

ERROR-PRONE DNA POLYMERASES: When Making a Mistake is the Only Way to Get Ahead¹

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■ **Abstract** Cells have high-fidelity polymerases whose task is to accurately replicate the genome, and low-fidelity polymerases with specialized functions. Although some of these low-fidelity polymerases are exceptional in their ability to replicate damaged DNA and restore the undamaged sequence, they are error prone on undamaged DNA. In fact, these error-prone polymerases are sometimes used in circumstances where the capacity to make errors has a selective advantage. The mutagenic potential of the error-prone polymerases requires that their expression, activity, and access to undamaged DNA templates be regulated. Here we review these specialized polymerases with an emphasis on their biological roles.

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INTRODUCTION

Several new DNA polymerases have been defined with specialized abilities to function on damaged DNA templates. It is perhaps an injustice that they have been called "error prone," which assumes that another polymerase could do better. Although these polymerases may be error prone on undamaged templates, some of them can use damaged templates that block the normal replicative polymerases. Indeed, some of these polymerases seem dedicated to synthesize across specific lesions restoring the undamaged sequence, and have therefore also been called translesion polymerases.

It is a well-entrenched dogma that cells must duplicate their genomes with high fidelity in order to pass on to their progeny the fruits of their evolutionary education. In fact, most cells duplicate their genome with less than ~ 1 base change per division (38a). For humans, that is less than one error per billion bases synthesized. The average human cell loses $\sim 10^4$ bases per day owing to spontaneous hydrolysis of glycosyl bonds alone (111). Cells also must deal with other types of DNA damage, such as the thousands of pyrimidine dimers produced in a sunburned human skin cell (179). Unrepaired lesions lead to mutagenesis, carcinogenesis, and aging. Fortunately, cells have evolved functionally redundant DNA repair pathways for dealing with the many different types of damage, such as Base Excision Repair (BER), Nucleotide Excision Repair (NER), Mismatch Repair (MMR), and Translesion Synthesis (TLS). Although TLS was hypothesized some time ago, DNA polymerases capable of TLS have only recently been unequivocally identified (reviewed in 56, 208).

Clearly, a major role of the error-prone polymerases is to provide a means to deal with environmental damage to the genome. Several of these polymerases were first identified by their roles in repair of damage from UV irradiation. Biochemical studies have provided fascinating insights into how the polymerases recognize their cognate lesions. The same properties that allow lesion bypass can also result in reduced stringency. As a result some of these polymerases have a much lower fidelity on undamaged DNA templates. Therefore it is important to understand how

cells recruit the error-prone polymerases to lesions when necessary, and conversely, how they limit access to undamaged templates. Sometimes these polymerases may be recruited to undamaged templates, perhaps as a consequence of balancing the benefits in damage repair with the potential mutagenic hazards. More recently, these polymerases have also been implicated in processes where, it can be argued, their lack of fidelity may provide a selective advantage to cells. We address these questions and the related literature in the section on Biological Roles. We first define the nomenclature and briefly discuss some of the details about each of the enzymes. This information is also summarized in the Tables.

Nomenclature

Although not deliberately designed to create confusion, the nomenclature used in this field is not welcoming: different names for the same polymerase, different names for closely related polymerases, and the use of gene names to refer to enzymes. For example, the polymerase responsible for the bulk of the spontaneous mutations in budding yeast is comprised of two subunit proteins encoded by the genes *REV3* (100) and *REV7* (103). Because it was the sixth polymerase identified, it is called Pol ζ (zeta, after the sixth letter of the Greek alphabet). However, Pol ζ is also designated *POLZ*, and sometimes called Pol zeta, Rev3, or Rev3p. In SWISSPROT it is referred to as DPOZ.YEAST. A recent attempt to standardize this nomenclature (26) settled on the use of the Greek alphabet as a reminder of the order of discovery of the enzyme, despite the problems it creates. Even so, Rev1 and TdT (terminal deoxynucleotidyl transferase) did not get Greek letters, presumably because they were named earlier. In Table 1, which is designed to guide readers through this nomenclature, the enzymes are organized according to families that reflect related phylogeny. Sometimes genes have been identified based on homology to a previously identified gene, and are often named after the original. Hence, there are human and mouse *REV1* genes identified by their relationship to the gene for the yeast Rev1 polymerase. In most cases, this reflects related enzymatic activities and biological roles, although similar in vitro enzymatic activities do not guarantee identical functions in different organisms. In *Escherichia coli*, the polymerases are designated by Roman numerals distinct from the eukaryotic polymerases, which are designated by Greek letters. We emphasize that *polA*, which encodes Pol I, is not related to Pol α and that *polB* is not related to *POLB* (Pol β).

In this review we refer to the enzymes by the Greek letter or Roman numeral designation (i.e., Pol I or Pol ζ). When gene names are used, we use the most common name for yeast genes, and the HUGO (Human Genome Organization) designation for mammalian genes. Table 1 lists the most commonly used gene designations for each polymerase. Because most of this research has been carried out in *E. coli*, *Saccharomyces cerevisiae*, and *Homo sapiens* or *Mus musculus*, we confine most of our discussion to these systems.

We provide a brief summary of the biochemical data for each of the polymerases in Tables 2 and 3, and in the text. For simplicity, we refer to “bulky adducts,”

TABLE 1 DNA Polymerase families

| Family ^a | Consensus sequence | <i>E. coli</i> ^b | <i>S. cerevisiae</i> ^b | <i>H. sapiens</i> ^c |
|---------------------|---|---|---|---|
| A | R-x(2)-[GSAV]-K-x(3)- [LIVMFY]-[AGQ]-x(2)- Y-x(2)-[GS]-x(3)-[LIVMA] ^d | <i>polA</i> /Pol I | <i>MIP1</i> /Pol γ | <i>POLG</i> /Pol γ <i>POLQ</i> /Pol θ |
| B | [YA]-[GLIVMSTAC]-D-T-D- [SG]-[LIVMFTC]-x- [LIVMSTAC] ^e | <i>polB/dinA</i> /Pol II | <i>POL1/CDC17</i> /Pol α <i>POL2/DUN2</i> /Pol ϵ <i>POL3/CDC2</i> /Pol δ <i>REV3/PSO1</i> /Pol ζ <i>POL5</i> /Pol ϕ | <i>POLA</i> /Pol α <i>POLE</i> /Pol ϵ <i>POLD1</i> /Pol δ <i>POLZ/REV3L</i> /Pol ζ |
| C | F-E-[RT]-F-[LMI]-[NSG]- [PF]-[DEKH]-[RG]-[KS]- [MLV]-P-D-[IF]-D ^f | <i>polC</i> /Pol III | None identified | None identified |
| D | [VIL]-[KR]-Y-[IL]-[VIC]- [IV]-A-G-D-[VLI]-V-D-G- [VI]-G-[VI]-Y-P-G-Q ^g | None identified | None identified | None identified |
| X | G-[SG]-[LFY]-X-R-[GE]- X(3)-[SGCL]-X-D- [LIVMFY](3)-X(2)-[SAP] ^h | None identified | <i>POL4/POLX</i> /Pol λ <i>TRF4</i> /(Pol κ)/Pol σ 1 <i>TRF5</i> /Pol σ 2 | <i>POLB</i> /Pol β <i>POLL/POLB2</i> /Pol λ <i>POLM</i> /Pol μ <i>TDT/DNNT</i> /TdT <i>POLS/hTRF4</i> /Pol σ |
| Y | [YFL]-x(2)-Y-x(3)-S-x(2)- [AIV]-x(2)-[IL]-[LFM]- x(2)-[YF]-x(3)-[IVF]-E- x(2)-[SG]-[ILF]-D-E-A- [YF]-[LV]-D-[ILV]-[ST] ⁱ | <i>DINB</i> /Pol IV <i>UMUC</i> /Pol V | <i>RAD30/DBH1</i> /Pol η <i>REV1</i> /Rev1 | <i>POLH/RAD30B</i> /Pol η <i>POLI/RAD30A</i> /Pol ι <i>POLK/DINB1</i> /Pol κ <i>REV1L</i> /Rev1 |

^aFrom (26).

