. [PubMed: 21952204]
- Aravind L, Koonin EV. Phosphoesterase domains associated with DNA polymerases of diverse origins. Nucleic Acids Res. 1998; 26(16):3746–3752. [PubMed: 9685491]

- Asturias FJ, Cheung IK, Sabouri N, Chilkova O, Wepplo D, Johansson E. Structure of *Saccharomyces cerevisiae* DNA polymerase epsilon by cryo-electron microscopy. *Nat Struct Mol Biol.* 2006; 13(1):35–43. [PubMed: 16369485]
- Baranovskiy AG, Babayeva ND, Liston VG, Rogozin IB, Koonin EV, Pavlov YI, Vassilyev DG, Tahirov TH. X-ray structure of the complex of regulatory subunits of human DNA polymerase delta. *Cell Cycle.* 2008; 7(19):3026–3036. [PubMed: 18818516]
- Baranovskiy AG, Lada AG, Siebler HM, Zhang Y, Pavlov YI, Tahirov TH. DNA polymerase delta and zeta switch by sharing accessory subunits of DNA polymerase delta. *J Biol Chem.* 2012; 287(21):17281–17287. [PubMed: 22465957]
- Bavoux C, Leopoldino AM, Bergoglio V, JOW, Ogi T, Bieth A, Judde JG, Pena SD, Poupon MF, Helleday T, Tagawa M, Machado C, Hoffmann JS, Cazaux C. Up-regulation of the error-prone DNA polymerase k promotes pleiotropic genetic alterations and tumorigenesis. *Cancer Res.* 2005; 65(1):325–330. [PubMed: 15665310]
- Bebenek K, Kunkel TA. Functions of DNA polymerases. *Adv Protein Chem.* 2004; 69:137–165. [PubMed: 15588842]
- Behmanesh M, Sakumi K, Abolhassani N, Toyokuni S, Oka S, Ohnishi YN, Tsuchimoto D, Nakabeppu Y. ITPase-deficient mice show growth retardation and die before weaning. *Cell Death and Differentiation.* 2009; 16(10):1315–1322. [PubMed: 19498443]
- Bermudez VP, Farina A, Raghavan V, Tappin I, Hurwitz J. Studies on human DNA polymerase epsilon and GINS complex and their role in DNA replication. *J Biol Chem.* 2011; 286(33):28963–28977. [PubMed: 21705323]
- Bierau J, Lindhout M, Bakker JA. Pharmacogenetic significance of inosine triphosphatase. *Pharmacogenomics.* 2007; 8(9):1221–1228. [PubMed: 17924837]
- Biertumpfel C, Zhao Y, Kondo Y, Ramon-Maiques S, Gregory M, Lee JY, Masutani C, Lehmann AR, Hanaoka F, Yang W. Structure and mechanism of human DNA polymerase eta. *Nature.* 2010; 465(7301):1044–1048. [PubMed: 20577208]
- Boeke JD, LaCrute F, Fink GR. A positive selection for mutants lacking orotidine-5'-phosphate decarboxylase activity in yeast: 5-fluoro-orotic acid resistance. *Mol Gen Genet.* 1984; 197(2):345–346. [PubMed: 6394957]
- Bradshaw JS, Kuzminov A. RdgB acts to avoid chromosome fragmentation in *Escherichia coli*. *Mol Microbiol.* 2003; 48(6):1711–1725. [PubMed: 12791149]
- Budke B, Kuzminov A. Production of clastogenic DNA precursors by the nucleotide metabolism in *Escherichia coli*. *Mol Microbiol.* 2009; 75(1):230–245. [PubMed: 19943897]
- Bullock PA, Seo YS, Hurwitz J. Initiation of simian virus 40 DNA synthesis *in vitro*. *Mol Cell Biol.* 1991; 11(5):2350–2361. [PubMed: 1673224]
- Burch LH, Yang Y, Sterling JF, Roberts SA, Chao FG, Xu H, Zhang L, Walsh J, Resnick MA, Mieczkowski PA, Gordenin DA. Damage-induced localized hypermutability. *Cell Cycle.* 2011; 10(7):1073–1085. [PubMed: 21406975]
- Burgers PM. Polymerase dynamics at the eukaryotic DNA replication fork. *J Biol Chem.* 2009; 284(7):4041–4045. [PubMed: 18835809]
- Burgis NE, Brucker JJ, Cunningham RP. Repair system for noncanonical purines in *Escherichia coli*. *J Bacteriol.* 2003; 185(10):3101–3110. [PubMed: 12730170]
- Burgis NE, Cunningham RP. Substrate specificity of RdgB protein, a deoxyribonucleoside triphosphate pyrophosphohydrolase. *J Biol Chem.* 2007; 282(6):3531–3538. [PubMed: 17090528]
- Cao H, Hegele RA. DNA polymorphisms in *ITPA* including basis of inosine triphosphatase deficiency. *Journal of Human Genetics.* 2002; 47(11):620–622. [PubMed: 12436200]
- Chabes A, Georgieva B, Domkin V, Zhao X, Rothstein R, Thelander L. Survival of DNA damage in yeast directly depends on increased dNTP levels allowed by relaxed feedback inhibition of ribonucleotide reductase. *Cell.* 2003; 112(3):391–401. [PubMed: 12581528]
- Chanet R, Heude M. Characterization of mutations that are synthetic lethal with *pol3-13*, a mutated allele of DNA polymerase delta in *Saccharomyces cerevisiae*. *Curr Genet.* 2003; 43(5):337–350. [PubMed: 12759774]
- Chang DJ, Cimprich KA. DNA damage tolerance: when it's OK to make mistakes. *Nat Chem Biol.* 2009; 5(2):82–90. [PubMed: 19148176]

- Chelico L, Pham P, Goodman MF. Stochastic properties of processive cytidine DNA deaminases AID and APOBEC3G. *Philos Trans R Soc Lond B Biol Sci.* 2009; 364(1517):583–593. [PubMed: 19022738]
- Chilkova O, Stenlund P, Isoz I, Stith CM, Grabowski P, Lundstrom EB, Burgers PM, Johansson E. The eukaryotic leading and lagging strand DNA polymerases are loaded onto primer-ends via separate mechanisms but have comparable processivity in the presence of PCNA. *Nucleic Acids Res.* 2007; 35(19):6588–6597. [PubMed: 17905813]
- Cleary JD, Pearson CE. Replication fork dynamics and dynamic mutations: the fork-shift model of repeat instability. *Trends Genet.* 2005; 21(5):272–280. [PubMed: 15851063]
- Clyman J, Cunningham RP. *Escherichia coli* K-12 mutants in which viability is dependent on *recA* function. *J Bacteriol.* 1987; 169(9):4203–4210. [PubMed: 2442140]
- Colussi C, Parlanti E, Degan P, Aquilina G, Barnes D, Macpherson P, Karran P, Crescenzi M, Dogliotti E, Bignami M. The mammalian mismatch repair pathway removes DNA 8-oxodGMP incorporated from the oxidized dNTP pool. *Curr Biol.* 2002; 12(11):912–918. [PubMed: 12062055]
- Conticello SG, Langlois MA, Neuberger MS. Insights into DNA deaminases. *Nat Struct Mol Biol.* 2007; 14(1):7–9. [PubMed: 17203067]
- Copeland WC, Wang TS. Catalytic subunit of human DNA polymerase alpha overproduced from baculovirus-infected insect cells. Structural and enzymological characterization. *J Biol Chem.* 1991; 266(33):22739–22748. [PubMed: 1939281]
- Copeland WC, Wang TS. Enzymatic characterization of the individual mammalian primase subunits reveals a biphasic mechanism for initiation of DNA replication. *J Biol Chem.* 1993; 268(35):26179–26189. [PubMed: 8253737]
- D'Errico M, Parlanti E, Dogliotti E. Mechanism of oxidative DNA damage repair and relevance to human pathology. *Mutat Res.* 2008; 659(1–2):4–14. [PubMed: 18083609]
- Daele DL, Mertz TM, Shcherbakova PV. A cancer-associated DNA polymerase delta variant modeled in yeast causes a catastrophic increase in genomic instability. *Proc Natl Acad Sci U S A.* 2010; 107(1):157–162. [PubMed: 19966286]
- Daigaku Y, Davies AA, Ulrich HD. Ubiquitin-dependent DNA damage bypass is separable from genome replication. *Nature.* 2010; 465(7300):951–955. [PubMed: 20453836]
- Dalhus B, Arvai AS, Rosnes I, Olsen OE, Backe PH, Alseth I, Gao H, Cao W, Tainer JA, Bjorås M. Structures of endonuclease V with DNA reveal initiation of deaminated adenine repair. *Nature Structural & Molecular Biology.* 2009; 16(2):138–143.
- Datta A, Schmeits JL, Amin NS, Lau PJ, Myung K, Kolodner RD. Checkpoint-dependent activation of mutagenic repair in *Saccharomyces cerevisiae* *pol3-01* mutants. *Mol Cell.* 2000; 6(3):593–603. [PubMed: 11030339]
- De Luca G, Russo MT, Degan P, Tiveron C, Zijno A, Meccia E, Ventura I, Mattei E, Nakabeppu Y, Crescenzi M, Pepponi R, Pezzola A, Popoli P, Bignami M. A role for oxidized DNA precursors in Huntington's disease-like striatal neurodegeneration. *PLoS Genetics.* 2008; 4(11):e1000266. [PubMed: 19023407]
- Dedon PC, Tannenbaum SR. Reactive nitrogen species in the chemical biology of inflammation. *Arch Biochem Biophys.* 2004; 423(1):12–22. [PubMed: 14989259]
- Deem A, Keszthelyi A, Blackgrove T, Vayl A, Coffey B, Mathur R, Chabes A, Malkova A. Break-induced replication is highly inaccurate. *PLoS Biol.* 2011; 9(2):e1000594. [PubMed: 21347245]
- Diamant N, Hendel A, Vered I, Carell T, Reissner T, de Wind N, Geaciov N, Livneh Z. DNA damage bypass operates in the S and G2 phases of the cell cycle and exhibits differential mutagenicity. *Nucleic Acids Res.* 2012; 40(1):170–180. [PubMed: 21908406]
- Dua R, Levy DL, Campbell JL. Role of the putative zinc finger domain of *Saccharomyces cerevisiae* DNA polymerase epsilon in DNA replication and the S/M checkpoint pathway. *J Biol Chem.* 1998; 273(45):30046–30055. [PubMed: 9792727]
- Dumstorf CA, Clark AB, Lin Q, Kissling GE, Yuan T, Kucherlapati R, McGregor WG, Kunkel TA. Participation of mouse DNA polymerase iota in strand-biased mutagenic bypass of UV photoproducts and suppression of skin cancer. *Proc Natl Acad Sci U S A.* 2006; 103(48):18083–18088. [PubMed: 17114294]

