Considering the cancer consequences of altered DNA polymerase function

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Our appreciation of the DNA transactions that replicate and maintain a stable human genome is changing rapidly due to recent discoveries indicating that eukaryotic cells contain many more DNA polymerases than previously thought. This review describes emerging information on the properties and functions of human DNA polymerases, with emphasis on connections between DNA polymerase functions and cancer.

Introduction

The integrity of the human genome depends on numerous processes that determine the fidelity of DNA replication. Several of these are responsible for providing the replication machinery with undamaged substrates with which to work (Figure 1, left). For example, the modified nucleotide 8-oxo-dGTP generated by oxidative stress is destroyed by enzymatic hydrolysis, thus preventing its mutagenic incorporation during replication (Colussi et al., 2002 and references therein). Endogenous cellular metabolism and physical or chemical insult from the external environment can damage the DNA. The resulting lesions come in many different forms, including strand breaks and base modifications that can alter or eliminate correct base pairing potential. These lesions are usually repaired by base excision repair, nucleotide excision repair, intrastrand crosslink repair, ssDNA break repair or, for dsDNA breaks, *n*on*h*omologous *e*nd *j*oining (NHEJ). These repair processes provide undamaged DNA that can be replicated accurately due to the high nucleotide selectivity and exonucleolytic proofreading activity of the replication machinery (Figure 1, center). However, because DNA repair processes are not perfect, the replication machinery occasionally encounters problematic substrates, including lesions that can block replication and can lead to double-strand DNA breaks. These encounters require specialized translesion DNA synthesis or homologous recombination to complete replication. Finally, modified and/or mismatched bases that are occasionally incorporated during replication can be corrected after replication (Figure 1, right) by specialized DNA repair processes that further reduce mutation rates.

The efficiency and fidelity with which these various DNA transactions operate can be key to the origins of cancer. This is beautifully illustrated by the affects of mutations that inactivate the human *MSH2* and *MLH1* genes. The products of both these genes are essential for DNA mismatch repair of replication errors, and loss of this activity alters cellular responses to DNA-damaging agents and strongly elevates mutation rates. Inactivating mutations in *MSH2* and *MLH1* are responsible for the majority of cases of hereditary non-polyposis colorectal cancer and also increase susceptibility to cancer in several other tissues. This linkage between mismatch repair gene mutation, elevated mutation rate, and cancer is consistent with the mutator hypothesis for cancer (reviewed in Loeb et al., 2003). This hypothesis suggests that an early event in tumorigenesis is a mutation that inactivates a gene that normally functions to maintain genome stability, resulting in an elevated mutation rate. This elevated mutation rate in turn results in additional mutations in other genes associated with multistage carcinogenesis, which then confer selective advantages that allow mutated cells to expand and achieve clonal dominance.