^bCatalytic subunit: *GENE NAME*(S)/Enzyme designation.

^cCatalytic subunit: *HUGO NAME*/OTHER *GENE NAME*/Enzyme designation.

^dA-family signature from Prosite <http://us.expasy.org/cgi-bin/nicedoc.pl?PDOC00412>

^eB-family signature from Prosite <http://us.expasy.org/cgi-bin/nicedoc.pl?PDOC00107>

^fPhP_N Block from Blocks <http://blocks.fhrc.org/blocks-bin/getblock.sh?IPB003141B>

^gMotif 2 from (29).

^hX-family signature from Prosite <http://us.expasy.org/cgi-bin/nicedoc.pl?PDOC00452>

ⁱMotif III of UmuC family signature from Prosite <http://us.expasy.org/cgi-bin/nicedoc.pl?PDOC50173>

keeping in mind that these encompass a wide variety of lesions (N-2 acetylaminofluorine, benzo[a]pyrene, 8-oxo-Guanine, etc.), and that not all enzymes work equally on all of these lesions. For further information see (56, 76).

DNA POLYMERASE FAMILIES

A Family

The A family is comprised of homologs of *E. coli* DNA Polymerase I encoded by the *polA* gene (Table 1). Pol I is a high-fidelity polypeptide with both 3' and 5' exonuclease activities. Only the 5' exonuclease is required for viability, because

TABLE 2 *E. coli* DNA polymerases

| Enzyme/ gene name ^a | Polymerase family | Additional enzymatic activities | Fidelity on undamaged DNA | Lesion bypass | Functions | Interactions |
|--------------------------------------|----------------------|---------------------------------------|------------------------------------|--|--|---|
| Pol I/ <i>polA</i> | A | 5'→3' exo 3'→5' exo | 10 ⁻⁵ –10 ⁻⁶ | | Okazaki maturation | β/γ |
| Pol II/ <i>polB</i> | B | 3'→5' Exo | 10 ⁻⁵ –10 ⁻⁶ | Bulky adducts | Replication restart | β/γ |
| Pol III/ <i>polC</i> | C | | 10 ⁻⁵ –10 ⁻⁶ | | Major replicative polymerase | β/γ |
| Pol IV/ <i>dinB</i> | Y | | 10 ⁻³ –10 ⁻⁴ | Bulky adducts Extend misaligned primers | TLS Replication restart Adaptive mutation SOS mutagenesis | β/γ |
| Pol V/ <i>umuC</i> | Y | | 10 ⁻³ –10 ⁻⁴ | UV dimers Abasic sites Bulky adducts Extend mispaired primers | TLS SOS mutagenesis | RecA UmuD' SSB β/γ PolIII (α, β, ε) |

^aGene refers to catalytic subunit.

it is necessary for the removal of RNA primers from Okazaki fragments during lagging strand synthesis. In eukaryotes (with the possible exception of plants; 26), mitochondrial DNA replication and repair requires the A family polymerase Pol γ (gamma). Pol γ is a high-fidelity enzyme with 3' exonuclease and dRPlyase (5' deoxyribose-5-phosphate lyase) activities. dRPlyases function during BER by removing the 5' dRP residue that remains after the combined activities of a DNA glycosylase and an AP endonuclease. A second eukaryotic member of the A family, Pol θ (theta), has been identified in humans by homology to a gene required for repair of intrastrand crosslinks (ICL) in *Drosophila melanogaster* (170). Although this is a high-fidelity enzyme with a 3' exonuclease, it can also readily replicate templates with abasic residues (114). Because little else is known about this enzyme it is not discussed further.

B Family

The B family is defined by proteins with homology to Pol II from *E. coli*. Pol II is a high-fidelity enzyme that plays a primary role in DNA replication restart of stalled forks (159). The major replicative polymerases of eukaryotes, Pol α (alpha), Pol δ (delta), and Pol ε (epsilon) are all members of the B family. For more information about these high-fidelity enzymes, see (76). Eukaryotic cells also have a lower fidelity B family polymerase, Pol ζ (zeta), that lacks the conserved 3' exonuclease domain (131; see below). A fifth eukaryotic B family member was identified in *S. cerevisiae* (Pol φ, phi). Although essential, the polymerase catalytic domain does

TABLE 3 Eukaryotic DNA polymerases

| Greek designation | Family | <i>S. cerevisiae</i> gene | HUGO name | Associated enzymatic activities | Fidelity on undamaged DNA | Lesion bypass | Protein interactions | Proposed primary function |
|-----------------------|--------|---------------------------|--------------|---|---------------------------|--|--|--------------------------------------|
| γ gamma | A | <i>MIP1</i> | <i>POLG</i> | 3'→5' Exo dRP lyase | 10^{-5} – 10^{-6} | | <i>POLG2</i> | Mitochondrial replication and repair |
| θ theta | A | | <i>POLQ</i> | 3'→5' Exo | 10^{-5} – 10^{-6} | Abasic sites | | ICL repair |
| α alpha | B | <i>POL1</i> | <i>POLA</i> | Primase | 10^{-4} – 10^{-5} | | RP-A | Replication priming |
| δ delta | B | <i>POL3</i> | <i>POLD1</i> | 3'→5' Exo | 10^{-5} – 10^{-6} | | PCNA | Chromosomal replication |
| ε epsilon | B | <i>POL2</i> | <i>POLE</i> | 3'→5' Exo | 10^{-5} – 10^{-6} | | PCNA RF-C | Chromosomal replication |
| ζ zeta | B | <i>REV3/REV7</i> | <i>POLG</i> | | 10^{-4} – 10^{-5} | Extend mispaired primers UV dimers Bulky adducts | REV7 REV1 | TLS DSBR SHM |
| ϕ phi | B | <i>POL5</i> | | | 10^{-5} – 10^{-6} | | | rRNA synthesis |
| β beta | X | | <i>POLB</i> | dRP lyase AP lyase Terminal transferase | 10^{-4} – 10^{-5} | Extend misaligned and mispaired primers Bulky adducts | ERCC1 + Lig3 PCNA Ligase I FEN1 | BER NER |
| λ lambda | X | <i>POL4</i> | <i>POLL</i> | dRP lyase | 10^{-4} – 10^{-5} | Abasic sites Extend mispaired primers | Lig4 + Lif1 PCNA | NHEJ Meiosis |

| | | | | | | | |
|-------------------------|---|---------------------------------|--------------------------------------|-------------------------|---|----------------------------|--|
| Terminal transferase | X | <i>TDT</i> | Terminal transferase 3'→5' exo | | | Ku XRCC4 + Lig4 | VDJ-N nucleotide insertion |
| μ mu | X | <i>POLM</i> | Terminal transferase | 10^{-4} – 10^{-5} | Bulky adducts UV dimer Extend mispaired primer | Ku XRCC4 + Lig4 | NHEJ |
| σ sigma | X | <i>TRF4/TRF5</i> <i>POLS</i> | Terminal transferase 3'→5' exo | 10^{-4} – 10^{-6} | | | Sister chromatid cohesion Chromosome condensation |
| Rev1 | Y | <i>REV1</i> | | | Abasic sites | Pol ζ | TLS |
| η eta | Y | <i>RAD30</i> <i>POLH</i> | | 10^{-2} – 10^{-3} | UV dimer Bulky adducts | PCNA | TLS SHM ICL repair |
| ι iota | Y | <i>POLI</i> | dRP lyase | $\sim 2 \times 10^{-1}$ | Deaminated cytosines UV dimers Bulky adducts | PCNA | TLS BER SHM |
| κ kappa | Y | <i>POLK</i> | | 10^{-3} – 10^{-4} | Abasic sites Bulky adducts Extend misaligned primers | PCNA RF-C RPA Ahr | TLS |

not appear to be required for viability (172), but instead seems to affect rRNA synthesis.