- Fellay J, Thompson AJ, Ge D, Gumbs CE, Urban TJ, Shianna KV, Little LD, Qiu P, Bertelsen AH, Watson M, Warner A, Muir AJ, Brass C, Albrecht J, Sulkowski M, McHutchison JG, Goldstein DB. ITPA gene variants protect against anaemia in patients treated for chronic hepatitis C. *Nature*. 2010; 464(7287):405–408. [PubMed: 20173735]
- Fijalkowska IJ, Jonczyk P, Tkaczyk MM, Bialoskorska M, Schaaper RM. Unequal fidelity of leading strand and lagging strand DNA replication on the *Escherichia coli* chromosome. *Proc Natl Acad Sci U S A*. 1998; 95(17):10020–10025. [PubMed: 9707593]
- Friedberg, EC. DNA repair and mutagenesis. Washington, D.C: ASM Press; 2006. p. xxixp. 1118
- Friedman KL, Brewer BJ. Analysis of replication intermediates by two-dimensional agarose gel electrophoresis. *Methods Enzymol*. 1995; 262:613–627. [PubMed: 8594382]
- Fukui T, Yamauchi K, Muroya T, Akiyama M, Maki H, Sugino A, Waga S. Distinct roles of DNA polymerases delta and epsilon at the replication fork in *Xenopus* egg extracts. *Genes Cells*. 2004; 9(3):179–191. [PubMed: 15005706]
- Furukohri A, Goodman MF, Maki H. A dynamic polymerase exchange with *Escherichia coli* DNA polymerase IV replacing DNA polymerase III on the sliding clamp. *J Biol Chem*. 2008; 283(17):11260–11269. [PubMed: 18308729]
- Gan GN, Wittschieben JP, Wittschieben BO, Wood RD. DNA polymerase zeta (pol zeta) in higher eukaryotes. *Cell Res*. 2008; 18(1):174–183. [PubMed: 18157155]
- Garg P, Stith CM, Sabouri N, Johansson E, Burgers PM. Idling by DNA polymerase delta maintains a ligatable nick during lagging-strand DNA replication. *Genes Dev*. 2004; 18(22):2764–2773. [PubMed: 15520275]
- Garg P, Burgers PM. DNA polymerases that propagate the eukaryotic DNA replication fork. *Crit Rev Biochem Mol Biol*. 2005; 40(2):115–128. [PubMed: 15814431]
- Garg P, Stith CM, Majka J, Burgers PM. Proliferating cell nuclear antigen promotes translesion synthesis by DNA polymerase zeta. *J Biol Chem*. 2005; 280(25):23446–23450. [PubMed: 15879599]
- Gari K, Ortiz AML, Borel V, Flynn H, Skehel JM, Boulton SJ. *MMS19* Links Cytoplasmic Iron-Sulfur Cluster Assembly to DNA Metabolism. *Science*. 2012; 337(6091):243–245. [PubMed: 22678361]
- Giot L, Chanet R, Simon M, Facca C, Faye G. Involvement of the yeast DNA polymerase delta in DNA repair in vivo. *Genetics*. 1997; 146(4):1239–1251. [PubMed: 9258670]
- Gloor JW, Balakrishnan L, Campbell JL, Bambara RA. Biochemical analyses indicate that binding and cleavage specificities define the ordered processing of human Okazaki fragments by Dna2 and FEN1. *Nucleic Acids Res*. 2012; 40(14):6774–6786. [PubMed: 22570407]
- Glover TW, Arlt MF, Casper AM, Durkin SG. Mechanisms of common fragile site instability. *Hum Mol Genet*. 2005; 14(Spec No 2):R197–205. [PubMed: 16244318]
- Goldsby RE, Lawrence NA, Hays LE, Olmsted EA, Chen X, Singh M, Preston BD. Defective DNA polymerase-delta proofreading causes cancer susceptibility in mice. *Nat Med*. 2001; 7(6):638–639. [PubMed: 11385474]
- Goldsby RE, Hays LE, Chen X, Olmsted EA, Slayton WB, Spangrude GJ, Preston BD. High incidence of epithelial cancers in mice deficient for DNA polymerase delta proofreading. *Proc Natl Acad Sci U S A*. 2002; 99(24):15560–15565. [PubMed: 12429860]
- Gomes XV, Burgers PM. Two modes of FEN1 binding to PCNA regulated by DNA. *EMBO J*. 2000; 19(14):3811–3821. [PubMed: 10899134]
- Gray FC, Pohler JR, Warbrick E, MacNeill SA. Mapping and mutation of the conserved DNA polymerase interaction motif (DPIM) located in the C-terminal domain of fission yeast DNA polymerase delta subunit Cdc27. *BMC Mol Biol*. 2004; 5(1):21. [PubMed: 15579205]
- Graziewicz MA, Longley MJ, Copeland WC. DNA polymerase gamma in mitochondrial DNA replication and repair. *Chem Rev*. 2006; 106(2):383–405. [PubMed: 16464011]
- Grollman AP, Moriya M. Mutagenesis by 8-oxoguanine: an enemy within. *Trends Genet*. 1993; 9(7):246–249. [PubMed: 8379000]
- Gu L, Hong Y, McCulloch S, Watanabe H, Li GM. ATP-dependent interaction of human mismatch repair proteins and dual role of PCNA in mismatch repair. *Nucleic Acids Res*. 1998; 26(5):1173–1178. [PubMed: 9469823]

- Guo JU, Su Y, Zhong C, Ming GL, Song H. Emerging roles of TET proteins and 5-hydroxymethylcytosines in active DNA demethylation and beyond. *Cell Cycle*. 2011; 10(16):2662–2668. [PubMed: 21811096]
- Gutierrez PJ, Wang TS. Genomic instability induced by mutations in *Saccharomyces cerevisiae* *POL1*. *Genetics*. 2003; 165(1):65–81. [PubMed: 14504218]
- Hakim IA, Harris RB, Brown S, Chow HH, Wiseman S, Agarwal S, Talbot W. Effect of increased tea consumption on oxidative DNA damage among smokers: a randomized controlled study. *J Nutr*. 2003; 133(10):3303S–3309S. [PubMed: 14519830]
- Harfe BD, Jinks-Robertson S. DNA mismatch repair and genetic instability. *Annu Rev Genet*. 2000; 34:359–399. [PubMed: 11092832]
- Herman TM, DePamphilis ML, Wassarman PM. Structure of chromatin at deoxyribonucleic acid replication forks: Okazaki fragments released from replicating SV40 chromosomes by single-strand specific endonucleases are not in nucleosomes. *Biochemistry*. 1979; 18(21):4563–4571. [PubMed: 227443]
- Herting G, Barber K, Zappala MR, Cunningham RP, Burgis NE. Quantitative in vitro and in vivo characterization of the human P32T mutant ITPase. *Biochimica et Biophysica Acta*. 2010; 1802(2):269–274. [PubMed: 19914375]
- Hicks WM, Kim M, Haber JE. Increased mutagenesis and unique mutation signature associated with mitotic gene conversion. *Science*. 2010; 329(5987):82–85. [PubMed: 20595613]
- Hiraga S, Hagihara-Hayashi A, Ohya T, Sugino A. DNA polymerases alpha, delta, and epsilon localize and function together at replication forks in *Saccharomyces cerevisiae*. *Genes Cells*. 2005; 10(4):297–309. [PubMed: 15773893]
- Hitomi Y, Cirulli ET, Fellay J, McHutchison JG, Thompson AJ, Gumbs CE, Shianna KV, Urban TJ, Goldstein DB. Inosine triphosphate protects against ribavirin-induced adenosine triphosphate loss by adenylosuccinate synthase function. *Gastroenterology*. 2011; 140(4):1314–1321. [PubMed: 21199653]
- Hochhauser SJ, Weiss B. *Escherichia coli* mutants deficient in deoxyuridine triphosphatase. *J Bacteriol*. 1978; 134(1):157–166. [PubMed: 148458]
- Hoege C, Pfander B, Moldovan GL, Pyrowolakis G, Jentsch S. RAD6-dependent DNA repair is linked to modification of PCNA by ubiquitin and SUMO. *Nature*. 2002; 419(6903):135–141. [PubMed: 12226657]
- Holbeck SL, Strathern JN. A role for *REV3* in mutagenesis during double-strand break repair in *Saccharomyces cerevisiae*. *Genetics*. 1997; 147(3):1017–1024. [PubMed: 9383049]
- Holmes AM, Haber JE. Double-strand break repair in yeast requires both leading and lagging strand DNA polymerases. *Cell*. 1999; 96(3):415–424. [PubMed: 10025407]
- Huang ME, de Calignon A, Nicolas A, Galibert F. POL32, a subunit of the *Saccharomyces cerevisiae* DNA polymerase delta, defines a link between DNA replication and the mutagenic bypass repair pathway. *Curr Genet*. 2000; 38(4):178–187. [PubMed: 11126776]
- Huang ME, Rio AG, Galibert MD, Galibert F. Pol32, a subunit of *Saccharomyces cerevisiae* DNA polymerase delta, suppresses genomic deletions and is involved in the mutagenic bypass pathway. *Genetics*. 2002; 160(4):1409–1422. [PubMed: 11973297]
- Hwang KY, Chung JH, Kim SH, Han YS, Cho Y. Structure-based identification of a novel NTPase from *Methanococcus jannaschii*. *Nat Struct Biol*. 1999; 6(7):691–696. [PubMed: 10404228]
- Iida T, Araki H. Noncompetitive counteractions of DNA polymerase epsilon and ISW2/yCHRAC for epigenetic inheritance of telomere position effect in *Saccharomyces cerevisiae*. *Mol Cell Biol*. 2004; 24(1):217–227. [PubMed: 14673157]
- Iwaki T, Kawamura A, Ishino Y, Kohno K, Kano Y, Goshima N, Yara M, Furusawa M, Doi H, Imamoto F. Preferential replication-dependent mutagenesis in the lagging DNA strand in *Escherichia coli*. *Mol Gen Genet*. 1996; 251(6):657–664. [PubMed: 8757396]
- Jackson AL, Loeb LA. The mutation rate and cancer. *Genetics*. 1998; 148(4):1483–1490. [PubMed: 9560368]
- Jaszczur M, Flis K, Rudzka J, Kraszewska J, Budd ME, Polaczek P, Campbell JL, Jonczyk P, Fijalkowska IJ. Dpb2p, a noncatalytic subunit of DNA polymerase epsilon, contributes to the

fidelity of DNA replication in *Saccharomyces cerevisiae*. *Genetics*. 2008; 178(2):633–647. [PubMed: 18245343]