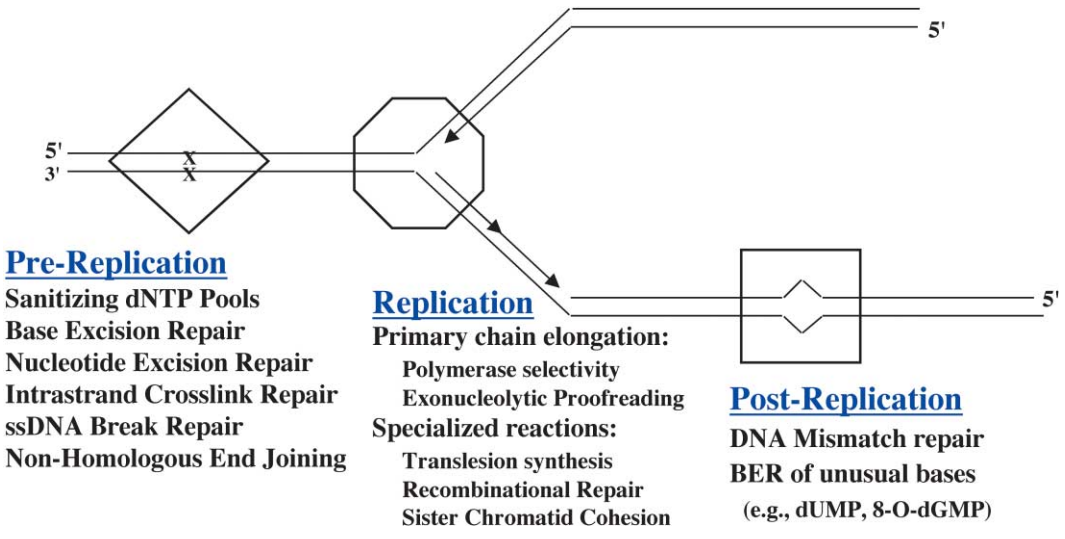

Figure 1. DNA polymerase-dependent processes that influence genome stability

See text for description. The Xs within the diamond indicate the presence of a lesion in DNA. ssDNA means single-stranded DNA and dNTP means deoxyribonucleoside triphosphate.

Table 1. DNA Template-dependent human DNA polymerases

DNA polymerase	Gene (alias)	Polymerase family	Chromosomal location	Associated activities
α (alpha)	*POLA*	B	Xp21.3–22.1	RNA primase
β (beta)	*POLB*	X	8p11.2	dRP lyase and AP lyase
γ (gamma)	*POLG*	A	15q25	3′ to 5′ exo, dRP lyase
δ (delta)	*POLD1*	B	19q13.3	3′ to 5′ exo
ε (epsilon)	*POLE*	B	12q24.3	3′ to 5′ exo
ζ (zeta)	*POLZ (REV3)*	B	6q21	
η (eta)	*POLH (XPV, RAD30A)*	Y	6p21.1	
θ (theta)	*POLQ*	A	3q13.3	
ι (iota)	*POLI (RAD30B)*	Y	18q21.1	dRP lyase
κ (kappa)	*POLK (DINB1)*	Y	5q13	
λ (lambda)	*POLL*	X	10q23	dRP lyase
μ (mu)	*POLM*	X	7p13	terminal transferase
σ (sigma)	*POLS (TRF4-1)*	X	5p15	3′ to 5′ exo
Rev1	*REV1*	Y	2q11.1–11.2	deoxycytidine transferase

Not listed is the template-independent enzyme terminal deoxynucleotide transferase, or telomerase, the ribonucleoprotein that adds TTAGGG repeats onto the ends of chromosomes.

The validity of the mutator hypothesis, and the number and types of cancers to which it may apply, is the subject of ongoing debate, and several alternative points of view should be carefully considered (e.g., see references in Loeb et al., 2003). Regardless, given the association between cancer and failure to correct DNA biosynthetic errors, it is interesting to note that the processes mentioned above involve the synthesis of DNA by various polymerases. Thus, it is logical to consider whether cancer is causally associated with mutations in DNA polymerase genes that lower the efficiency of repair to leave potentially mutagenic damage in DNA, or that reduce the fidelity of DNA synthesis associated with replication, repair, or recombination. Investigating relationships between cancer and polymerase defects has become a much greater challenge due to the discovery of a large number of DNA polymerases that were unknown only a few years ago.

A veritable plethora of polymerases

Organisms from bacteria to man have long been known to contain more than one DNA polymerase. The first mammalian DNA polymerase was identified in 1960 and was later designated with the first letter of the Greek alphabet as DNA polymerase α. DNA polymerase α was at one time believed to be THE nuclear replicative enzyme, but that view changed with the discovery of additional polymerases. Through 1995, five mammalian DNA polymerases had claimed Greek letters, DNA polymerases α, β, γ, δ, and ε. Amazingly, it has taken only seven more years to discover nine additional DNA template-dependent DNA polymerases, all but one of which now has a Greek letter designation (Table 1). This review focuses on very recent literature on these polymerases and emerging relationships between DNA polymerase dysfunction and cancer. For additional citations related to the seminal discoveries and characterizations of these DNA polymerases, interested readers can consult several other reviews (e.g., Hübscher et al., 2002; Goodman, 2002; Friedberg et al., 2002; Shcherbakova et al., 2003).