C and D Families

The major replicative polymerase of *E. coli*, Pol III, and its homologs comprise the C family. For more information about Pol III, see (133) and references therein. The D family has only been found in archaeobacteria (29) and is not discussed here.

X Family

The X family is a large family of nucleotidyl transferase proteins many of which are not DNA polymerases (i.e., antibiotic nucleotidyl transferases; 3). Six different X-family DNA polymerases have been identified in eukaryotes. Two of the enzymes [Pol β (beta; 202) and Pol λ (lambda; 2, 50)] have a dRPlyase activity. TdT (17), Pol μ (mu; 38), and Pol σ 1 (sigma; 198) are capable of template-independent polymerization, although Pol μ and Pol σ 1 also carry out template-dependent DNA synthesis. Pol σ 1 and Pol σ 2 are most closely related to the cytoplasmic PolyA polymerases, which also belong to the X family (3).

Y Family

The Y family of polymerases is diverged from the other polymerase families, but shares some common structural features (208). The founding members of this family were the UmuC and DinB proteins from *E. coli*, and the Rev1 and Rad30 proteins from *S. cerevisiae* (141). Four members of this family have been identified in mammals (Table 1). These polymerases appear to be specific to the type of lesion that they can bypass (see below).

TRANSLESION SYNTHESIS IN *E. COLI*

Five DNA polymerases have been isolated from *E. coli* (Table 2). Pol I is required for the maturation of lagging strands, and Pol III is the major replicative polymerase. The high fidelity of Pol III and its inability to use damaged DNA templates creates a need for translesion synthesis or damage-avoidance pathways. Upon exposure to DNA damage, *E. coli* responds by mounting the SOS damage response that induces the expression of more than 30 genes involved in DNA repair (180; see Polymerase Regulation). Among the induced genes are three nonessential DNA polymerases: Pol II, Pol IV, and Pol V.

Pol II

Pol II is a high-fidelity polymerase (27) required for error-free replication restart of UV-induced lesions. Replication ceases when Pol III encounters a blocking lesion. The replication fork then regresses, and the newly replicated sister-chromatid

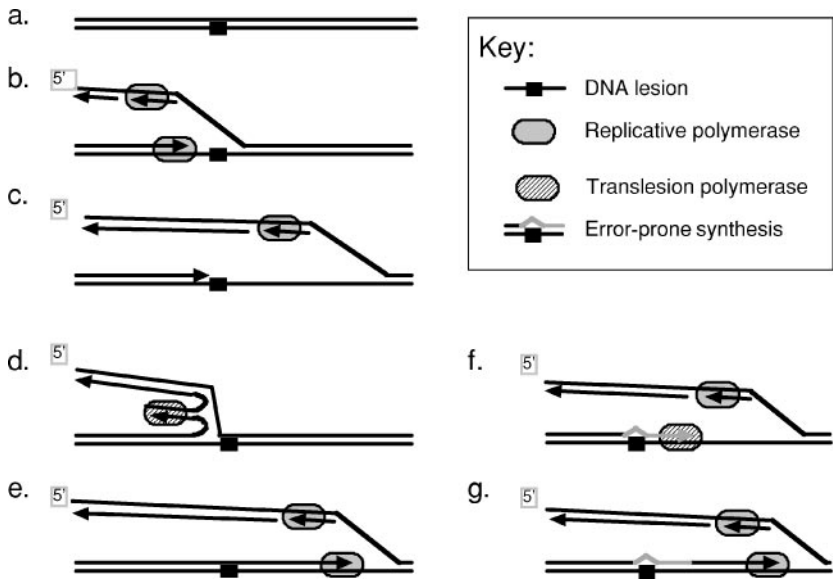


Figure 1 Lesion avoidance versus translesion synthesis. (a) DNA with lesion shown as black box. (b) Replication fork stalled at DNA lesion. (c) Uncoupled lagging strand synthesis. (d) and (e) Lesion avoidance by replication restart copying first off lagging strand then reestablishing replication fork. (f) and (g) Translesion synthesis and then reestablishing replication fork.

strand serves as a template for further synthesis (Figure 1a–e). The extent of synthesis is limited to the distance already replicated on the undamaged strand. Once the replication proceeds beyond the site of the damage, the replication fork is re-established by reverse branch migration, thereby effectively bypassing the lesion without correcting it. The lesion may then be repaired after the completion of replication by an alternative mechanism (159). Perhaps unique features of the regressed fork are involved in the recruitment of Pol II. *E. coli* cells that lack Pol II are only slightly sensitive to UV, but become hypersensitive if Pol V is also absent (159), indicating that Pol II can help to bypass some UV-induced lesions. In vitro, Pol II can synthesize across an abasic site (183), and can bypass some bulky adducts by a primer misalignment mechanism (195). Primer misalignment requires a repeated sequence that allows the template and the primer to slip relative to one another. The bypassed base(s) is looped out, leaving a paired base adjacent to the incoming nucleotide, resulting in a frameshift mutation.

Pol IV

Although the first *dinB* (damage inducible) mutants were isolated about 20 years ago (113), the gene was only recently shown to encode a DNA polymerase, Pol IV

(196). Pol IV is normally abundant but is further induced around tenfold by the SOS response (87). Pol IV is incapable of bypassing UV-induced pyrimidine dimers (181), but it can bypass some bulky lesions (171) and it can also extend misaligned primers (87, 196). Pol IV may function in the reinitiation of stalled forks by extending aberrant ends that cannot be removed by proofreading (56). *dinB* mutants do not have any obvious DNA repair defects, but they are defective in untargeted mutagenesis, whereby more mutations are seen among the progeny of bacteriophage lambda propagated on irradiated cells than on unirradiated cells (23; see Untargeted Mutagenesis). Pol IV is also responsible for the majority (~85%) of *lacZ*(-1) frameshift "adaptive" mutations (46, 91, 125; see Selection Induced Mutagenesis).

Pol V

Mutant strains of *E. coli* that are unmutable by UV light (*umu*) identified the *umuC* and *umuD* genes (85, 176), which are now known to encode DNA polymerase V (161, 182). Pol V is proficient at bypassing both UV-induced pyrimidine dimers and abasic sites in vitro (161, 182). The in vivo spectrum of SOS-induced mutants, with a preference for T→C transitions from thymine dimers (99, 105), correlates with the in vitro bypass specificity of Pol V (161, 182). Pol V is highly regulated both transcriptionally and posttranscriptionally, consistent with the expectation that unfettered low-fidelity DNA replication is not a good thing (see Polymerase Regulation).

TRANSLESION SYNTHESIS IN EUKARYOTES

To date, 16 different DNA polymerases have been identified in eukaryotes (Table 3). Three of these, Pol α , Pol δ , and Pol ϵ , are required for chromosomal replication and are members of the B family (reviewed in 76). Pol γ , an A family member, is required for mitochondrial replication and repair. The remaining 12 polymerases appear to have specialized functions in DNA repair or chromosome stability.

B Family

The B family has highly conserved motifs including the catalytic aspartic acid residues, residues that contact the template and primer, residues involved in binding the incoming dNTP, and the 3' exonuclease domain.

Pol ζ REV3 (107) and REV7 (103), encoding the two subunits of Pol ζ , were first identified from screens for mutants defective in UV-induced reversion of auxotrophic mutations (*reversionless*) in *S. cerevisiae*. Pol ζ is required for the majority of both spontaneous and damage-induced mutagenesis (101, 163) in yeast, and overexpression of Pol ζ results in increased mutagenesis of UV-irradiated cells if Rev1 is also overexpressed (157; see below). REV3 is highly conserved,

and is present in all eukaryotes examined to date (104). Human cells deficient in Pol ζ grow normally, but have reduced UV-induced mutagenesis (211). Deletion of *REV3* in mice results in midgestation embryonic lethality, suggesting that Pol ζ is essential for development (39, 134, 204). The Rev3 protein includes all of the conserved B family motifs except for the 3' exonuclease (131). The key properties distinguishing Pol ζ from Pol α , Pol δ , and Pol ϵ are its ability to copy over damaged DNA and to extend mispaired primers. In vitro, Pol ζ can synthesize across a *cis*-syn TT dimer (58, 109, 131), a thymine glycol (82), and several different bulky adducts (9, 57, 173). However, a more important function of Pol ζ may be its ability to extend mispaired primers (Figure 2d), as it does this with ~ 100 -fold greater efficiency than misincorporation opposite a lesion (152; see More Than One Polymerase). The function of Rev7 is not known, but it stimulates the polymerase activity of Rev3 by ~ 30 -fold (131).