- Jaszczur M, Rudzka J, Kraszewska J, Flis K, Polaczek P, Campbell JL, Fijalkowska JJ, Jonczyk P. Defective interaction between Pol2p and Dpb2p, subunits of DNA polymerase epsilon, contributes to a mutator phenotype in *Saccharomyces cerevisiae*. *Mutat Res*. 2009; 669(1–2):27–35. [PubMed: 19463834]
- Jin YH, Obert R, Burgers PM, Kunkel TA, Resnick MA, Gordenin DA. The 3'→5' exonuclease of DNA polymerase delta can substitute for the 5' flap endonuclease Rad27/Fen1 in processing Okazaki fragments and preventing genome instability. *Proc Natl Acad Sci U S A*. 2001; 98(9):5122–5127. [PubMed: 11309502]
- Jin YH, Clark AB, Slebos RJ, Al-Refai H, Taylor JA, Kunkel TA, Resnick MA, Gordenin DA. Cadmium is a mutagen that acts by inhibiting mismatch repair. *Nat Genet*. 2003; 34(3):326–329. [PubMed: 12796780]
- Jin YH, Garg P, Stith CM, Al-Refai H, Sterling JF, Murray LJ, Kunkel TA, Resnick MA, Burgers PM, Gordenin DA. The multiple biological roles of the 3'→5' exonuclease of *Saccharomyces cerevisiae* DNA polymerase delta require switching between the polymerase and exonuclease domains. *Mol Cell Biol*. 2005; 25(1):461–471. [PubMed: 15601866]
- Johansson E, Garg P, Burgers PM. The Pol32 subunit of DNA polymerase delta contains separable domains for processive replication and proliferating cell nuclear antigen (PCNA) binding. *J Biol Chem*. 2004; 279(3):1907–1915. [PubMed: 14594808]
- Johansson E, Macneill SA. The eukaryotic replicative DNA polymerases take shape. *Trends Biochem Sci*. 2010; 35(6):339–347. [PubMed: 20163964]
- Johnson RE, Kondratieff CM, Prakash S, Prakash L. *hRAD30* mutations in the variant form of Xeroderma pigmentosum. *Science*. 1999; 285(5425):263–265. [PubMed: 10398605]
- Johnson RE, Washington MT, Haracska L, Prakash S, Prakash L. Eukaryotic polymerases iota and zeta act sequentially to bypass DNA lesions. *Nature*. 2000; 406(6799):1015–1019. [PubMed: 10984059]
- Johnson RE, Prakash L, Prakash S. Pol31 and Pol32 subunits of yeast DNA polymerase delta are also essential subunits of DNA polymerase zeta. *Proc Natl Acad Sci U S A*. 2012; 109(31):12455–12460. [PubMed: 22711820]
- Jokela M, Raki M, Heikkinen K, Sepponen K, Eskelinen A, Syvaöja JE. The screening of expression and purification conditions for replicative DNA polymerase associated B-subunits, assignment of the exonuclease activity to the C-terminus of archaeal pol D DP1 subunit. *Protein Expr Purif*. 2005; 43(1):73–84. [PubMed: 15979340]
- Kadyrov FA, Dzantiev L, Constantin N, Modrich P. Endonucleolytic function of MutLalpha in human mismatch repair. *Cell*. 2006; 126(2):297–308. [PubMed: 16873062]
- Kadyrova LY, Blanko ER, Kadyrov FA. CAF-I-dependent control of degradation of the discontinuous strands during mismatch repair. *Proc Natl Acad Sci U S A*. 2011; 108(7):2753–2758. [PubMed: 21282622]
- Kamiya H. Mutagenicity of oxidized DNA precursors in living cells: Roles of nucleotide pool sanitization and DNA repair enzymes, and translesion synthesis DNA polymerases. *Mutation Research*. 2010; 703(1):32–36. [PubMed: 20542139]
- Kao HI, Bambara RA. The protein components and mechanism of eukaryotic Okazaki fragment maturation. *Crit Rev Biochem Mol Biol*. 2003; 38(5):433–452. [PubMed: 14693726]
- Karthikeyan R, Vonarx EJ, Straffon AF, Simon M, Faye G, Kunz BA. Evidence from mutational specificity studies that yeast DNA polymerases delta and epsilon replicate different DNA strands at an intracellular replication fork. *J Mol Biol*. 2000; 299(2):405–419. [PubMed: 10860748]
- Kesti T, Flick K, Keranen S, Syvaöja JE, Wittenberg C. DNA polymerase epsilon catalytic domains are dispensable for DNA replication, DNA repair, and cell viability. *Mol Cell*. 1999; 3(5):679–685. [PubMed: 10360184]
- Khromov-Borisov NN, Rogozin IB, Pegas Henriques JA, de Serres FJ. Similarity pattern analysis in mutational distributions. *Mutat Res*. 1999; 430(1):55–74. [PubMed: 10592318]

- Kim N, Huang SN, Williams JS, Li YC, Clark AB, Cho JE, Kunkel TA, Pommier Y, Jinks-Robertson S. Mutagenic processing of ribonucleotides in DNA by yeast topoisomerase I. *Science*. 2011; 332(6037):1561–1564. [PubMed: 21700875]
- Kim N, Jinks-Robertson S. Transcription as a source of genome instability. *Nat Rev Genet*. 2012; 13(3):204–214. 210.1038/nrg3152. [PubMed: 22330764]
- Klinge S, Hirst J, Maman JD, Krude T, Pellegrini L. An iron-sulfur domain of the eukaryotic primase is essential for RNA primer synthesis. *Nat Struct Mol Biol*. 2007; 14(9):875–877. [PubMed: 17704817]
- Klinge S, Nunez-Ramirez R, Llorca O, Pellegrini L. 3D architecture of DNA Pol alpha reveals the functional core of multi-subunit replicative polymerases. *EMBO J*. 2009; 28(13):1978–1987. [PubMed: 19494830]
- Koonin EV. Temporal order of evolution of DNA replication systems inferred by comparison of cellular and viral DNA polymerases. *Biol Direct*. 2006; 1:39. [PubMed: 17176463]
- Kornberg, A.; Baker, T. DNA Replication. W.H.Freeman & Co; 1991. p. 931
- Korona DA, Lecompte KG, Pursell ZF. The high fidelity and unique error signature of human DNA polymerase epsilon. *Nucleic Acids Res*. 2011; 39(5):1763–1773. [PubMed: 21036870]
- Kovtun IV, McMurray CT. Trinucleotide expansion in haploid germ cells by gap repair. *Nat Genet*. 2001; 27(4):407–411. [PubMed: 11279522]
- Kovtun IV, Liu Y, Bjoras M, Klungland A, Wilson SH, McMurray CT. OGG1 initiates age-dependent CAG trinucleotide expansion in somatic cells. *Nature*. 2007; 447(7143):447–452. [PubMed: 17450122]
- Kozmin S, Slezak G, Reynaud-Angelin A, Elie C, de Rycke Y, Boiteux S, Sage E. UVA radiation is highly mutagenic in cells that are unable to repair 7,8-dihydro-8-oxoguanine in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci U S A*. 2005; 102(38):13538–13543. [PubMed: 16157879]
- Kozmin SG, Schaaper RM, Shcherbakova PV, Kulikov VN, Noskov VN, Guetsova ML, Alenin VV, Rogozin IB, Makarova KS, Pavlov YI. Multiple antimutagenesis mechanisms affect mutagenic activity and specificity of the base analog 6-N-hydroxylaminopurine in bacteria and yeast. *Mutation Research*. 1998; 402(1–2):41–50. [PubMed: 9675240]
- Kozmin SG, Pavlov YI, Dunn RL, Schaaper RM. Hypersensitivity of *Escherichia coli* D(uvrB-bio) mutants to 6-hydroxylaminopurine and other base analogs is due to a defect in molybdenum cofactor biosynthesis. *J Bacteriol*. 2000; 182(12):3361–3367. [PubMed: 10852865]
- Kozmin SG, Schaaper RM. Molybdenum cofactor-dependent resistance to N-hydroxylated base analogs in *Escherichia coli* is independent of MobA function. *Mutat Res*. 2007; 619(1–2):9–15. [PubMed: 17349664]
- Kozmin SG, Leroy P, Pavlov YI, Schaaper RM. YcbX and yjiM, two novel determinants for resistance of *Escherichia coli* to N-hydroxylated base analogues. *Molecular Microbiology*. 2008; 68(1):51–65. [PubMed: 18312271]
- Kuchta RD, Stengel G. Mechanism and evolution of DNA primases. *Biochim Biophys Acta*. 2010; 1804(5):1180–1189. [PubMed: 19540940]
- Kujoth GC, Hiona A, Pugh TD, Someya S, Panzer K, Wohlgemuth SE, Hofer T, Seo AY, Sullivan R, Jobling WA, Morrow JD, Van Remmen H, Sedivy JM, Yamasoba T, Tanokura M, Weindruch R, Leeuwenburgh C, Prolla TA. Mitochondrial DNA mutations, oxidative stress, and apoptosis in mammalian aging. *Science*. 2005; 309(5733):481–484. [PubMed: 16020738]
- Kulikov VV, Derkatch IL, Noskov VN, Tarunina OV, Chernoff YO, Rogozin IB, Pavlov YI. Mutagenic specificity of the base analog 6-N-hydroxylaminopurine in the *LYS2* gene of yeast *Saccharomyces cerevisiae*. *Mutat Res*. 2001; 473(2):151–161. [PubMed: 11166033]
- Kumar D, Abdulovic AL, Viberg J, Nilsson AK, Kunkel TA, Chabes A. Mechanisms of mutagenesis in vivo due to imbalanced dNTP pools. *Nucleic Acids Res*. 2011; 39(4):1360–1371. [PubMed: 20961955]
- Kunkel TA, Bebenek K. DNA replication fidelity. *Annu Rev Biochem*. 2000; 69:497–529. [PubMed: 10966467]
- Kunkel TA. DNA replication fidelity. *J Biol Chem*. 2004; 279(17):16895–16898. [PubMed: 14988392]

- Kunkel TA, Erie DA. DNA mismatch repair. *Annu Rev Biochem.* 2005; 74:681–710. [PubMed: 15952900]
- Kunkel TA, Burgers PM. Dividing the workload at a eukaryotic replication fork. *Trends Cell Biol.* 2008; 18(11):521–527. [PubMed: 18824354]
- Kunz BA. Mutagenesis and deoxyribonucleotide pool imbalance. *Mutat Res.* 1988; 200(1–2):133–147. [PubMed: 3292903]
- Lada AG, Waisertreiger IS, Grabow CE, Prakash A, Borgstahl GE, Rogozin IB, Pavlov YI. Replication protein A (RPA) hampers the processive action of APOBEC3G cytosine deaminase on single-stranded DNA. *PLoS One.* 2011; 6(9):e24848. [PubMed: 21935481]
- Lahue RS, Slater DL. DNA repair and trinucleotide repeat instability. *Front Biosci.* 2003; 8:s653–665. [PubMed: 12700078]
- Lamantea E, Tiranti V, Bordoni A, Toscano A, Bono F, Servidei S, Papadimitriou A, Spelbrink H, Silvestri L, Casari G, Comi GP, Zeviani M. Mutations of mitochondrial DNA polymerase gamma are a frequent cause of autosomal dominant or recessive progressive external ophthalmoplegia. *Ann Neurol.* 2002; 52(2):211–219. [PubMed: 12210792]
- Lang GI, Murray AW. Mutation rates across budding yeast chromosome VI are correlated with replication timing. *Genome Biol Evol.* 2011; 3:799–811. Epub 2011 Jun 2010. [PubMed: 21666225]
- Lange SS, Wittschleben JP, Wood RD. DNA polymerase zeta is required for proliferation of normal mammalian cells. *Nucleic Acids Res.* 2012; 40(10):4473–4482. [PubMed: 22319213]
- Larrea AA, Lujan SA, Nick McElhinny SA, Mieczkowski PA, Resnick MA, Gordenin DA, Kunkel TA. Genome-wide model for the normal eukaryotic DNA replication fork. *Proc Natl Acad Sci U S A.* 2010; 107(41):17674–17679. [PubMed: 20876092]
- Lawrence CW, Gibbs PE, Murante RS, Wang XD, Li Z, McManus TP, McGregor WG, Nelson JR, Hinkle DC, Maher VM. Roles of DNA polymerase zeta and Rev1 protein in eukaryotic mutagenesis and translesion replication. *Cold Spring Harbor Symposia on Quantitative Biology.* 2000; 65:61–69.
- Lawrence CW. Cellular roles of DNA polymerase zeta and Rev1 protein. *DNA Repair (Amst).* 2002; 1(6):425–435. [PubMed: 12509231]
- Lee GH, Matsushita H. Genetic linkage between Pol iota deficiency and increased susceptibility to lung tumors in mice. *Cancer Sci.* 2005; 96(5):256–259. [PubMed: 15904465]
- Lee GS, Savage EA, Ritzel RG, von Borstel RC. The base-alteration spectrum of spontaneous and ultraviolet radiation-induced forward mutations in the *URA3* locus of *Saccharomyces cerevisiae*. *Mol Gen Genet.* 1988; 214(3):396–404. [PubMed: 3063945]
- Lemoine FJ, Degtyareva NP, Lobachev K, Petes TD. Chromosomal translocations in yeast induced by low levels of DNA polymerase a model for chromosome fragile sites. *Cell.* 2005; 120(5):587–598. [PubMed: 15766523]
- Li F, Martienssen R, Cande WZ. Coordination of DNA replication and histone modification by the Rik1-Dos2 complex. *Nature.* 2011; 475(7355):244–248. [PubMed: 21725325]
- Li L, Murphy KM, Kanevets U, Reha-Krantz LJ. Sensitivity to phosphonoacetic acid: a new phenotype to probe DNA polymerase d in *Saccharomyces cerevisiae*. *Genetics.* 2005; 170(2):569–580. [PubMed: 15802517]
- Li Z, Zhang H, McManus TP, McCormick JJ, Lawrence CW, Maher VM. hREV3 is essential for error-prone translesion synthesis past UV or benzo[a]pyrene diol epoxide-induced DNA lesions in human fibroblasts. *Mutat Res.* 2002; 510(1–2):71–80. [PubMed: 12459444]
- Lill R. Function and biogenesis of iron-sulphur proteins. *Nature.* 2009; 460(7257):831–838. [PubMed: 19675643]
- Lill R, Hoffmann B, Molik S, Pierik AJ, Rietzschel N, Stehling O, Uzarska MA, Webert H, Willbrecht C, Muhlenhoff U. The role of mitochondria in cellular iron-sulfur protein biogenesis and iron metabolism. *Biochim Biophys Acta.* 2012; 15:15.
- Lin S, McLennan AG, Ying K, Wang Z, Gu S, Jin H, Wu C, Liu W, Yuan Y, Tang R, Xie Y, Mao Y. Cloning, expression, and characterization of a human inosine triphosphate pyrophosphatase encoded by the *ITPA* gene. *J Biol Chem.* 2001a; 276(22):18695–18701. [PubMed: 11278832]