Table 2. Error rates of mammalian DNA polymerases

		Error rate × 10^{-5}	
DNA polymerase	Proofreading exonuclease	Substitutions	–1 deletions
Pol δ	Yes	~1	2
Pol ε	Yes	≤1	≤0.5
Pol γ	Yes	≤1	0.6
Pol α	No	16	3
Pol β	No	67	13
Pol κ	No	580	180
Pol η	No	3500	240
Pol ι	No	72,000 (T•dGTP)	—

Shown are average single base error rates per nucleotide incorporated for gap filling DNA synthesis in vitro, as measured using M13mp2 fidelity assays. For further details, see references in Kunkel et al., 2003.

Defective proofreading by a replicative DNA polymerase and cancer

The majority of DNA synthesis performed in a human cell is replication of the six billion nucleotide nuclear genome. The bulk of this polymerization is likely to be catalyzed by DNA polymerases δ, DNA polymerase ε, or both. These enzymes and certain other polymerases in the B family insert correct nucleotides onto correctly aligned primer templates while only rarely generating mismatches that can lead to base substitution and frameshift mutations. This high nucleotide selectivity reflects dNTP-induced conformational changes in the DNA polymerase and template primer that assemble a solvent-inaccessible dNTP binding pocket. This pocket snugly accommodates nascent base pairs having normal Watson-Crick geometry while excluding those that do not (Kunkel and Bebenek, 2000; Kool, 2002). The catalytic subunits of DNA polymerases δ and ε also have intrinsic 3′ to 5′ proofreading (PR) exonuclease activities that can excise base-base and insertion/deletion mismatches generated by the polymerase. The combination of nucleotide selectivity and proofreading accounts for the remarkably high fidelity of DNA polymerases δ and ε (Table 2), a property that is obviously key for faithful replication of the large nuclear genome. Current evidence suggests that, in addition to their roles in replication, DNA polymerases δ and ε fill gaps in DNA generated during nucleotide repair (NER), mismatch repair (MMR), and possibly base excision repair (BER).

The important role for the 3′ to 5′ exonuclease of DNA polymerase δ in suppressing tumorigenesis is clearly illustrated by the phenotypes of mice with a *POLD1* gene mutation that inactivates the exonuclease activity of DNA polymerase δ (Goldsby et al., 2002). This mutation results in loss of PR, a recessive

mutator phenotype, and a recessive cancer phenotype characterized by reduced life span (median survival, 10 months) and several different tumor types of epithelial cell origin. This implies that DNA polymerase errors that escape correction by PR contribute to carcinogenesis, preferentially in epithelial tissues. Thus, loss of proofreading during replication, like loss of MMR after replication, generally conforms to the mutator hypothesis for cancer. Interestingly, PR-deficient and MMR-deficient mice develop different types of tumors arising from somewhat different cell populations. These differences may reflect the sequence composition of the target genes that are mutated upon loss of these two distinctly different error correction processes. For example, a well-known mutational signature for loss of MMR is microsatellite instability (MSI). This form of instability likely initiates with DNA strand slippage. When this occurs during replication of long repetitive sequences, the misaligned intermediate can be stabilized by numerous correct base pairs, and the unpaired bases are far removed from the primer terminus. This effectively promotes polymerization, which requires a duplex primer terminus, and reduces the efficiency proofreading because the exonuclease digests single-stranded DNA (reviewed in Bebenek and Kunkel, 2000). This leaves MMR as the major guardian against MSI. The risk that a target gene involved in multistage carcinogenesis will be altered by a frameshift mutation in an MMR-deficient cell will increase as the repetitive sequence content of its open reading frame increases. However, the relationship between mutational risk and loss of proofreading should be quite different. This is because PR removes replication errors that are directly at or within very few base pairs of the primer terminus and can therefore more readily enter the exonuclease active site as single-stranded DNA. Thus a PR defect in a major replicative DNA polymerase places all genes at increased risk, especially for base substitutions and for frameshifts in short repeats. The increase in risk due to a proofreading defect will vary somewhat depending on the nucleotide selectivity of the DNA polymerase that generated the mismatch, the type of mismatch (12 base-base mismatches are possible), and the local sequence environment (for further details, see Kunkel and Bebenek, 2000). Thus, the fact that PR and MMR are distinct processes whose efficiencies depend on different parameters naturally places different genes at risk for mutations involved in multistage carcinogenesis.