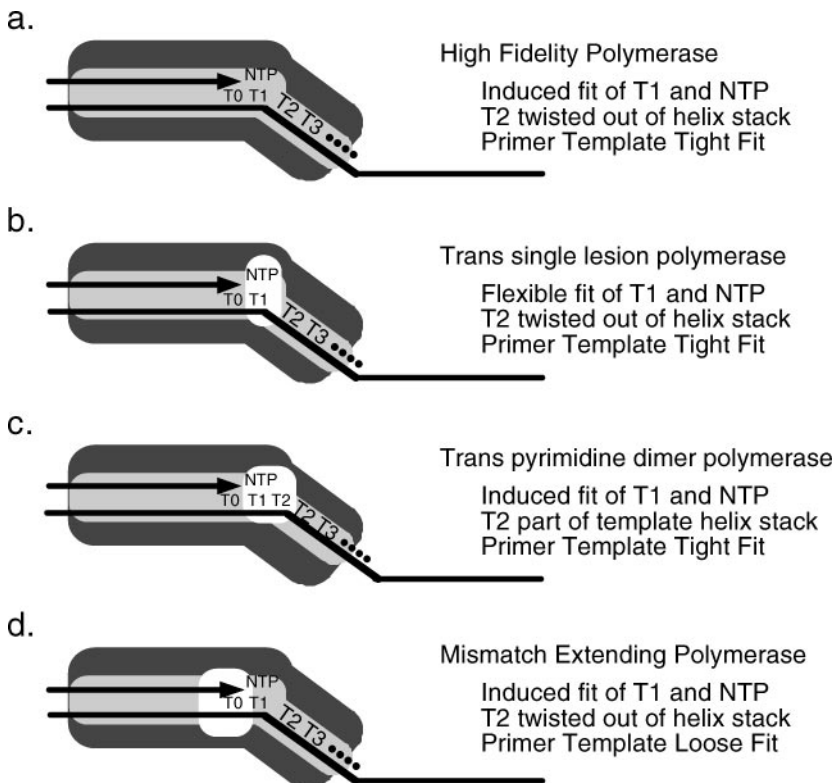


Figure 2 Tailored polymerase fidelity. (a) High-fidelity polymerase. (b) *Trans* single lesion polymerase with relaxed fit in the site for template and/or in coming dNTP (shown as white region). (c) *Trans* pyrimidine dimer polymerase with room in template site for dimer (shown as white region). (d) Mismatch extending polymerase with extra room at position of 3' end of primer (shown as white region).

X Family

Pol β Pol β can catalyze two important enzymatic steps during BER; the dR-Plyase activity can remove the 5' dRP residue remaining after AP endonuclease cleavage of an abasic residue, and the DNA polymerase can replace the excised base (174, 202). Pol β is also required for long patch BER (2–6 nt; 36, 150) where the flap endonuclease, Fen1, is required for removal of the displaced strand (89). Pol β is found in complexes with either DNA ligase I (153), or XRCC1-DNA ligase III (95). In vitro Pol β makes deletion and insertion errors at a high rate (10^{-3} ; 96) due to primer misalignment. Pol β is overexpressed in a number of different tumor types (28, 168, 175) and leads to increased aneuploidy when overexpressed in CHO cells (14, 28). Mouse cells deficient in Pol β or defective in the dR-Plyase activity are hypersensitive to some DNA-methylating agents (174). Mice lacking Pol β have severe growth retardation, and die immediately after birth (178).

Pol λ Pol λ is closely related to Pol β , and was originally called *POLB2* (128). Like Pol β , it has a dR-Plyase activity and has \sim fivefold greater DNA synthesis fidelity than Pol β (158) due to an increased affinity for dNTPs. Pol λ can replicate DNA templates with abasic residues (158). Although the gene is expressed ubiquitously, expression is particularly high in testis, suggesting a possible role in meiosis. Knockout mice are reported to either have immotile cilia syndrome (90) or no obvious defects (15).

The yeast *POL4* gene, most closely related to Pol λ (Table 3), is upregulated in meiosis (24), and null mutants exhibit increased meiotic intragenic recombination (106). It is important for nonhomologous end joining (NHEJ) when either 5' or 3' terminal mismatches must be removed (203). Yeast Pol λ physically interacts with DNA ligase IV, but not with other NHEJ proteins such as the Mre11/Rad50/Xrs2 complex or Lif4 (190).

TdT TdT is responsible for addition of nontemplated (N) nucleotides at V(D)J antigen receptor coding joints in lymphoid cells in vivo (53, 92). It is only found in immature thymocytes, bone marrow cells, transformed pre-B and pre-T cell lines, and leukemia cells (54, 97). TdT has two splice variants that differ by only 60 nucleotides; the short variant encodes TdT polymerase, and the long variant encodes a 3' exonuclease. These two enzymes probably function in concert to limit the level of N nucleotide addition (184). TdT is a template-independent polymerase that can catalyze the addition of dNTPs or rNTPs in vitro (18). It also interacts with Ku and XRCC4-Ligase IV, consistent with a role in end joining (118, 119).

Pol μ Pol μ , a TdT homolog, is expressed predominantly in lymphoid tissues (2, 38). Knockout mice have a slight reduction in B-cells from peripheral lymphoid tissues but no defect in somatic hypermutation (15). In vitro Pol μ is a dNTP or rNTP transferase (132), which is strongly stimulated by the presence of template (38). It is highly error prone, with a preference for incorporating pyrimidines (212).

Pol μ correctly bypasses *cis*-syn TT dimers (212), and can also bypass many different types of bulky adducts by primer misalignment (71, 212, 213). A role for Pol μ in NHEJ is suggested by its association with Ku and XRCC4-LigaseIV in extracts and by its ability to stimulate their end-joining activity (119).

Pol σ Pol σ (originally named Pol κ ; 198) is required for the establishment of sister chromatid cohesion in yeast, possibly by coordinating the loading of cohesion proteins during replication (30). Cells deficient in Pol σ 1 (*trf4* mutants) are cold sensitive. Loss of Pol σ 1 is lethal when combined with *top1* (topoisomerase) mutants (166), but overexpression of the closely related gene *TRF5* can overcome this synthetic lethality (197). In vitro, Pol σ 1 is a processive enzyme with a 3' exonuclease proofreading activity (198). However, Pol σ 1 can also catalyze template-independent extension of oligonucleotides (198). A human homolog of Pol σ has been identified; the gene is in a chromosomal region that is often amplified in small cell lung tumor lines and high-grade ovarian tumors (197).

Y Family

The discovery of this new family of DNA polymerases sparked considerable interest because these polymerases can copy damaged DNA templates and have a high error rate when they copy undamaged DNA templates (Table 3). Structurally, Y family polymerases have a much more open catalytic site that can accommodate damaged bases and/or tolerate mispairing of the incoming nucleotide (208).

Rev1 REV1 was originally identified as a gene required for UV-induced mutagenesis in yeast (101). It is important for the increased UV-induced mutagenesis seen when Pol ζ is overexpressed (157). The ability of Rev1 to insert C opposite abasic sites was the first demonstration of a nucleotidyl transferase activity by a Y-family member (130). More recently, it was found that Rev1 can insert all four nucleotides on an undamaged template, but the enzyme prefers a poly G template (64). Rev1 may also have a structural role, independent of its nucleotidyl transferase activity, in promoting the ability of Pol ζ to bypass some lesions (129) and to extend from correctly paired termini (10; see More Than One Polymerase). The human gene, cloned by homology, can also incorporate C opposite abasic sites (110). Overexpression of antisense REV1 results in reduced UV mutagenesis, suggesting that Rev1 promotes error-free bypass of UV damage in humans, as it does in yeast (52).