- Lin S, McLennan AG, Ying K, Wang Z, Gu S, Jin H, Wu C, Liu W, Yuan Y, Tang R, Xie Y, Mao Y. Cloning, expression, and characterization of a human inosine triphosphate pyrophosphatase encoded by the ITPA gene. *J Biol Chem*. 2001b; 276(22):18695–18701. [PubMed: 11278832]
- Liu M, Schatz DG. Balancing AID and DNA repair during somatic hypermutation. *Trends Immunol*. 2009; 30(4):173–181. Epub 2009 Mar 18. [PubMed: 19303358]
- Liu VF, Bhaumik D, Wang TS. Mutator phenotype induced by aberrant replication. *Mol Cell Biol*. 1999; 19(2):1126–1135. [PubMed: 9891047]
- Liu Y, Kao HI, Bambara RA. Flap endonuclease 1: a central component of DNA metabolism. *Annu Rev Biochem*. 2004; 73:589–615. [PubMed: 15189154]
- Loeb LA, Springgate CF, Battula N. Errors in DNA replication as a basis of malignant changes. *Cancer Res*. 1974; 34(9):2311–2321. [PubMed: 4136142]
- Loeb LA. Human cancers express mutator phenotypes: origin, consequences and targeting. *Nat Rev Cancer*. 2011; 11(6):450–457. [PubMed: 21593786]
- Longhese MP, Jovine L, Plevani P, Lucchini G. Conditional mutations in the yeast DNA primase genes affect different aspects of DNA metabolism and interactions in the DNA polymerase alpha-primase complex. *Genetics*. 1993; 133(2):183–191. [PubMed: 8436268]
- Longley MJ, Pierce AJ, Modrich P. DNA polymerase delta is required for human mismatch repair *in vitro*. *J Biol Chem*. 1997; 272(16):10917–10921. [PubMed: 9099749]
- Lukas L, Kuzminov A. Chromosomal fragmentation is the major consequence of the *rdgB* defect in *Escherichia coli*. *Genetics*. 2006; 172(2):1359–1362. [PubMed: 16322510]
- MacNeill SA, Baldacci G, Burgers PM, Hubscher U. A unified nomenclature for the subunits of eukaryotic DNA polymerase delta. *Trends Biochem Sci*. 2001; 26(1):16–17. [PubMed: 11165510]
- Madhusudan S, Middleton MR. The emerging role of DNA repair proteins as predictive, prognostic and therapeutic targets in cancer. *Cancer Treat Rev*. 2005; 31(8):603–617. [PubMed: 16298073]
- Maeda T, Sumi S, Ueta A, Ohkubo Y, Ito T, Marinaki AM, Kurono Y, Hasegawa S, Togari H. Genetic basis of inosine triphosphate pyrophosphohydrolase deficiency in the Japanese population. *Mol Genet Metab*. 2005; 85(4):271–279. [PubMed: 15946879]
- Maga G, Stucki M, Spadari S, Hubscher U. DNA polymerase switching: I. Replication factor C displaces DNA polymerase alpha prior to PCNA loading. *J Mol Biol*. 2000; 295(4):791–801. [PubMed: 10656791]
- Magni GE, von Borstel RC. Different rates of spontaneous mutation during mitosis and meiosis in yeast. *Genetics*. 1962; 47:1097–1108. [PubMed: 17248123]
- Maki H, Sekiguchi M. MutT protein specifically hydrolyses a potent mutagenic substrate for DNA synthesis. *Nature*. 1992; 355(6357):273–275. [PubMed: 1309939]
- Maki H. Origins of spontaneous mutations: specificity and directionality of base-substitution, frameshift, and sequence-substitution mutageneses. *Annu Rev Genet*. 2002; 36:279–303. [PubMed: 12429694]
- Maki S, Hashimoto K, Ohara T, Sugino A. DNA polymerase II (epsilon) of *Saccharomyces cerevisiae* dissociates from the DNA template by sensing single-stranded DNA. *J Biol Chem*. 1998; 273(33):21332–21341. [PubMed: 9694894]
- Makiniemi M, Pospiech H, Kilpelainen S, Jokela M, Vihinen M, Syvaioja JE. A novel family of DNA-polymerase-associated B subunits. *Trends Biochem Sci*. 1999; 24(1):14–16. [PubMed: 10087916]
- Marinaki AM, Duley JA, Arenas M, Ansari A, Sumi S, Lewis CM, Shobowale-Bakre M, Fairbanks LD, Sanderson J. Mutation in the ITPA gene predicts intolerance to azathioprine. *Nucleosides, Nucleotides & Nucleic acids*. 2004; 23(8–9):1393–1397.
- Marsh S, King CR, Ahluwalia R, McLeod HL. Distribution of ITPA P32T alleles in multiple world populations. *Journal of Human Genetics*. 2004; 49(10):579–581. [PubMed: 15322947]
- Masutani C, Kusumoto R, Yamada A, Dohmae N, Yokoi M, Yuasa M, Araki M, Iwai S, Takio K, Hanaoka F. The XPV (xeroderma pigmentosum variant) gene encodes human DNA polymerase eta. *Nature*. 1999; 399(6737):700–704. [PubMed: 10385124]
- Mathews CK, Ji J. DNA precursor asymmetries, replication fidelity, and variable genome evolution. *Bioessays*. 1992; 14(5):295–301. [PubMed: 1637360]

- Mathews CK. DNA precursor metabolism and genomic stability. The FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 2006; 20(9): 1300–1314.
- McCulloch SD, Kunkel TA. The fidelity of DNA synthesis by eukaryotic replicative and translesion synthesis polymerases. Cell Res. 2008; 18(1):148–161. [PubMed: 18166979]
- McHenry CS. Chromosomal replicases as asymmetric dimers: studies of subunit arrangement and functional consequences. Mol Microbiol. 2003; 49(5):1157–1165. [PubMed: 12940977]
- Menezes MR, Waisertreiger IS, Lopez-Bertoni H, Luo X, Pavlov YI. Pivotal role of inosine triphosphate pyrophosphatase in maintaining genome stability and the prevention of apoptosis in human cells. PLoS One. 2012; 7(2):e32313. [PubMed: 22384212]
- Michaels ML, Miller JH. The GO system protects organisms from the mutagenic effect of the spontaneous lesion 8-hydroxyguanine (7,8-dihydro-8-oxoguanine). J Bacteriol. 1992; 174(20): 6321–6325. [PubMed: 1328155]
- Mirkin SM. Toward a unified theory for repeat expansions. Nat Struct Mol Biol. 2005; 12(8):635–637. [PubMed: 16077722]
- Mirkin SM. DNA structures, repeat expansions and human hereditary disorders. Curr Opin Struct Biol. 2006; 16(3):351–358. [PubMed: 16713248]
- Miyabe I, Kunkel TA, Carr AM. The major roles of DNA polymerases epsilon and delta at the eukaryotic replication fork are evolutionarily conserved. PLoS Genet. 2011; 7(12):e1002407. [PubMed: 22144917]
- Mizuno T, Yamagishi K, Miyazawa H, Hanaoka F. Molecular architecture of the mouse DNA polymerase alpha-primase complex. Mol Cell Biol. 1999; 19(11):7886–7896. [PubMed: 10523676]
- Modrich P, Lahue R. Mismatch repair in replication fidelity, genetic recombination, and cancer biology. Annu Rev Biochem. 1996; 65:101–133. [PubMed: 8811176]
- Moe A, Ringvoll J, Nordstrand LM, Eide L, Bjoras M, Seeberg E, Rognes T, Klungland A. Incision at hypoxanthine residues in DNA by a mammalian homologue of the *Escherichia coli* antimutator enzyme endonuclease V. Nucleic Acids Research. 2003; 31(14):3893–3900. [PubMed: 12853604]
- Morrison A, Araki H, Clark AB, Hamatake RK, Sugino A. A third essential DNA polymerase in *S. cerevisiae*. Cell. 1990; 62(6):1143–1151. [PubMed: 2169349]
- Morrison A, Bell JB, Kunkel TA, Sugino A. Eukaryotic DNA polymerase amino acid sequence required for 3'→5' exonuclease activity. Proc Natl Acad Sci U S A. 1991; 88(21):9473–9477. [PubMed: 1658784]
- Morrison A, Johnson AL, Johnston LH, Sugino A. Pathway correcting DNA replication errors in *Saccharomyces cerevisiae*. EMBO J. 1993; 12(4):1467–1473. [PubMed: 8385605]
- Morrison A, Sugino A. The 3'→5' exonucleases of both DNA polymerases delta and epsilon participate in correcting errors of DNA replication in *Saccharomyces cerevisiae*. Mol Gen Genet. 1994; 242(3):289–296. [PubMed: 8107676]
- Mossi R, Keller RC, Ferrari E, Hubscher U. DNA polymerase switching: II. Replication factor C abrogates primer synthesis by DNA polymerase alpha at a critical length. J Mol Biol. 2000; 295(4):803–814. [PubMed: 10656792]
- Murakumo Y, Roth T, Ishii H, Rasio D, Numata S, Croce CM, Fishel R. A human *REV7* homolog that interacts with the polymerase zeta catalytic subunit *hREV3* and the spindle assembly checkpoint protein *hMAD2*. J Biol Chem. 2000; 275(6):4391–4397. [PubMed: 10660610]
- Muzi-Falconi M, Giannattasio M, Foiani M, Plevani P. The DNA polymerase alpha-primase complex: multiple functions and interactions. Scientific World Journal. 2003; 3:21–33. [PubMed: 12806117]
- Nakamura M, Nabetani A, Mizuno T, Hanaoka F, Ishikawa F. Alterations of DNA and chromatin structures at telomeres and genetic instability in mouse cells defective in DNA polymerase alpha. Mol Cell Biol. 2005; 25(24):11073–11088. [PubMed: 16314528]
- Negishi K, Bessho T, Hayatsu H. Nucleoside and nucleobase analog mutagens. Mutat Res. 1994; 318(3):227–238. [PubMed: 7527486]