The tissue specificity of cancers due to loss of MMR is suggested to reflect both an increased mutation rate and altered cellular responses to DNA damage. In a similar manner, differences in the cancer phenotypes of PR-deficient mice may reflect loss of other functions performed by 3′ to 5′ exonucleases. For example, the mutator phenotype observed in yeast upon inactivation of the 3′ to 5′ exonuclease of DNA polymerase δ depends on the S phase checkpoint (Datta et al., 2000). Also, the 3′ to 5′ exonuclease activity of DNA polymerase δ can excise displaced DNA flaps during lagging strand replication, and failure to do so can result in duplications (Jin et al., 2001). For these reasons, it will be interesting to investigate the carcinogenic consequences of concomitant loss of PR and MMR. Since these operate in series to correct DNA polymerase errors and stabilize the genome, polymorphisms in proofreading or MMR genes that have little or no effect alone may have serious consequences if combined.

Defective translesion DNA synthesis and skin cancer

Human DNA polymerase η is one of four human polymerases (Table 1) in the Y family. Pol η is encoded by the *XPV* (*PolH*) gene (reviewed in Prakash and Prakash, 2002), and persons with mutations that inactivate polymerase η suffer from *Xeroderma pigmentosum*, one symptom of which is greatly increased susceptibility to sunlight-induced skin cancer. This relationship is thought to reflect the ability of DNA polymerase η to efficiently copy a DNA template containing a *c*yclobutane *p*yrimidine *d*imer (CPD), a major sunlight-induced lesion that distorts DNA. This *t*rans*l*esion *s*ynthesis (TLS) ability of DNA polymerase η is remarkable because highly efficient bypass of bulky lesions was not seen in many years of studying other DNA polymerases in families A, B, and X. The current hypothesis for the connection between skin cancer and loss of DNA polymerase η in *XPV* patients is that, unlike certain other UV photoproducts, CPDs are slowly removed by excision repair and therefore sometimes encountered by the replication machinery. CPDs block DNA synthesis by accurate replicative polymerases like Pol δ or ε, while Pol η efficiently performs TLS that avoids generating CPD-induced mutations. In the absence of Pol η, as is the case in *XPV* patients, CPDs are bypassed in a manner that generates the mutations that lead to skin cancer. Two enzymes suggested to catalyze this backup bypass reaction are DNA polymerases ι and ζ (Table 1), neither of which has a proofreading activity. DNA polymerase ι has several interesting properties, among which is the ability to efficiently insert nucleotides opposite certain lesions, but without efficiently extending the resulting primer terminus. However, DNA polymerase ζ, a family B member encoded by the *REV3* gene (Lawrence and Maher, 2001), can extend mismatched primer termini efficiently, and genetic evidence reveals that *REV3* is required for ultraviolet light-induced UV mutagenesis. This has led to a "two-polymerase" bypass model (see Woodgate, 2001; Prakash and Prakash, 2002; Bresson and Fuchs, 2002, and references therein), wherein the first enzyme inserts a nucleotide opposite a lesion and a second enzyme extends that substrate to complete lesion bypass.