Pol η The yeast *RAD30* gene, encoding Pol η (eta), was identified by homology with the *E. coli* *dinB* and *umuC* genes (123, 165). Yeast cells lacking Pol η are slightly sensitive to UV, and only weakly affect UV mutagenesis unless cells have additional repair defects (123, 165). Humans with a variant form of xeroderma pigmentosum (XP-V) have Pol η defects caused by mutations (primarily frameshifts) in the *POLH* gene (78, 121). XP-V individuals have a greatly increased probability

of getting skin cancer (32), and cells from XP-V individuals are very UV sensitive (156). Despite the different phenotypes observed in yeast and humans, the biochemistry of the two enzymes is remarkably similar, and the increased incidence of skin cancer is explained by the fact that Pol η can bypass UV-induced cyclobutane thymine dimers accurately and efficiently (Figure 2c) (79, 120). Presumably, these lesions lead to mutations in cells lacking Pol η . Pol η can also accurately bypass some bulky adducts (66, 210), but is error prone when copying other bulky adducts (i.e., cisplatin; 77, 192), or on undamaged DNA templates (199). However, Pol η does not efficiently extend mispaired termini (Figure 2b) (201), which limits its ability to introduce errors.

Pol ι Mammals have another homolog of *RAD30*, Pol ι (124), which appears to be specialized for error-free bypass of deaminated cytosines. Although Pol ι is error prone on undamaged DNA and preferentially inserts G opposite T or U templates, it replicates A templates relatively accurately and can extend a mispaired base (48, 81, 186, 193, 215). Pol ι also has a dRPlyase activity (191), and can substitute for Pol β for BER in vitro in the presence of Uracil N-glycosylase (UNG), AP endonuclease, and DNA ligase I (11). Although ubiquitously expressed, there is good evidence that Pol ι plays an important role in somatic hypermutation (SHM), because cells lacking this enzyme have reduced levels of SHM (41; see Somatic Hypermutation).

Pol κ Pol κ is another mammalian Y-family member (112, 139), originally also named Pol θ (80). Pol κ is expressed ubiquitously, but is further induced by the arylhydrocarbon receptor (AhR; 137). AhR is a ligand-activated transcription factor that mediates the activation of the common environmental polycyclic aromatic hydrocarbon benzo[a]pyrene into the potent carcinogen BPDE (137). Indeed, Pol κ can efficiently and accurately bypass BPDE lesions (138). Pol κ preferentially inserts A residues across abasic residues (200), and can accurately bypass thymine glycols (44) and some bulky adducts (140, 214). However, Pol κ is error prone when copying other bulky adducts (140, 214) or undamaged templates and can also extend mispaired bases (63, 2003).

BIOLOGICAL ROLES

Given the ability of error-prone polymerases to generate mutations when copying undamaged DNA, cells must be able to limit the access of these polymerases to undamaged templates, and conversely, to recruit the correct polymerase to a damaged template when necessary. The very redundancy of DNA damage-repair mechanisms requires that cells regulate the way in which substrates are directed to each of the pathways. The balance between pathway usage is different in different organisms, or even in diversified cells within an organism. This regulation may reflect changes in enzyme concentrations, or in the mechanisms by which the

polymerases are recruited to damaged substrates. Disruption of one pathway may shift the balance of the other enzymes or pathways used. Below we discuss situations where error-prone polymerases appear to have a biological effect (also see 96a).

Spontaneous Mutation

It is not easy to determine whether the spontaneous mutation rate reflects the fidelity of replicative polymerases or the processing of accumulated spontaneous DNA damage. The increased mutation rate observed in MMR-defective strains may reveal the inherent replication error rate, but the MMR pathway also has a role in detection of DNA damage. The reduction in spontaneous mutation in *S. cerevisiae* cells defective in Pol ζ (75, 163) suggests that most spontaneous mutations are dependent on that enzyme. These mutations might reflect the action of Pol ζ on spontaneous DNA lesions or, alternatively, the recruitment of Pol ζ to undamaged templates, perhaps extending errors made by replicative polymerases. For example, variants of Pol δ (*pol3-Y708A*) have a mutator phenotype dependent on Pol ζ and MMR (145). These results are consistent with the view that mistakes made by Pol δ are subject to a step (probably extension) that requires Pol ζ , and that the misincorporations are subject to MMR. Similarly, the spontaneous mutation rate is reduced in *E. coli* with a defect in Pol IV (108).

It is unlikely that the spontaneous mutation rate directly reflects the fidelity of the replicative DNA polymerases, making it impossible to determine their contribution to the spontaneous mutation rate. Synthesis of leading and lagging strands may differ in their fidelity or TLS, as suggested by the observation of orientation-dependent differences in mutation spectrum and frequency (4). The fidelity of the pool of DNA polymerases in a population of cells reflects the accuracy with which the individual polymerase molecules were synthesized. For example, the molecular basis of the mutator phenotype of *mutA*, a miscoding tRNA, is due to a reduced fidelity of Pol III isolated from *E. coli mutA* cells, presumably due to amino acid substitutions made in Pol III subunits by the miscoding tRNA (1). Importantly, the mutator phenotype in *mutA* cells is independent of Pol IV or Pol V, suggesting that some of the defective Pol III molecules can extend the errors they introduce.

Polymerase Regulation

The expression of many error-prone polymerases is under transcriptional regulation. In *E. coli*, Pol II, Pol IV, and Pol V are constitutively expressed at low levels, but are induced by the DNA damage SOS response (87, 154, 205). LexA is the normal repressor of these functions. SOS induction occurs when RecA binds ssDNA and becomes activated (RecA*). RecA* then interacts with the LexA repressor, resulting in a conformational change that promotes LexA self-cleavage (98). Inactivation of LexA leads to induced expression of more than 30 genes, most of which are involved in DNA repair (180). It is not clear how cells avoid

using the constitutive levels of Pol II and Pol IV inappropriately in the absence of DNA damage.

Pol V provides an example of how cells limit expression of potentially mutagenic proteins until needed (reviewed in 55). Active Pol V is composed of the catalytic subunit UmuC and two subunits of activated UmuD (designated UmuD'₂). Uncleaved UmuD cannot associate with UmuC, and both proteins are rapidly degraded by the Lon protease. Activation of UmuD requires a co-protease cleavage reaction with RecA*. However, UmuD dimers are preferentially processed to produce UmuD/D' heterodimers in which the UmuD' subunit is subject to rapid proteolysis by ClpXP. Thus, the UmuD'₂ dimer only accumulates and associates with UmuC to form an enzymatically active Pol V enzyme when high levels of RecA* are present.

Similar levels of regulation probably exist for the eukaryotic translesion polymerases. For example, transcription of *RAD30* (Pol η) is induced by UV light in yeast (123, 165) and in humans (84). In *S. pombe*, Pol κ is activated both transcriptionally and posttranscriptionally in response to the DNA-damage checkpoint arrest (83), and in humans, the expression of Pol κ is induced by activated AhR (137). Pol μ mRNA appears to be regulated by splicing upon treatment with DNA-damaging agents (2, 119), and Pol λ is downregulated after exposure to DNA-damaging agents (2). Some polymerases appear to be under tissue-specific transcriptional control; for example, TdT is only present in immature thymocytes and bone marrow cells (97), Pol μ is found predominantly in lymphoid tissues (2, 38), and Pol λ is highly expressed in testis (2, 51).

There is evidence that overexpression of some of the translesion polymerases is associated with oncogenesis. For example, elevated levels of Pol κ have been seen in human lung cancer (135), ovarian, and prostate cancer tissues (13). Increased levels of Pol β are seen in ovarian (28), prostate, breast, and colon cancer tissues (175). Supportive evidence linking the overexpression of Pol β with oncogenesis is provided by the finding of increased aneuploidy in CHO cells that overexpress ectopically introduced Pol β (14, 28).