- Nelson JR, Lawrence CW, Hinkle DC. Thymine-thymine dimer bypass by yeast DNA polymerase ζ . *Science*. 1996; 272(5268):1646–1649. [PubMed: 8658138]
- Netz DJ, Stumpfig M, Dore C, Muhlenhoff U, Pierik AJ, Lill R. Tah18 transfers electrons to Dre2 in cytosolic iron-sulfur protein biogenesis. *Nat Chem Biol*. 2010; 6(10):758–765. [PubMed: 20802492]
- Netz DJ, Pierik AJ, Stumpfig M, Bill E, Sharma AK, Pallesen LJ, Walden WE, Lill R. A bridging [4Fe-4S] cluster and nucleotide binding are essential for function of the Cfd1-Nbp35 complex as a scaffold in iron-sulfur protein maturation. *J Biol Chem*. 2012a; 287(15):12365–12378. [PubMed: 22362766]
- Netz DJ, Stith CM, Stumpfig M, Kopf G, Vogel D, Genau HM, Stodola JL, Lill R, Burgers PM, Pierik AJ. Eukaryotic DNA polymerases require an iron-sulfur cluster for the formation of active complexes. *Nat Chem Biol*. 2012b; 8(1):125–132. [PubMed: 22119860]
- Nick McElhinny SA, Stith CM, Burgers PM, Kunkel TA. Inefficient proofreading and biased error rates during inaccurate DNA synthesis by a mutant derivative of *Saccharomyces cerevisiae* DNA polymerase δ . *J Biol Chem*. 2007; 282(4):2324–2332. [PubMed: 17121822]
- Nick McElhinny SA, Gordenin DA, Stith CM, Burgers PM, Kunkel TA. Division of labor at the eukaryotic replication fork. *Mol Cell*. 2008; 30(2):137–144. [PubMed: 18439893]
- Nick McElhinny SA, Kumar D, Clark AB, Watt DL, Watts BE, Lundstrom EB, Johansson E, Chabes A, Kunkel TA. Genome instability due to ribonucleotide incorporation into DNA. *Nat Chem Biol*. 2010; 6(10):774–781. [PubMed: 20729855]
- Niimi A, Limsirichaikul S, Yoshida S, Iwai S, Masutani C, Hanaoka F, Kool ET, Nishiyama Y, Suzuki M. Palm mutants in DNA polymerases α and ϵ alter DNA replication fidelity and translesion activity. *Mol Cell Biol*. 2004; 24(7):2734–2746. [PubMed: 15024063]
- Nik-Zainal S, Alexandrov LB, Wedge DC, Van Loo P, Greenman CD, Raine K, Jones D, Hinton J, Marshall J, Stebbings LA, Menzies A, Martin S, Leung K, Chen L, Leroy C, Ramakrishna M, Rance R, Lau KW, Mudie LJ, Varela I, McBride DJ, Bignell GR, Cooke SL, Shlien A, Gamble J, Whitmore I, Maddison M, Tarpey PS, Davies HR, Papaemmanuil E, Stephens PJ, McLaren S, Butler AP, Teague JW, Jonsson G, Garber JE, Silver D, Miron P, Fatima A, Boyault S, Langerod A, Tutt A, Martens JW, Aparicio SA, Borg A, Salomon AV, Thomas G, Borresen-Dale AL, Richardson AL, Neuberger MS, Futreal PA, Campbell PJ, Stratton MR. Mutational Processes Molding the Genomes of 21 Breast Cancers. *Cell*. 2012; 149(5):979–993. [PubMed: 22608084]
- Northam MR, Garg P, Baitin DM, Burgers PM, Shcherbakova PV. A novel function of DNA polymerase ζ regulated by PCNA. *EMBO J*. 2006; 25(18):4316–4325. [PubMed: 16957771]
- Northam MR, Robinson HA, Kochenova OV, Shcherbakova PV. Participation of DNA polymerase ζ in replication of undamaged DNA in *Saccharomyces cerevisiae*. *Genetics*. 2010; 184(1):27–42. [PubMed: 19841096]
- Noskov V, Negishi K, Ono A, Matsuda A, Ono B, Hayatsu H. Mutagenicity of 5-bromouracil and N6-hydroxyadenine studied by yeast oligonucleotide transformation assay. *Mutat Res*. 1994; 308(1):43–51. [PubMed: 7516485]
- Noskov VN, Staak K, Shcherbakova PV, Kozmin SG, Negishi K, Ono BC, Hayatsu H, Pavlov YI. *HAM1*, the gene controlling 6-N-hydroxylaminopurine sensitivity and mutagenesis in the yeast *Saccharomyces cerevisiae*. *Yeast*. 1996; 12(1):17–29. [PubMed: 8789257]
- Nunez-Ramirez R, Klinge S, Sauguet L, Melero R, Recuero-Checa MA, Kilkenny M, Perera RL, Garcia-Alvarez B, Hall RJ, Nogales E, Pellegrini L, Llorca O. Flexible tethering of primase and DNA Pol α in the eukaryotic primosome. *Nucleic Acids Res*. 2011; 39(18):8187–8199. [PubMed: 21715379]
- Nuutinen T, Tossavainen H, Fredriksson K, Pirila P, Permi P, Pospiech H, Syvaöja JE. The solution structure of the amino-terminal domain of human DNA polymerase epsilon subunit B is homologous to C-domains of AAA+ proteins. *Nucleic Acids Res*. 2008; 36(15):5102–5110. [PubMed: 18676977]
- O-Wang J, Kawamura K, Tada Y, Ohmori H, Kimura H, Sakiyama S, Tagawa M. DNA polymerase κ , implicated in spontaneous and DNA damage-induced mutagenesis, is overexpressed in lung cancer. *Cancer Res*. 2001; 61(14):5366–5369. [PubMed: 11454676]

- Ogawa M, Limsirichaikul S, Niimi A, Iwai S, Yoshida S, Suzuki M. Distinct function of conserved amino acids in the fingers of *Saccharomyces cerevisiae* DNA polymerase alpha. *J Biol Chem*. 2003; 278(21):19071–19078. [PubMed: 12637557]
- Ohkumo T, Kondo Y, Yokoi M, Tsukamoto T, Yamada A, Sugimoto T, Kanao R, Higashi Y, Kondoh H, Tatematsu M, Masutani C, Hanaoka F. UV-B radiation induces epithelial tumors in mice lacking DNA polymerase eta and mesenchymal tumors in mice deficient for DNA polymerase iota. *Mol Cell Biol*. 2006; 26(20):7696–7706. [PubMed: 17015482]
- Ohya T, Kawasaki Y, Hiraga S, Kanbara S, Nakajo K, Nakashima N, Suzuki A, Sugino A. The DNA polymerase domain of pol epsilon is required for rapid, efficient, and highly accurate chromosomal DNA replication, telomere length maintenance, and normal cell senescence in *Saccharomyces cerevisiae*. *J Biol Chem*. 2002; 277(31):28099–28108. [PubMed: 12015307]
- Okada Y, Nakamura K, Hiromura K, Nojima Y, Horiuchi R, Yamamoto K. Pro32Thr polymorphism of inosine triphosphate pyrophosphatase gene predicts efficacy of low-dose azathioprine for patients with systemic lupus erythematosus. *Clinical Pharmacology and Therapeutics*. 2009; 85(5):527–530. [PubMed: 19129747]
- Pang B, McFaline JL, Burgis NE, Dong M, Taghizadeh K, Sullivan MR, Elmquist CE, Cunningham RP, Dedon PC. Defects in purine nucleotide metabolism lead to substantial incorporation of xanthine and hypoxanthine into DNA and RNA. *Proc Natl Acad Sci U S A*. 2012; 109(7):2319–2324. [PubMed: 22308425]
- Pavlov Iu I. Mutants of *Saccharomyces cerevisiae* supersensitive to the mutagenic effect of 6-N-hydroxylaminopurine. *Genetika (USSR)*. 1986; 22(9):2235–2243.
- Pavlov YI, Noskov VN, Lange EK, Moiseeva EV, Pshenichnov MR, Khromov-Borisov NN. The genetic activity of N6-hydroxyadenine and 2-amino-N6-hydroxyadenine in *Escherichia coli*, *Salmonella typhimurium* and *Saccharomyces cerevisiae*. *Mutat Res*. 1991; 253(1):33–46. [PubMed: 1870608]
- Pavlov YI, Shcherbakova PV, Kunkel TA. In vivo consequences of putative active site mutations in yeast DNA polymerases alpha, epsilon, delta, and zeta. *Genetics*. 2001; 159(1):47–64. [PubMed: 11560886]
- Pavlov YI, Newlon CS, Kunkel TA. Yeast origins establish a strand bias for replicational mutagenesis. *Mol Cell*. 2002a; 10(1):207–213. [PubMed: 12150920]
- Pavlov YI, Rogozin IB, Galkin AP, Aksenova AY, Hanaoka F, Rada C, Kunkel TA. Correlation of somatic hypermutation specificity and A-T base pair substitution errors by DNA polymerase eta during copying of a mouse immunoglobulin k light chain transgene. *Proc Natl Acad Sci U S A*. 2002b; 99(15):9954–9959. [PubMed: 12119399]
- Pavlov YI, Mian IM, Kunkel TA. Evidence for preferential mismatch repair of lagging strand DNA replication errors in yeast. *Curr Biol*. 2003; 13(9):744–748. [PubMed: 12725731]
- Pavlov YI, Maki S, Maki H, Kunkel TA. Evidence for interplay among yeast replicative DNA polymerases alpha, delta and epsilon from studies of exonuclease and polymerase active site mutations. *BMC Biol*. 2004; 2(1):11. [PubMed: 15163346]
- Pavlov YI, Frahm C, McElhinny SA, Niimi A, Suzuki M, Kunkel TA. Evidence that errors made by DNA polymerase alpha are corrected by DNA polymerase delta. *Curr Biol*. 2006a; 16(2):202–207. [PubMed: 16431373]
- Pavlov YI, Shcherbakova PV, Rogozin IB. Roles of DNA polymerases in replication, repair, and recombination in eukaryotes. *Int Rev Cytol*. 2006b; 255:41–132. [PubMed: 17178465]
- Pavlov YI, Shcherbakova PV. DNA polymerases at the eukaryotic fork-20 years later. *Mutat Res*. 2010; 685(1–2):45–53. [PubMed: 19682465]
- Pearson CE, Edamura KN, Cleary JD. Repeat instability: mechanisms of dynamic mutations. *Nat Rev Genet*. 2005; 6(10):729–742. [PubMed: 16205713]
- Pham P, Smolka MB, Calabrese P, Landolph A, Zhang K, Zhou H, Goodman MF. Impact of phosphorylation and phosphorylation-null mutants on the activity and deamination specificity of activation-induced cytidine deaminase. *J Biol Chem*. 2008; 283(25):17428–17439. [PubMed: 18417471]