The multipolymerase TLS models are likely to be relevant to other human Y family members implicated in TLS, including DNA polymerase κ and REV1 (Table 1). Like DNA polymerase η, DNA polymerase κ alone can efficiently bypass certain bulky lesions that distort DNA, but its TLS specificity is somewhat different than that of DNA polymerase η (see references in Shcherbakova et al., 2003). For example, DNA polymerase κ can bypass a benzo[*a*]pyrene diol epoxide (BPDE) adduct on the N2 of guanine. This bypass primarily involves insertion of dCMP, an event that would avoid benzo[*a*]pyrene-induced mutations. This is interesting because expression of the mouse *PolK* gene is under the control of the arylhydrocarbon receptor (AhR), a crucial factor for activation of benzo[*a*]pyrene into BPDE in mammalian cells. This has led to the suggestion that DNA polymerase κ participates in bypassing lesions generated by polycyclic aromatic hydrocarbons (PAH) in a manner that avoids mutations (see Ogi et al., 2002 and references therein). Perhaps also relevant to the two-polymerase model is REV1, a partially template-dependent DNA polymerase that preferentially adds dCMP to primer termini. This protein interacts with REV3 and it is required for mutagenesis induced by some agents that induce DNA damage (Lawrence and Maher, 2001; Goodman, 2002).

The fact that 3 (Pols α, δ, and ε) of the 14 enzymes listed in Table 1 are required for normal replication of undamaged nuclear DNA and that at least 5 others (Pols ζ, η, κ, ι, and REV1) are implicated in TLS has led to the "factory model"

(Goodman, 2002). The hypothesis is that many polymerases may be present in a large replication complex, and the TLS enzymes are only called upon when the major replicative polymerases are blocked, either by a lesion or a naturally occurring undamaged barrier to replication, such as an unusual DNA structure. After performing their tasks, the TLS enzymes leave the template primer to allow resumption of synthesis by the major replicative DNA polymerases. Whether present in a factory or dispersed throughout the nucleus (e.g., see Kannouche et al., 2001), we do not yet know exactly how polymerases can switch as needed. Switching will undoubtedly require interactions between a polymerase catalytic subunit or accessory subunit (Hübscher et al., 2002) and other proteins (e.g., PCNA, RPA). These interactions can involve domains of DNA polymerases not required for polymerase activity per se (e.g., see Kannouche et al., 2002). Striking examples of catalytic subunits of human DNA polymerases that are much larger than needed to encode only their polymerization activity include Pol ε (255 kDa) and Pol ζ (353 kDa). Indeed, carboxy-terminal residues well beyond those encoding the polymerase and exonuclease activities of DNA polymerase ε are essential for cell cycle checkpoint control (see Datta et al., 2000 and Shcherbakova et al., 2003 for further discussion of polymerases and checkpoints). At least theoretically, such residues are potential targets for mutations leading to loss of checkpoint control in tumors. Analyses of protein-protein interactions and structure-function studies of the polymerase, exonuclease, AP lyase, and dRP lyase activities of DNA polymerases have now matured to the point where it is feasible to efficiently investigate possible associations between cancer and putative functional polymorphisms in the genes encoding DNA polymerases (e.g., see http://www.niehs.nih.gov/envgenom/snpsdb.htm).