Untargeted Mutagenesis

Untargeted mutagenesis refers to the increased mutagenesis of undamaged DNA when it is introduced into cells that have been exposed to DNA damage. For example, UV irradiation of *E. coli* cells increases the mutation frequency of bacteriophage lambda progeny introduced after the UV treatment (23, 113). This process normally depends on transcriptional induction of translesion polymerases by the SOS pathway, and can be mimicked by overexpressing Pol IV in *E. coli* (86, 91). In yeast, increased reversion of mutations present in unirradiated cells is observed when these cells are mated to irradiated cells (102). Untargeted mutagenesis is also seen when Pol ζ and Rev1 are overexpressed in yeast (157), or when Pol κ is overexpressed in mouse cells (136). Thus, it appears that overexpression is sufficient to allow error-prone polymerases access to undamaged DNA. This may

reflect enhanced processing of spontaneous lesions into an error-prone pathway, or elevated access by error-prone polymerase to undamaged templates.

The Meiotic Effect

Magni & von Borstel (117) observed that the mutation rate in meiosis is higher than in mitotic cells. This “meiotic effect” could reflect untargated mutagenesis related to the high levels of DSB-initiated recombination events in meiotic cells. In fact, the meiotic mutations are associated with nearby recombination events (116). This relationship to DNA damage is more direct than can be demonstrated for untargated mutagenesis, although it cannot be determined whether the mutation provoked the recombination or the recombination elevated the probability of a mutation. Over 40 years later we might wonder whether this seminal observation reflects elevated expression of error-prone polymerases during meiosis in yeast (24) and presaged the recent observations that translesion polymerases are highly expressed in germ cells: Pol ζ (194), Pol η (124, 207), Pol κ (136, 169), and Pol λ (2, 51).

Selection-Induced Mutagenesis

Cells exposed to stress that is not obviously genotoxic nonetheless have elevated mutation rates. There is a lively debate as to whether this mutagenesis is random and is subsequently selected, or whether it is directed to that class of genes most likely to provide relief from the selection (20, 46, 125). There is evidence that transcription elevates spontaneous mutation rates (34, 126, 206), and one might anticipate that the genes required to pass a selection are preferentially transcribed. Paradoxically, transcribed DNA is subjected to repair of DNA damage more readily than silent DNA (59). In the best studied example, *lacZ* frameshift reversion in *E. coli*, much of the selection-induced mutagenesis (adaptive mutation) is dependent on Pol IV (25, 91), and on recombination functions that imply a need for DNA ends (47, 69). The requirement for an end could reflect a recruitment mechanism for Pol IV (25), and suggests a link with recombination-associated mutagenesis (discussed below). Additional data support the conclusion that a subpopulation of cells undergoing selection-induced mutagenesis are hypermutable (164, 188) owing to a deficiency in MMR-related functions (43, 67). One might also wonder whether a change in transcriptional or translational fidelity as discussed above for *mutA* strains (8) might also contribute to selection-induced mutagenesis.

Recruitment of Translesion Polymerases

One mechanism by which cells can regulate the access of error-prone polymerases to damaged DNA is by specifically recruiting them when and where needed. All five of the polymerases in *E. coli* interact with the β -clamp processivity factor, and mutations in the β -clamp interacting domain abolish the ability of Pol II, Pol IV,

Recruitment Mechanisms

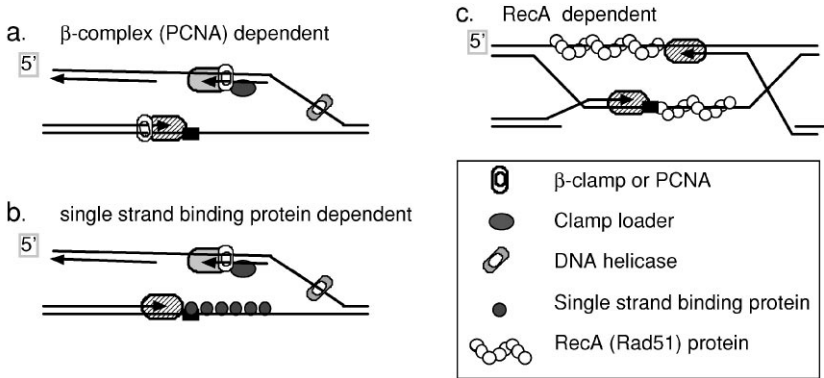


Figure 3 Recruitment mechanisms. (a) Translesion polymerase binds to β clamp or PCNA at stalled replication fork. (b) Translesion polymerase binds to single-strand binding protein at primer template junction. (c) Translesion polymerase binds to recA and related proteins.

and Pol V to promote translesion synthesis (Figure 3a) (Table 2) (12). Similarly, Pol η (60, 62), Pol ι (61), Pol κ (65), and Pol λ (115) interact with the eukaryotic β -clamp counterpart, PCNA (proliferating cell nuclear antigen; Table 3).

Recent studies in *S. cerevisiae* demonstrate that PCNA can be differentially modified, suggesting a mechanism for recruitment of different repair factors. PCNA can be sumoylated, monoubiquitinated, or polyubiquitinated on Lys164 (72). The type and level of modification depends on the position within the cell cycle or the level of exogenous DNA damage. Interestingly, polyubiquitination requires Rad5, Ubc13, and Mms2. In the absence of these proteins, repair is error prone. Thus, PCNA modification may well be involved in recruiting the translesion polymerases for different repair pathways.

Other *E. coli* proteins that may help to recruit polymerases to the sites of DNA damage are SSB (single-strand binding protein) and RecA. In the presence of SSB and the β -clamp, Pol V is recruited to a 3' primer end and carries out distributive DNA synthesis (Figure 3b). When RecA is also present, binding of Pol V to the 3' primer is stimulated, and the DNA synthesis is highly processive (Figure 3c). However, RecA must be removed from the ssDNA template in order for synthesis to proceed. Removal of RecA ahead of Pol V has been likened to a cowcatcher on a locomotive, stripping the RecA from the template as synthesis proceeds (149). Notably, in a two-ended invasion event, RecA filament formation is limited to the region between the invading strands (Figure 3c). In this case, the disassembly of RecA filaments from both ends would aid the reannealing of the undamaged template duplex. It is not yet known whether Rad51 (the eukaryotic RecA homolog) plays a similar role in recruiting the error-prone polymerases to damaged DNA in eukaryotes.

More Than One Polymerase

Biochemical studies indicate that translesion replication sometimes requires the sequential action of two polymerases. For example, Rev1 inserts a C residue opposite an abasic site, and extension is then carried out by Pol ζ (130). Pol η correctly and efficiently inserts two A residues across from a *cis-syn* TT dimer (79). In contrast, when Pol η synthesizes on a (6-4) pyrimidine dimer, it only inserts a single G opposite the 3' pyrimidine (77), but Pol ζ can accurately and efficiently extend the G residue inserted by Pol η (77). Thus, for two of the three (6-4) photoproducts, the sequential action of Pol η and Pol ζ result in error-free bypass (152).

What are the properties of the polymerases that allow them to recognize and act upon the different lesions? For high-fidelity polymerases there is a very tight fit near the active site that encompasses a few bases of duplex DNA, the templating base, and the incoming dNTP; the next untemplated base is flipped out of the active site. This tight fit at the active site imposes a high degree of base selectivity for the incoming base (Figure 2a). Interestingly, Pol ζ is more efficient at extending mispaired bases than it is at misincorporating bases (81), suggesting that the fit at the position of the previously incorporated base is somewhat looser, but maintains a high degree of selectivity for the incoming base (Figure 2d). Pol η , on the other hand, efficiently incorporates two A residues across from *cis-syn* TT dimers. The structure of these TT dimers does not permit the 5'T to rotate out of the active site (Figure 2c). Examination of the crystal structure of Pol η shows that the active site is open, and can readily accommodate the templating residue as well as the next 5' unpaired residue (189) (Figure 2c). Although Pol η can efficiently accommodate pyrimidine dimers, it can only incorporate a single residue opposite the more highly distorted (6-4) pyrimidine dimers. However, once Pol η has incorporated one residue, Pol ζ can accommodate the bulkier lesion, and can extend the primer by correctly inserting a nucleotide opposite the 5' pyrimidine (Figure 2c, d).