- Polidori MC, Mecocci P, Stahl W, Sies H. Cigarette smoking cessation increases plasma levels of several antioxidant micronutrients and improves resistance towards oxidative challenge. *Br J Nutr.* 2003; 90(1):147–150. [PubMed: 12844386]
- Poloumienko A, Dershowitz A, De J, Newlon CS. Completion of replication map of *Saccharomyces cerevisiae* chromosome III. *Mol Biol Cell.* 2001; 12(11):3317–3327. [PubMed: 11694569]
- Porta J, Kolar C, Kozmin SG, Pavlov YI, Borgstahl GE. Structure of the orthorhombic form of human inosine triphosphate pyrophosphatase. *Acta Crystallograph Sect F Struct Biol Cryst Commun.* 2006; 62(Pt 11):1076–1081.
- Pospiech H, Syvaoja JE. DNA polymerase epsilon - more than a polymerase. *Scientific World Journal.* 2003; 3:87–104. [PubMed: 12806123]
- Prakash S, Johnson RE, Prakash L. Eukaryotic translesion synthesis DNA polymerases: specificity of structure and function. *Annu Rev Biochem.* 2005; 74:317–353. [PubMed: 15952890]
- Prelich G, Tan CK, Kostura M, Mathews MB, So AG, Downey KM, Stillman B. Functional identity of proliferating cell nuclear antigen and a DNA polymerase-delta auxiliary protein. *Nature.* 1987; 326(6112):517–520. [PubMed: 2882424]
- Pursell ZF, Isoz I, Lundstrom EB, Johansson E, Kunkel TA. Yeast DNA polymerase epsilon participates in leading-strand DNA replication. *Science.* 2007a; 317(5834):127–130. [PubMed: 17615360]
- Pursell ZF, Isoz I, Lundstrom EB, Johansson E, Kunkel TA. Regulation of B family DNA polymerase fidelity by a conserved active site residue: characterization of M644W, M644L and M644F mutants of yeast DNA polymerase epsilon. *Nucleic Acids Res.* 2007b; 35(9):3076–3086. [PubMed: 17452367]
- Pursell ZF, Kunkel TA. DNA polymerase epsilon: a polymerase of unusual size (and complexity). *Prog Nucleic Acid Res Mol Biol.* 2008; 82:101–145. [PubMed: 18929140]
- Pustovalova Y, Bezsonova I, Korzhnev DM. The C-terminal domain of human Rev1 contains independent binding sites for DNA polymerase eta and Rev7 subunit of polymerase zeta. *FEBS Lett.* 2012 Jul 22. Epub ahead of print.
- Ransom M, Dennehey BK, Tyler JK. Chaperoning histones during DNA replication and repair. *Cell.* 2010; 140(2):183–195. [PubMed: 20141833]
- Rattray AJ, Shafer BK, McGill CB, Strathern JN. The roles of *REV3* and *RAD57* in double-strand-break-repair-induced mutagenesis of *Saccharomyces cerevisiae*. *Genetics.* 2002; 162(3):1063–1077. [PubMed: 12454056]
- Reijns MA, Rabe B, Rigby RE, Mill P, Astell KR, Lettice LA, Boyle S, Leitch A, Keighren M, Kilanowski F, Devenney PS, Sexton D, Grimes G, Holt IJ, Hill RE, Taylor MS, Lawson KA, Dorin JR, Jackson AP. Enzymatic removal of ribonucleotides from DNA is essential for Mammalian genome integrity and development. *Cell.* 2012; 149(5):1008–1022. [PubMed: 22579044]
- Reynolds N, Watt A, Fantes PA, MacNeill SA. Cdm1, the smallest subunit of DNA polymerase d in the fission yeast *Schizosaccharomyces pombe*, is non-essential for growth and division. *Curr Genet.* 1998; 34(4):250–258. [PubMed: 9799358]
- Roberts JD, Izuta S, Thomas DC, Kunkel TA. Mismatch-, site-, and strand-specific error rates during simian virus 40 origin-dependent replication in vitro with excess deoxythymidine triphosphate. *J Biol Chem.* 1994; 269(3):1711–1717. [PubMed: 8294419]
- Roberts SA, Sterling J, Thompson C, Harris S, Mav D, Shah R, Klimczak LJ, Kryukov GV, Malc E, Mieczkowski PA, Resnick MA, Gordenin DA. Clustered mutations in yeast and in human cancers can arise from damaged long single-strand DNA regions. *Mol Cell.* 2012; 46(4):424–435. [PubMed: 22607975]
- Rogozin IB, Malyarchuk BA, Pavlov YI, Milanese L. From context-dependence of mutations to molecular mechanisms of mutagenesis. *Pac Symp Biocomput.* 2005:409–420. [PubMed: 15759646]
- Roy A, Fuchs RP. Mutational spectrum induced in *Saccharomyces cerevisiae* by the carcinogen N-2-acetylaminofluorene. *Mol Gen Genet.* 1994; 245(1):69–77. [PubMed: 7845359]

- Rumbaugh JA, Henricksen LA, DeMott MS, Bambara RA. Cleavage of substrates with mismatched nucleotides by Flap endonuclease-1. Implications for mammalian Okazaki fragment processing. *J Biol Chem.* 1999; 274(21):14602–14608. [PubMed: 10329652]
- Sakiyama T, Kohno T, Mimaki S, Ohta T, Yanagitani N, Sobue T, Kunitoh H, Saito R, Shimizu K, Hirama C, Kimura J, Maeno G, Hirose H, Eguchi T, Saito D, Ohki M, Yokota J. Association of amino acid substitution polymorphisms in DNA repair genes *TP53*, *POL1*, *REVI* and *LIG4* with lung cancer risk. *Int J Cancer.* 2005; 114(5):730–737. [PubMed: 15609317]
- Sakumi K, Abolhassani N, Behmanesh M, Iyama T, Tsuchimoto D, Nakabeppu Y. ITPA protein, an enzyme that eliminates deaminated purine nucleoside triphosphates in cells. *Mutat Res.* 2010; 703(1):43–50. [PubMed: 20601097]
- Sale JE, Lehmann AR, Woodgate R. Y-family DNA polymerases and their role in tolerance of cellular DNA damage. *Nat Rev Mol Cell Biol.* 2012; 13(3):141–152. [PubMed: 22358330]
- Sanchez Garcia J, Ciufu LF, Yang X, Kearsey SE, MacNeill SA. The C-terminal zinc finger of the catalytic subunit of DNA polymerase delta is responsible for direct interaction with the B-subunit. *Nucleic Acids Res.* 2004; 32(10):3005–3016. [PubMed: 15173383]
- Sanchez Garcia J, Baranovskiy AG, Knatko EV, Gray FC, Tahirov TH, MacNeill SA. Functional mapping of the fission yeast DNA polymerase delta B-subunit Cdc1 by site-directed and random pentapeptide insertion mutagenesis. *BMC Mol Biol.* 2009; 10:82. [PubMed: 19686603]
- Saparbaev M, Mani JC, Laval J. Interactions of the human, rat, *Saccharomyces cerevisiae* and *Escherichia coli* 3-methyladenine-DNA glycosylases with DNA containing dIMP residues. *Nucleic Acids Res.* 2000; 28(6):1332–1339. [PubMed: 10684927]
- Sasaki S, Mello CC, Shimada A, Nakatani Y, Hashimoto S, Ogawa M, Matsushima K, Gu SG, Kasahara M, Ahsan B, Sasaki A, Saito T, Suzuki Y, Sugano S, Kohara Y, Takeda H, Fire A, Morishita S. Chromatin-associated periodicity in genetic variation downstream of transcriptional start sites. *Science.* 2009; 323(5912):401–404. [PubMed: 19074313]
- Saugar I, Parker JL, Zhao S, Ulrich HD. The genome maintenance factor Mgs1 is targeted to sites of replication stress by ubiquitylated PCNA. *Nucleic Acids Res.* 2012; 40(1):245–257. [PubMed: 21911365]
- Schaaper RM. Base selection, proofreading, and mismatch repair during DNA replication in *Escherichia coli*. *J Biol Chem.* 1993; 268(32):23762–23765. [PubMed: 8226906]
- Schopf B, Bregenhorn S, Quivy JP, Kadyrov FA, Almouzni G, Jiricny J. Interplay between mismatch repair and chromatin assembly. *Proc Natl Acad Sci U S A.* 2012; 109(6):1895–1900. Epub 2012 Jan 1899. [PubMed: 22232658]
- Sekiguchi M, Tsuzuki T. Oxidative nucleotide damage: consequences and prevention. *Oncogene.* 2002; 21(58):8895–8904. [PubMed: 12483507]
- Sharma NM, Kochenova OV, Shcherbakova PV. The non-canonical protein binding site at the monomer-monomer interface of yeast proliferating cell nuclear antigen (PCNA) regulates the Rev1-PCNA interaction and Polzeta/Rev1-dependent translesion DNA synthesis. *J Biol Chem.* 2011; 286(38):33557–33566. [PubMed: 21799021]
- Shcherbakova PV, Pavlov YI. Mutagenic specificity of the base analog 6-N-hydroxylaminopurine in the *URA3* gene of the yeast *Saccharomyces cerevisiae*. *Mutagenesis.* 1993; 8(5):417–421. [PubMed: 8231822]
- Shcherbakova PV, Noskov VN, Pshenichnov MR, Pavlov YI. Base analog 6-N-hydroxylaminopurine mutagenesis in the yeast *Saccharomyces cerevisiae* is controlled by replicative DNA polymerases. *Mutation research.* 1996; 369(1–2):33–44. [PubMed: 8700180]
- Shcherbakova PV, Pavlov YI. 3'→5' exonucleases of DNA polymerases ϵ and δ correct base analog induced DNA replication errors on opposite DNA strands in *Saccharomyces cerevisiae*. *Genetics.* 1996; 142(3):717–726. [PubMed: 8849882]
- Shcherbakova, PV.; Bebenek, K.; Kunkel, TA. [26 February 2003] Functions of eukaryotic DNA polymerases. *Science's SAGE KE.* 2003a. 2003/8/re3: <http://sageke.sciencemag.org/cgi/content/full/sageke>
- Shcherbakova PV, Pavlov YI, Chilkova O, Rogozin IB, Johansson E, Kunkel TA. Unique error signature of the four-subunit yeast DNA polymerase epsilon. *J Biol Chem.* 2003b; 278(44):43770–43780. [PubMed: 12882968]