Exonuclease-deficient DNA polymerases with specialized functions

Given the importance of proofreading to genome stability and the consequences of losing 3′ to 5′ exonuclease activity mentioned above, it is interesting that the majority of the human DNA polymerases listed in Table 1 actually lack intrinsic 3′ to 5′ exonuclease activity and cannot proofread the errors they make. Current evidence indicates that these DNA polymerases have specialized functions involving synthesis of relatively short DNA chains (reviewed further in Shcherbakova et al., 2003; Kunkel et al., 2003). For example, DNA polymerase α is a member of family B with an associated primase that is used to start replication of Okazaki fragments. Even without proofreading, the nucleotide selectivity of DNA polymerase α alone is sufficient for relatively accurate DNA synthesis (Table 2). It is also possible that an exonuclease encoded by a different gene may proofread errors made by exonuclease-deficient DNA polymerases. For example, the average base substitution error rate of DNA polymerase β alone (Table 2) is sufficient to introduce more than one mismatch per day into the genome during repair of the ≥10,000 damaged bases estimated to arise in 24 hr. However, the apurinic endonuclease that incises the DNA backbone following DNA glycosylase removal of a damaged base contains an intrinsic 3′ to 5′ exonuclease that can preferentially excise mismatched bases from primer termini (Chou and Cheng, 2002). Thus, errors made by DNA polymerase β during BER might be proofread by this exonuclease. This could be important for preventing the accumulation of mismatches in the human genome, perhaps especially for quiescent cells subject to stresses that result in damage repaired by BER, e.g., oxidative stress.

To fulfill its specialized function in BER that replaces a single damaged nucleotide (e.g., removal of U from a G-U mismatch arising from cytosine deamination), DNA polymerase β has an intrinsic dRP lyase activity that excises the 5′-deoxyribosephosphate group produced by APE incision. DNA polymerase γ has a dRP lyase activity that could serve a similar role in repair of the mitochondrial genome. Curiously, human DNA polymerases ι and λ also have an intrinsic dRP lyase activity. This implies that repair that requires removal of a sugar-phosphate may be performed by more than one DNA polymerase, with the choice of which polymerase to use perhaps depending on the cell type, damaged substrate, or timing of DNA repair. For example, DNA polymerase ι may participate in removing uracil from DNA when it is incorporated opposite adenine during replication using dUTP pools (Kunkel et al., 2003). In addition to functional redundancy, quite different functions may be performed by even closely related human DNA polymerases. For example, DNA polymerase λ is an X family enzyme with homology to and some properties in common with DNA polymerase β. DNA polymerase λ is also homologous to yeast DNA polymerase IV, which participates in NHEJ of double-stranded breaks in DNA (Tseng and Tomkinson, 2002 and references therein). This has cancer implications because defects in NHEJ are associated with cancer (Tlsty, 2002). Thus, human polymerases not yet directly implicated in disease may eventually be found to be relevant to cancer by virtue of possible functions such as repair of DNA crosslinks by DNA polymerase θ (reviewed in Shcherbakova et al., 2003) or participation in sister chromatid cohesion by DNA polymerase σ (Carson and Christman, 2001).

Amazingly inaccurate DNA polymerases

In contrast to the other exonuclease-deficient DNA polymerases listed in Table 2, members of the Y family that also lack proofreading have much lower base substitution and frameshift fidelity when copying undamaged templates (Table 2). In fact, DNA polymerase η error rates are similar to what was predicted over 20 years ago if a polymerase acted as a mere zipper without closely checking for correct base pair geometry. The fidelity of DNA polymerase κ is almost as low, and DNA polymerase ι has the truly amazing ability to generate some mismatches (e.g., T-dGTP, Table 2) at rates similar to or even higher than correct base pairs. The low fidelity of the Y family DNA polymerases that are implicated in TLS suggests that relaxed geometric selectivity may be important for efficient bypass of template lesions that distort helix geometry. Consistent with this, *Sulfolobus sulfataricus* DNA polymerase 4, a homolog of human Pol κ with similarly low fidelity, has an open and solvent accessible active site that can even accommodate two template bases at once, one of which is unpaired (Ling et al., 2001). Structural studies of Y family DNA polymerases (reviewed in Boudsocq et al., 2002; Friedberg et al., 2001) also reveal a fourth polymerase subdomain not found in polymerases in families A, B, and X, which interacts with DNA and may be critical for TLS efficiency and specificity. The existence of several human polymerases with very low fidelity suggests the need for careful regulation of their functions by protein-protein interactions, transcriptional and/or translational control. That permanent or transient loss of regulation of a DNA polymerase may be relevant to human cancer is suggested by the tumorigenic consequences of ectopic expression of DNA polymerase β in mice (Bergoglio et al., 2002 and references therein).