Some polymerases, for example Pol IV and Pol κ , can extend misaligned, but not mispaired, primers (87, 136, 200). These polymerases must be able to accommodate a bulge from the unpaired bases in either the template or the primer. TdT, Pol μ , and Pol σ can all extend oligonucleotides in the absence of the template (5, 38, 198). Clearly, in these cases, contact with the template strand must not be necessary. In this context, the mode that Pol σ binds to the primer is intriguing, because it is reported to carry out both template-independent extension as well as high-fidelity template-dependent extension (198).

Recombination-Associated Mutagenesis

Recombination is considered an error-free mechanism of DNA repair; however, the pathways leading to spontaneous recombination and mutagenesis are coupled. For example, genotoxic treatments are both mutagenic and recombinogenic. As has already been discussed, the elevated mutation seen in meiosis is associated with recombination. Similar observations have been made demonstrating an association of mutagenesis with mitotic recombination (40). More recently, we

found that homologous recombination causes mutations by a pathway that involves error-prone polymerases (73, 177). When recombination is initiated by making a site-specific double-strand break (DSB), there is a 100–1000-fold increase in the mutation rate of genes adjacent to the DSB. By using a forward mutation assay in a counter-selectable gene, it was possible to determine the spectrum of mutations associated with DSB repair (160). The majority of these mutations are point mutations (BRIMs, *break repair induced mutations*); there is a slight excess of deletions over base substitutions. Although Pol ζ is not required for efficient DSB repair, the majority of the base substitutions require Pol ζ . In contrast, the frequency of single base deletions remains elevated among the recombinants in Pol ζ –defective cells. The polymerase responsible for the frameshift mutations has not been identified.

The mechanism that recruits Pol ζ to recombination intermediates has not been determined. Perhaps the recombination pathway involves intermediates (i.e., ssDNA) that are more susceptible to spontaneous damage that would require translesion polymerases. A greater susceptibility of ssDNA to damage is suggested by the finding that cytosine is deaminated >100-fold more frequently in ssDNA than in dsDNA (42), and that transcribed DNA is more mutable (34, 206). The observation that *rad55* and *rad57* mutations elevate BRIMs (160) may reflect the role for these gene products in promoting formation and stability of the RecA-like filament formed by Rad51. The Rad51 filament may form a protective environment for single-strand intermediates, reducing spontaneous DNA damage. Alternatively, the recombination intermediates themselves may provide a recruiting mechanism for error-prone polymerases (149, 162), as discussed above for the *recA* protein, and in so doing, enhance the error rate on undamaged DNA templates (Figure 3*b, c*).

Nonhomologous End Joining

Error-prone polymerases may also play a role in NHEJ (Figure 4). DNA ligase is sufficient to covalently join ends that are blunt, or complementary to each other provided they have the correct phosphate and hydroxyl moieties. However, DNA ends often have to be processed by nucleases and/or polymerases before they are ligatable. Directly repeated sequences flanking a DSB site can anneal resulting in loss of the intervening DNA (Figure 4*a–d*). Removal of the intervening DNA requires the activities of enzymes that have roles in recombination and NER. For example, removal of the nonhomologous 3' end is accomplished by the Rad1/Rad10 complex (Figure 4*c*) (45). The intermediate in Figure 4*d* is similar in structure to the substrates in Figure 3 and might recruit error-prone polymerases. Notably, elevated mutation rates are seen adjacent to sites of recombination between direct repeats (74).

Many NHEJ joints are due to interactions between microhomologous sequences. In yeast, Pol λ appears to be important for NHEJ, promoting the joining of DNA ends with terminal mismatches (203). We wonder whether error-prone polymerases might also serve to extend microhomologies (Figure 4*e*). Extension would stabilize the interaction, and the intermediates could then be resolved by recombination,

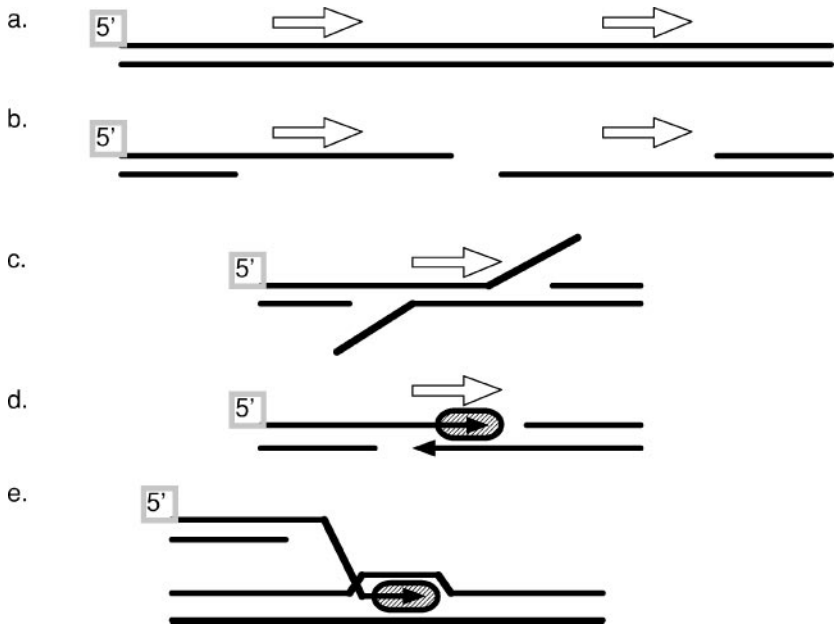


Figure 4 Error-prone polymerases in nonhomologous end joining. (a) DNA with a direct repeat shown as an open arrow. (b) DSB between repeats is resected to uncover homology. (c) Pairing at homology. (d) Trimming to create paired primer and extension by polymerase to fill in single-strand gaps. (e) Invasion of an end at a microhomology followed by extension by an error-prone polymerase.

replication, and/or ligation-mediated processes. A hallmark that might indicate the involvement of error-prone polymerases in NHEJ would be the presence of untemplated bases or base substitutions close to deletion junctions. Such untemplated additions are common in Ig and T cell receptor gene rearrangements, and a role for TdT in their formation is well documented (35, 92).

Somatic Hypermutation

The enormous selective pressure for the ability to mount a highly variable immune response has resulted in the evolution of marvelously complicated pathways for genome alterations in Ig-expressing cells. Such cells are a great place to look for exceptions to the rule that cells should be able to replicate their genomes with high fidelity (68). Mouse and human Ig genes are assembled into their active form by site-specific recombination of reiterated pieces. Further diversification of the assembled genes occurs when B cells that recognize an antigen are induced to proliferate and to undergo very high levels of mutagenesis in the portion of the Ig gene that encodes the antigen-interacting domain. This process is known as somatic hypermutation (SHM).

Several important aspects of this hypermutation process have been demonstrated. (a) The gene must be transcribed (6, 49, 147). Although the promoter itself can be replaced with any active promoter, the presence of the Ig enhancer is critical for targeting mutations to the variable region or reporter genes placed under its control (7, 16, 88, 94). (b) SHM depends upon AID (activation-induced deaminase), which is closely related to cytosine deaminase (127). This homology suggested that AID should act on mRNA; however, AID was recently purified and shown to deaminate cytosines on ssDNA (19, 31). (c) Both ssDNA nicks and dsDNA breaks are seen in cells undergoing SHM (21, 93, 142). (d) Three error-prone polymerases have been implicated in generating the mutations Pol ζ (37, 211), Pol ι (41, 151), and Pol η (122, 144, 209).