- Sheaff RJ, Kuchta RD. Misincorporation of nucleotides by calf thymus DNA primase and elongation of primers containing multiple noncognate nucleotides by DNA polymerase alpha. *J Biol Chem*. 1994; 269(30):19225–19231. [PubMed: 8034683]
- Sheaff RJ, Kuchta RD, Ilsley D. Calf thymus DNA polymerase alpha-primase: “communication” and primer-template movement between the two active sites. *Biochemistry*. 1994; 33(8):2247–2254. [PubMed: 8117681]
- Shishkin AA, Voineagu I, Matera R, Cherng N, Chernet BT, Krasilnikova MM, Narayanan V, Lobachev KS, Mirkin SM. Large-scale expansions of Friedreich’s ataxia GAA repeats in yeast. *Mol Cell*. 2009; 35(1):82–92. [PubMed: 19595718]
- Simandan T, Sun J, Dix TA. Oxidation of DNA bases, deoxyribonucleosides and homopolymers by peroxy radicals. *Biochem J*. 1998; 335(Pt 2):233–240. [PubMed: 9761719]
- Smith DJ, Whitehouse I. Intrinsic coupling of lagging-strand synthesis to chromatin assembly. *Nature*. 2012; 483(7390):434–438. 410.1038/nature10895. [PubMed: 22419157]
- Stehling O, Vashisht AA, Mascarenhas J, Jonsson ZO, Sharma T, Netz DJA, Pierik AJ, Wohlschlegel JA, Lill R. MMS19 Assembles Iron-Sulfur Proteins Required for DNA Metabolism and Genomic Integrity. *Science*. 2012; 337(6091):195–199. [PubMed: 22678362]
- Stelter P, Ulrich HD. Control of spontaneous and damage-induced mutagenesis by SUMO and ubiquitin conjugation. *Nature*. 2003; 425(6954):188–191. [PubMed: 12968183]
- Stenmark P, Kursula P, Flodin S, Graslund S, Landry R, Nordlund P, Schuler H. Crystal structure of human inosine triphosphatase. Substrate binding and implication of the inosine triphosphatase deficiency mutation P32T. *J Biol Chem*. 2007; 282(5):3182–3187. [PubMed: 17138556]
- Stepchenkova EI, Kozmin SG, Alenin VV, Pavlov YI. Genome-wide screening for genes whose deletions confer sensitivity to mutagenic purine base analogs in yeast. *BMC Genet*. 2005; 6(1):31. [PubMed: 15932646]
- Stepchenkova EI, Koz’min SG, Alenin VV, Pavlov Iu I. Genetic control of metabolism of mutagenic purine base analogs 6-hydroxylaminopurine and 2-amino-6-hydroxylaminopurine in yeast *Saccharomyces cerevisiae*. *Genetika (Russia)*. 2009a; 45(4):471–477.
- Stepchenkova EI, Tarakhovskaya ER, Spitler K, Frahm C, Menezes MR, Simone PD, Kolar C, Marky LA, Borgstahl GE, Pavlov YI. Functional study of the P32T ITPA variant associated with drug sensitivity in humans. *Journal of Molecular Biology*. 2009b; 392(3):602–613. [PubMed: 19631656]
- Stocco G, Cheok MH, Crews KR, Dervieux T, French D, Pei D, Yang W, Cheng C, Pui CH, Relling MV, Evans WE. Genetic polymorphism of inosine triphosphate pyrophosphatase is a determinant of mercaptopurine metabolism and toxicity during treatment for acute lymphoblastic leukemia. *Clinical Pharmacology and Therapeutics*. 2009; 85(2):164–172. [PubMed: 18685564]
- Strathern JN, Shafer BK, McGill CB. DNA synthesis errors associated with double-strand-break repair. *Genetics*. 1995; 140(3):965–972. [PubMed: 7672595]
- Sumi S, Marinaki AM, Arenas M, Fairbanks L, Shobowale-Bakre M, Rees DC, Thein SL, Ansari A, Sanderson J, De Abreu RA, Simmonds HA, Duley JA. Genetic basis of inosine triphosphate pyrophosphohydrolase deficiency. *Hum Genet*. 2002; 111(4–5):360–367. [PubMed: 12384777]
- Swan MK, Johnson RE, Prakash L, Prakash S, Aggarwal AK. Structural basis of high-fidelity DNA synthesis by yeast DNA polymerase delta. *Nat Struct Mol Biol*. 2009; 16(9):979–986. [PubMed: 19718023]
- Sweasy JB. Fidelity mechanisms of DNA polymerase beta. *Prog Nucleic Acid Res Mol Biol*. 2003; 73:137–169. [PubMed: 12882517]
- Sweasy JB, Lauper JM, Eckert KA. DNA polymerases and human diseases. *Radiat Res*. 2006; 166(5):693–714. [PubMed: 17067213]
- Tahirov TH, Makarova KS, Rogozin IB, Pavlov YI, Koonin EV. Evolution of DNA polymerases: an inactivated polymerase-exonuclease module in Pol epsilon and a chimeric origin of eukaryotic polymerases from two classes of archaeal ancestors. *Biol Direct*. 2009; 4:11. [PubMed: 19296856]
- Tahirov, TH. Structure and Function of Eukaryotic DNA Polymerase delta. In: Neill, M., editor. *The Eukaryotic Replisome: a Guide to Protein Structure and Function*. Dordrecht: Springer Science; 2012. in press

- Takada-Takayama R, Suzuki M, Enomoto T, Hanaoka F, Ui M. Purification and characterization of mouse DNA polymerase α devoid of primase activity. *FEBS Lett.* 1990; 273(1–2):27–30. [PubMed: 2226860]
- Takayama Y, Kamimura Y, Okawa M, Muramatsu S, Sugino A, Araki H. GINS, a novel multiprotein complex required for chromosomal DNA replication in budding yeast. *Genes Dev.* 2003; 17(9): 1153–1165. [PubMed: 12730134]
- Tanaka S, Cao K, Niimi A, Limsirichaikul S, Miao HQ, Nakamura N, Murate T, Hasegawa Y, Takahashi T, Suzuki M. Functions of base selection step in human DNA polymerase α . *DNA Repair (Amst).* 2010; 9(5):534–541. [PubMed: 20202915]
- Tishkoff DX, Filosi N, Gaida GM, Kolodner RD. A novel mutation avoidance mechanism dependent on *S. cerevisiae* *RAD27* is distinct from DNA mismatch repair. *Cell.* 1997; 88(2):253–263. [PubMed: 9008166]
- Trakselis MA, Mayer MU, Ishmael FT, Roccasacca RM, Benkovic SJ. Dynamic protein interactions in the bacteriophage T4 replisome. *Trends Biochem Sci.* 2001; 26(9):566–572. [PubMed: 11551794]
- Tran HT, Gordenin DA, Resnick MA. The 3'→5' exonucleases of DNA polymerases δ and ϵ and the 5'→3' exonuclease Exo1 have major roles in postreplication mutation avoidance in *Saccharomyces cerevisiae*. *Mol Cell Biol.* 1999; 19(3):2000–2007. [PubMed: 10022887]
- Tsubota T, Maki S, Kubota H, Sugino A, Maki H. Double-stranded DNA binding properties of *Saccharomyces cerevisiae* DNA polymerase ϵ and of the Dpb3p-Dpb4p subassembly. *Genes Cells.* 2003; 8(11):873–888. [PubMed: 14622139]
- Tsurimoto T, Melendy T, Stillman B. Sequential initiation of lagging and leading strand synthesis by two different polymerase complexes at the SV40 DNA replication origin. *Nature.* 1990; 346(6284):534–539. [PubMed: 2165567]
- Tsurimoto T, Stillman B. Replication factors required for SV40 DNA replication in vitro. II. Switching of DNA polymerase α and δ during initiation of leading and lagging strand synthesis. *J Biol Chem.* 1991a; 266(3):1961–1968. [PubMed: 1671046]
- Tsurimoto T, Stillman B. Replication factors required for SV40 DNA replication in vitro. I. DNA structure-specific recognition of a primer-template junction by eukaryotic DNA polymerases and their accessory proteins. *J Biol Chem.* 1991b; 266(3):1950–1960. [PubMed: 1671045]
- Uchiyama M, Wang TS. The B-subunit of DNA polymerase α -primase associates with the origin recognition complex for initiation of DNA replication. *Mol Cell Biol.* 2004; 24(17):7419–7434. [PubMed: 15314153]
- Ulrich HD. Timing and spacing of ubiquitin-dependent DNA damage bypass. *FEBS Lett.* 2011; 585(18):2861–2867. Epub 2011 May 28. [PubMed: 21605556]
- Vaisman A, Lehmann AR, Woodgate R. DNA polymerases ϵ and ι . *Adv Protein Chem.* 2004; 69:205–228. [PubMed: 15588844]
- Vaithiyalingam S, Warren EM, Eichman BF, Chazin WJ. Insights into eukaryotic DNA priming from the structure and functional interactions of the 4Fe-4S cluster domain of human DNA primase. *Proc Natl Acad Sci U S A.* 2010; 107(31):13684–13689. [PubMed: 20643958]
- Van Goethem G, Dermaut B, Lofgren A, Martin JJ, Van Broeckhoven C. Mutation of *POLG* is associated with progressive external ophthalmoplegia characterized by mtDNA deletions. *Nat Genet.* 2001; 28(3):211–212. [PubMed: 11431686]
- Veaute X, Fuchs RP. Greater susceptibility to mutations in lagging strand of DNA replication in *Escherichia coli* than in leading strand. *Science.* 1993; 261(5121):598–600. [PubMed: 8342022]
- Venkatesan RN, Hsu JJ, Lawrence NA, Preston BD, Loeb LA. Mutator phenotypes caused by substitution at a conserved motif A residue in eukaryotic DNA polymerase δ . *J Biol Chem.* 2006; 281(7):4486–4494. [PubMed: 16344551]
- Verhoef VL, Fuller SA, Morris AJ. Individual variation of nucleoside triphosphate pyrophosphohydrolase activity in human erythrocytes, granulocytes, lymphocytes, and platelets. *Biochemical Genetics.* 1980; 18(3–4):235–245. [PubMed: 6108768]
- von Borstel RC, Lee GS. Base sequence alterations induced by ultraviolet radiation in the *URA3* locus in yeast. *Prog Clin Biol Res.* 1986; 209A:449–456. [PubMed: 3529108]