Promiscuous polymerases, somatic hypermutation, cytosine deamination, and cancer

In the field of cancer research, mutations are considered to be detrimental to health. However, normal human B cells in germinal centers are thought to conduct an inaccurate DNA transaction that results in mutations beneficial to health, somatic hypermutation (SHM) of DNA encoding the variable (antigen binding) regions of the heavy and light chain immunoglobulin genes. SHM occurs at a rate that is much higher than the overall rate of spontaneous mutation rate in the nuclear genome, suggesting that targeted, highly inaccurate DNA synthesis is occurring. The human Y family polymerases are prime candidates for contributing to SHM due to their low fidelity, as is Pol ζ, by virtue of its promiscuous ability to extend mismatches. Indeed, several recent observations suggest the possible involvement of DNA polymerases ζ, η, and ι in SHM (see Kunkel et al., 2003 and numerous references therein).

The types of replication and/or repair transactions possibly involved in SHM are the subject of active investigation and considerable speculation. SHM and class switch recombination (CSR), a second process involved in immune system development, require *a*ctivation-*i*nduced cytidine *d*eaminase (AID). AID belongs to a family of proteins that includes the apolipoprotein B mRNA editing catalytic subunit 1 (APOBEC1). APOBEC1 edits mRNA in gastrointestinal tissues by deaminating a particular cytosine to uracil to create a stop codon. It was recently discovered that AID (Petersen-Mahrt et al., 2002), APOBEC1, and two related Apobec proteins (Harris et al., 2002) are all mutagenic when expressed in *E. coli*. The induced mutations are C•G to T•A transitions whose frequencies are substantially higher in cells lacking uracil DNA glycosylase, indicating that these proteins enzymatically deaminate cytosine to produce uracil in DNA. The mutagenic potential of uracil is particularly high because, unlike the blocking lesions mentioned above that require TLS polymerases, uracil can be efficiently replicated like normal thymine to yield C to T base substitution mutations. What might this have to do with cancer? In addition to beneficial functions like SHM and RNA editing, AID and Apobec could potentially produce widespread genomic instability if enzymatic deamination is not controlled properly. This is an alternative to aberrant mRNA editing for explaining why transgenic expression of APOBEC1 in mice leads to liver cancer (Yamanaka et al., 1995). Indeed, Neuberger and colleagues report examples wherein expression of Apobec-1 and Apobec3G is high in tumor tissue but not in normal tissue (Harris et al., 2002). Thus, unregulated expression of cytidine deaminase family members could possibly result in cytosine deamination-dependent mutagenesis that contributes to carcinogenesis, again consistent with the mutator hypothesis for multistage carcinogenesis.

Closing thoughts

Cancer is now associated with mutagenic replication of damaged DNA resulting from loss of Pol η, with defective proofreading by Pol δ and with defective mismatch repair of errors made by replicative DNA polymerases. These three examples have in common the loss of a replication fidelity function that elevates the mutation rate without loss of the replication efficiency needed for clonal expansion of mutant cells to give rise to a tumor. Given the existence of many DNA polymerases whose fidelity can vary over a 10,000-fold range (Table 2), and given the multiple and complex transactions that all require DNA synthesis to maintain genome stability, there are probably many more ways to generate mutant cells that thrive despite reduced DNA synthesis fidelity. Thus, the current list of associations between defective DNA synthesis fidelity and cancer is almost certainly incomplete. How cells regulate which DNA polymerase is used for which DNA transaction and substrate, the accessory protein partnerships, and mechanisms used for polymerase switching or to prevent catalysis at the wrong place or time, and whether additional polymerase gene mutations or functional polymorphisms exist that are relevant to carcinogenesis are issues for the future. The opportunity and the challenge are great, given the number of polymerases, the sequence complexity of the human genome, and the large number of different DNA adducts that can result from endogenous cellular metabolism and from external exposure to physical and chemical carcinogens.

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