One model proposes that transcription plus some activity of the Ig enhancer target AID. The role of the enhancer may be to recruit proteins that stabilize a transcription-dependent secondary structure exposing ssDNA as a substrate for AID (Figure 5c). Evidence supporting enhancer-dependent extended RNA/DNA hybrids in the Ig gene has been presented (185). AID deaminates cytosine to uridine in the ssDNA bubble created by transcription. The modified DNA is either replicated, generating C \rightarrow U \rightarrow T transitions (Figure 5d), or processed by UNG into an abasic site. The abasic site can be copied by translesion polymerases allowing other base substitutions (Figure 5e) or further processed by AP endonuclease into a ssDNA break. The break provides additional opportunities to recruit error-prone polymerases (Figure 5f), or it can lead to DSB formation and subsequent repair by recombination. As described above, recombination can itself be mutagenic, or provide the gene conversion pathway for importing variants from related sequences (see below). Support for each of these steps or branches is discussed below.

Many of the mutations are found at hot spot motifs (WRCY or its complement RGYW). Normal mice can make transition or transversion substitutions at this motif, but UNG-deficient mice have primarily C \rightarrow T transitions (155), consistent with the view that the primary event is deamination of cytosine by AID. A similar mutational pattern is seen in Pol ι -deficient BL2 cell lines activated to undergo SHM, suggesting that Pol ι makes the transversion substitutions at the abasic sites (41). Pol ι could act directly by copying over the abasic site, or it could function in BER by removing the dRP residue after the activity of AP endonuclease, preparing the template for a different error-prone polymerase.

Another hot spot motif during SHM is WA or TW. Mutations at these sites are mediated by Pol η since XP-V individuals lack these mutations (209). Because the mutations are still present in UNG-deficient mice (155) and in Pol ι -deficient cell lines (41), the intermediate that recruits Pol η is unknown. Finally, Pol ζ also appears to be important in SHM, because SHM is reduced by $\sim 70\%$ in cell lines treated with antisense oligonucleotides to *REV3* (211), and mice expressing antisense *REV3* RNA fail to generate high-affinity antibodies (37). Whether the role for Pol ζ is direct, or as the second polymerase in a two-step error-prone repair pathway remains to be determined.

DNA breaks are found in cells undergoing Ig class switching and SHM (21, 93, 142). A role for AID in promoting DSB formation is supported by the

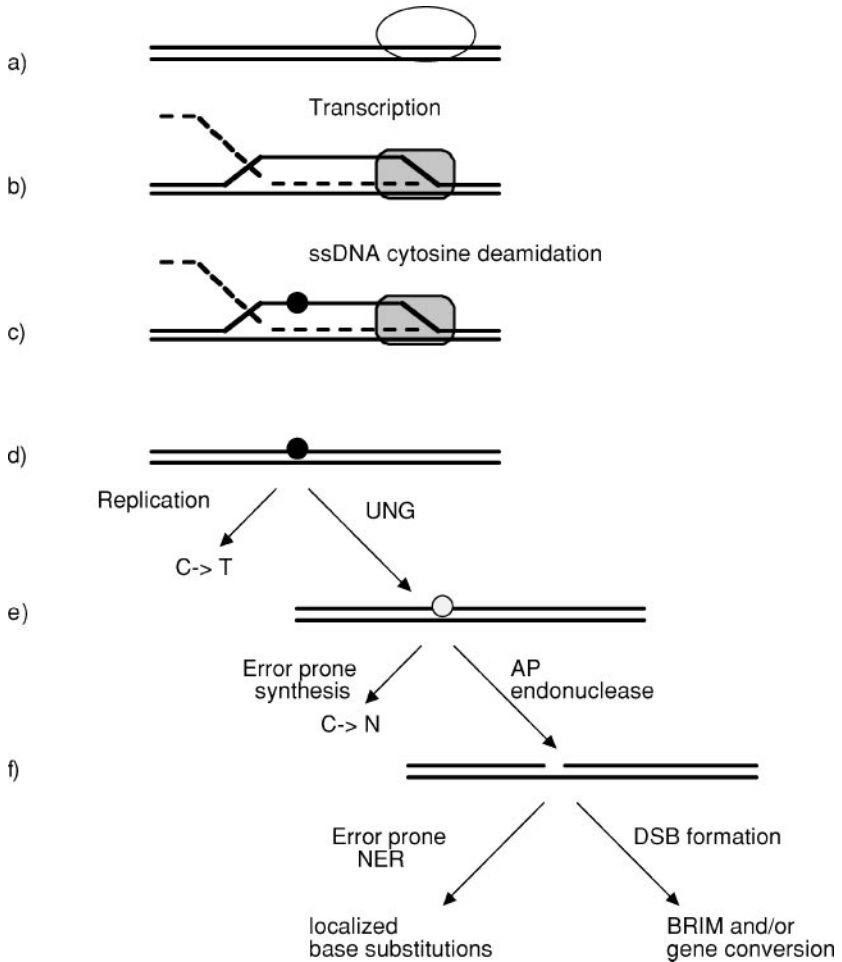


Figure 5 Somatic hypermutation. (a) Enhancer-specific modification of transcription. (b) Transcription-dependent secondary structure creating ssDNA. (c) ssDNA cytosine deamination by AID creates uridine (*closed circle*). (d) Transition mutations made by copying uridine, or creation of an abasic site by UNG (*open circle*). (e) Base substitution mutations made by Pol ι (and Pol ζ ?), or creation of a single-strand gap. (f) DSB initiated BRIM and/or gene conversion.

finding that H2AX foci, presumed to be associated with DSBs, are not formed in the absence of AID (148). However, recent data suggest that DSBs are still detected in cells deficient in AID activity (22, 143). In chicken, B-cells undergo gene conversion (Figure 5f) rather than somatic hypermutation (although some point mutations are detected among the recombinants), and AID is necessary for this gene conversion (70). Rabbits, cattle, and pigs use related gene conversion

pathways for adaptive immunity (reviewed in 68). Chicken B cells with defects in recombination caused by mutations in *XRCC2*, *XRCC3*, or *RAD51B* use a pathway that leads to high levels of point mutations (167). This is reminiscent of studies in yeast, where increased BRIMs are seen in the absence of *rad55* or *rad57* (160), and suggests that decreased efficiency of recombination may lead to increased error-prone DNA synthesis.

CONCLUDING REMARKS

The biological roles of the recently identified error-prone DNA polymerases are beginning to emerge. A major role for these polymerases is to decrease the potentially lethal effects of DNA damage. Some of these polymerases are dedicated to function on specific types of DNA lesions, and in many cases, to repair these lesions by restoring the original sequence. In other cases, the translesion synthesis involves a polymerase of such relaxed specificity that mutations may be introduced. Cells limit the danger posed by these error-prone polymerases by regulating their expression and their access to undamaged DNA. We look forward to additional research aimed at understanding how polymerases are activated and targeted.

The selective use of error-prone polymerases also provides organisms with the opportunity to accelerate evolution. Organisms must balance the risks and benefits of low-fidelity DNA synthesis. Single-celled organisms (or mobile DNAs) often survive and compete in hostile environments, so the ability to modulate the mutation rate when stressful situations arise is an obvious benefit. It is probably not an accident that many wide host range transposons and plasmids carry with them a copy of the *dinB* gene (146). Might higher eukaryotes use a similar strategy? The generation of immunologic diversity by somatic hypermutation provides a clear example of how organisms target error-prone DNA synthesis to specific genes. The elevated expression of error-prone polymerases in cancer cells increases the probability that a cell can acquire the multiple changes required for oncogenesis. Although these are examples of events that arise in somatic cells, the same enzymes are elevated in germ cells and the meiotic effect in yeast reminds us that meiosis is associated with elevated mutation rate. Might error-prone polymerases be targeted to specific genes in higher eukaryotes to promote diversity or accelerated evolution? Some genes or genomic regions clearly evolve at much higher rates than others, including mammalian sperm proteins, olfactory receptors, and cone snail toxins (33, 187). We look forward to learning whether the molecular basis of gene-specific evolution rates involves a role for error-prone polymerases.

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