- von Borstel RC, Ord RW, Stewart SP, Ritzel RG, Lee GS, Hennig UG, Savage EA. The mutator mut7-1 of *Saccharomyces cerevisiae*. *Mutat Res.* 1993; 289(1):97–106. [PubMed: 7689169]
- Waga S, Stillman B. Anatomy of a DNA replication fork revealed by reconstitution of SV40 DNA replication in vitro. *Nature.* 1994; 369(6477):207–212. [PubMed: 7910375]
- Waga S, Stillman B. The DNA replication fork in eukaryotic cells. *Annu Rev Biochem.* 1998; 67:721–751. [PubMed: 9759502]
- Waisertreiger IS, Menezes MR, Randazzo J, Pavlov YI. Elevated Levels of DNA Strand Breaks Induced by a Base Analog in the Human Cell Line with the P32T ITPA Variant. *J Nucleic Acids.* 2010; 2010
- Wang X, Ira G, Tercero JA, Holmes AM, Diffley JF, Haber JE. Role of DNA replication proteins in double-strand break-induced recombination in *Saccharomyces cerevisiae*. *Mol Cell Biol.* 2004; 24(16):6891–6899. [PubMed: 15282291]
- Wang Z. DNA damage-induced mutagenesis : a novel target for cancer prevention. *Mol Interv.* 2001; 1(5):269–281. [PubMed: 14993366]
- Wardle J, Burgers PM, Cann IK, Darley K, Heslop P, Johansson E, Lin LJ, McGlynn P, Sanvoisin J, Stith CM, Connolly BA. Uracil recognition by replicative DNA polymerases is limited to the archaea, not occurring with bacteria and eukarya. *Nucleic Acids Res.* 2008; 36(3):705–711. [PubMed: 18032433]
- Waters LS, Minesinger BK, Wilttrout ME, D'Souza S, Woodruff RV, Walker GC. Eukaryotic translesion polymerases and their roles and regulation in DNA damage tolerance. *Microbiol Mol Biol Rev.* 2009; 73(1):134–154. [PubMed: 19258535]
- Williams JS, Clausen AR, Nick McElhinny SA, Watts BE, Johansson E, Kunkel TA. Proofreading of ribonucleotides inserted into DNA by yeast DNA polymerase epsilon. *DNA Repair (Amst).* 2012; 11(8):649–656. [PubMed: 22682724]
- Xie Z, Braithwaite E, Guo D, Zhao B, Geacintov NE, Wang Z. Mutagenesis of benzo[a]pyrene diol epoxide in yeast: requirement for DNA polymerase zeta and involvement of DNA polymerase eta. *Biochemistry.* 2003; 42(38):11253–11262. [PubMed: 14503875]
- Yang J, Chen Z, Liu Y, Hickey RJ, Malkas LH. Altered DNA polymerase iota expression in breast cancer cells leads to a reduction in DNA replication fidelity and a higher rate of mutagenesis. *Cancer Res.* 2004; 64(16):5597–5607. [PubMed: 15313897]
- Yang W, Woodgate R. What a difference a decade makes: insights into translesion DNA synthesis. *Proc Natl Acad Sci U S A.* 2007; 104(40):15591–15598. [PubMed: 17898175]
- Zelinkova Z, Derijks LJ, Stokkers PC, Vogels EW, van Kampen AH, Curvers WL, Cohn D, van Deventer SJ, Hommes DW. Inosine triphosphate pyrophosphatase and thiopurine s-methyltransferase genotypes relationship to azathioprine-induced myelosuppression. *Clin Gastroenterol Hepatol.* 2006; 4(1):44–49. [PubMed: 16431304]
- Zhang S, Zhou Y, Trusa S, Meng X, Lee EY, Lee MY. A novel DNA damage response: rapid degradation of the p12 subunit of DNA polymerase delta. *J Biol Chem.* 2007; 282(21):15330–15340. [PubMed: 17317665]
- Zhang Y, Yuan F, Presnell SR, Tian K, Gao Y, Tomkinson AE, Gu L, Li GM. Reconstitution of 5'-directed human mismatch repair in a purified system. *Cell.* 2005; 122(5):693–705. [PubMed: 16143102]
- Zheng L, Shen B. Okazaki fragment maturation: nucleases take centre stage. *J Mol Cell Biol.* 2011; 3(1):23–30. [PubMed: 21278448]
- Zhong X, Garg P, Stith CM, Nick McElhinny SA, Kissling GE, Burgers PM, Kunkel TA. The fidelity of DNA synthesis by yeast DNA polymerase zeta alone and with accessory proteins. *Nucleic Acids Res.* 2006; 34(17):4731–4742. [PubMed: 16971464]

Figure 1. Nucleic acid metabolism and the origin of mutations

New RNA and DNA are made on undamaged templates with ribo- (rN) and deoxy (dN) nucleosidetriphosphates. The rN are predominantly used by transcription apparatus, but are needed for RNA priming by primase (insert below, illustrating sequential action of primase, DNA Pol α and Pol δ). They can also be rarely incorporated into DNA by DNA Pols. N stands for any normal but incorrect nucleotide symbolizing a change from the initial DNA sequence. Star-like shapes represent modified bases in nucleotide pools or in nucleic acid. Mutation in duplex DNA is represented by dN/dNc, where dNc is a nucleotide complementary to dN.

Figure 2. Two types of mutagens: misrepair vs. misreplication

Spot-test for induction of *can1* mutants in wild-type yeast 8C-YUNI101 strain [Pavlov et al., 2001] and its *rev3::LEU2* disruption derivative. UV light is strongly mutagenic only in the wild-type strain, while HAP spotted in the center of the plates induces mutations with equal efficiency in both strains.

Figure 3. Subunit structure of human DNA polymerases of the B-family Pals are color-coded. Pol δ and Pol ζ have two common subunits

The catalytic subunits are called POLA1 or p180, POLD1 or p125, POLE or p261 and REV3L or p353 in humans. In budding yeast, the subunits are called Pol1, Pol2, Pol3 and Rev3, respectively. The B-subunits of Pol α, Pol δ and Pol ε in humans and in yeast (in brackets) are POLA2 or p70 (Pol12), POLD2 or p50 (Pol31) and POLE2 or p59 (Dpb2), respectively. The second subunit of Pol ζ is MAD2L2 or hREV7 or p30 (Rev7). The third or C-subunit of Pol δ is POLD3 or p66 (Pol32). The smaller subunit of Pol δ is POLD4 or p12 (absent as a separate protein in budding yeast, called Cdm1 in *S. pombe*). Small subunits of Pol ε are POIE3 or p17 (Dpb3) and POLE4 or p12 (Dpb4).

Figure 4. Pathways of base analogs

A. Inter-conversion of adenine (black) and base analog hypoxanthine and HAP (blue).

B. Salvage pathways and nucleotide biosynthesis *de novo* as a source of IMP-related base analogs in DNA.

Base analogs and their derivatives are in blue font. ADE, adenine; HYP, hypoxanthine; GUA, guanine; SAMP, succinyl-AMP;

IMP, inosine-5'-monophosphate; HAPMP, HAP-5'-monophosphate; HAPTP, HAP-5'-triphosphate; and dHAPTP, HAP-2'-deoxyriboside-5'-triphosphate.

* Gene inactivation leads to an increase of HYP and xanthine in DNA or in sensitivity to the mutagenic or toxic effect of HAP in bacteria and yeast [Stepchenkova et al., 2005; Stepchenkova et al., 2009a; Pang et al., 2012]. *AHH1* in yeast encodes for adenine aminohydrolase; *ADE12* (*ADSSL* in humans) - for SAMP synthase; *ADE13* (*ADSL* in humans) - adenylosuccinate lyase; *HAM1* (*rdgB* in *E. coli* and *ITPA* in humans) – inosine triphosphate pyrophosphohydrolase.

Biosynthesis *de novo* (red arrows) generates IMP, then the pathway splits into branches leading to AMP and GMP. A series of well studied reactions (black arrows pointing down, we do not specify participating enzymes for simplicity) lead to dATP and dGTP, respectively. IMP, AMP and GMP could also be generated by salvage pathway by direct activation of hypoxanthine, adenine, and guanine bases, respectively, and by corresponding phosphoribosyl transferases (black arrows). The exact sequence of events leading to the conversion of IMP and XMP directly to dIMP and dXTP is not known; presumably it happens due to some promiscuity of enzymes activating AMP and GMP (red dotted arrows). HAP activation to dHAPTP (blue pathway on the left) most likely utilized the pathway of conversion of adenine [Stepchenkova et al., 2009a].

Figure 5. Specificity of HAP mutagenesis in the *URA3* gene at different distances from the replication origin is independent from the orientation of the gene

A. Position and orientations of the reporter gene and the ratios of G to A versus C to T transitions (based on the sequencing of 40 mutants in each position).

B. To generate a G to A transition, read on non-transcribed strand, dHAPTP is misincorporated opposite C in the leading strand in the forward (F) orientation of the reporter and into the lagging strand in reverse orientation (R).

C. Statistical analysis of the differences between mutation spectra in different orientations. The analysis was performed as described in [Khromov-Borisov et al., 1999].

Figure 6. Mutagenesis associated with the patches of DNA synthesized by inaccurate Pol α at the 5' ends of Okazaki fragments

A. Analysis of the timing of origin activation in strains with the insertion of a reporter cassette. Left panel - Schematic presentation of 2-D gel patterns anticipated for molecules with an active origin of replication. Fragments containing ARS306 of approximately the same length were analyzed: 7,140 bp *XbaI-DrdI* fragment for control Δ1 strain with no insertion; 7,222 bp *PvuII-PvuII* fragment for n306; 7194 bp *PvuII-PvuII* fragment for 306 L; 7220 bp *PvuII-PvuII* fragment for 306 R; and 7213 bp *PvuII-PvuII* fragment for agp1.

Right panel - Images of the 2-D gels for different strains. Lines connect the gel pattern to the schemes of molecule distribution. Δ1 – control strain, n306 – insertion of the *URA3 LEU2* cassette to the left to the origin 2 kb from it, 306L – insertion to the left but very close to the origin, 306R – insertion very close to the right from the origin, 306R *pol1-L868M* – the same but in the *pol1-L868M* strain, 306R *pol1-L868M* + HAP – the same, but with the inclusion of HAP in the medium, and agp1 – insertion at 2 Kb to the right from the origin. Cells were released synchronously into the S phase from an α-factor block with the addition of 50mg/ml pronase into fresh YPD media. Samples were taken at the indicated times after release and processed as described [Friedman and Brewer, 1995]. Neutral/neutral two-dimensional gel analysis was used to monitor replication. The 6-*N*-Hydroxylaminopurine (HAP) in a concentration of 0.1mg/ml was added to YPD medium along with pronase.

B. The distribution of mutations along the *URA3* gene caused by inaccurate Pol α is similar to the distribution in the *rad27* strains and is different from the distribution of mutations induced by UV light.

The proportion of mutations falling into arbitrary chosen regions of 100 nucleotides is shown. The *URA3* is in forward orientation very close to the right side of ARS306.

Left top (purple-brown bars) - mutations induced by Cd^{2+} in the *pol1-L868M* strain 2 (n=85).

Left bottom (blue bars) – 5' ends of duplications in the *rad27* strain (n=17).

Right top (red bars) - UV light induced base pair changes (n=25).

Right bottom (green bars) – detectable position in the genes (based on the analysis of 151 mutations induced under various conditions).

C. A schematic representation of experimental design - positions of hotspots of mutations associated with the boundaries of Okazaki fragments when the reporter gene is moved.

D. Constant and moving hotspots of *ura3* mutations in the three locations and two orientations of the *URA3* gene in respect to replication origin.

DNA fragment containing the *URA3* gene with its own promoter and terminator was PCR amplified with primers allowing for integration of the reporter at the desired locations. All sequences are available on demand. In the closest to the origin forward orientation (top diagram of the chromosome with *ARS* and the *URA3* reporter, where the numbers above the rectangle representing the *URA3* gene correspond to the distance in base pairs from the start of the gene) the ATG codon is 278 bp from the right side of *ARS306*. The next two diagrams represent the reporter moved 75 bp and 150 bp away from the initial location. In the closest to the origin reverse orientation, the TAA codon is 193 bp from the right side of *ARS306* (diagram four). Then the reporter was moved away by the same increments as before. The independent *ura3* mutants arising in the resulting strains while MMR was inhibited by the presence of cadmium (Cd^{2+}) were sequenced to build the mutation spectra. The mutation rate was almost two orders of magnitude higher in the presence of Cd^{2+} as described previously [Pavlov et al., 2006a] and similar in all strains. The numbers in brackets are the number of mutations in the each spectrum. The mutation hotspots were detected as described [Rogozin et al., 2005]. Black dotted lines represent the hotspots that are unique for a given spectrum (moving hotspots), yellow dotted lines are the hotspots that are present in two out of three spectra in the same orientation. Red dotted lines represent hotspots present in all three spectra (constant hotspots, positions 26, 98, 368, 743 and 764 in the forward orientation; positions 200–210, 434–436, 646 and 679 in the reverse orientation). Vertical pink rectangles represent areas where moving hotspots are found in at least one forward spectrum and at least one reverse spectrum. The distances between the centers of these zones are at the bottom of the figure and we hypothesize that they correspond to the sizes of the Okazaki fragments.