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Visions & Reflections

Family growth: the eukaryotic DNA polymerase revolution

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Our appreciation of the rich complexity of DNA transactions needed to replicate and maintain genomes has grown dramatically in the past few years with the discovery of a number of DNA polymerases that have remarkable properties. Historically, mammalian DNA polymerases have been designated with letters of the Greek alphabet. When DNA polymerase α was discovered more than four decades ago, it was thought to be THE polymerase responsible for replicating the six billion nucleotides of the human genome. How things have changed! We now know that human cells contain more than a dozen distinct DNA polymerases belonging to four different families (A, B, X, and Y) based on sequence alignments (table 1). Four of the first five are essential for accurately replicating the nuclear (pols α, δ, and ε) or mitochnodrial genome (pol γ), while pol β participates in excision repair of damaged bases. Other enzymatic activities intrinsic to or associated with the polymerase catalytic subunits allow them to start replication, to continue replication accurately and processively, or to excise baseless sugar-phosphates during repair. Current excitement now stems not only from the sheer number of polymerases recently added to the list but also from unanticipated properties that imply highly specialized functions.

Table 1. Eukaryotic DNA polymerases.

Name	Alias	Family	Amino acids	$3' \rightarrow 5'$ exo	Associated activities	Proposed main function
Pol α	yPol I	B	1462	no	primase	initiates replication
Pol β		X	335	no	dRP lyase, AP lyase	base excision repair
Pol γ	yMIP1	A	1239	yes	dRP lyase	mitochondrial DNA replication
Pol δ	yPol III	B	1107	yes		nuclear DNA replication
Pol ε	yPol II	B	3000	yes		nuclear DNA replication, checkpoint control
Pol ζ	yREV3	B	3130	no		mutageneic synthesis, other?
Pol η	XPV, yRad30	Y	713	no		translesion replication
Pol θ	Mus308	A	2724	?	helicase?	repair of cross-links?
Pol ι	RAD30B	Y	715	no	dRP lyase	specialized base excision repair?
Pol κ	DinB1, pol θ	Y	870	no		unknown, Translesion synthesis
Pol λ	Pol β2, yPol IV	X	575	no	dRP lyase	base excision repair, meiotic DNA transactions
Pol μ		X	495	no	TdT activity	ds DNA break repair, Ig hypermutation
Pol σ	TRF4, Pol κ	X	543	?		sister chromatid cohesion
REV1	yREV1	Y	1251	no	adds dCMP	mutagenic translesion DNA synthesis

dRP, deoxyribose-5-phosphate; AP, apurinic/apyrimindinic; TdT, deoxynucleotidyl transferase.

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Welcome to our families

New additions to the list of eukaryotic DNA polymerases include an enzyme in the B family, pol ζ. Recent studies suggest that pol ζ may participate in mutagenic translesion synthesis, by its ability to incorporate nucleotides opposite sites of DNA damage and by efficiently extending aberrant primer termini [1]. Pol ζ is thought to work in partnership with other proteins, including Rev7 and Rev1. Rev1 is itself a specialized polymerase with deoxycytidyl transferase activity [1–3]. Studies from three different laboratories have revealed that disruption of the pol ζ gene in mice results in early embryonic lethality, indicating that pol ζ has a critical function in mammalian development [4–6].

The X family has also recently expanded by three – pols λ, μ, and σ (table 1). Pol λ is a close homologue of pol β that is predicted to have a similar structural organization [7–9]. Like pol β, pol λ lacks an intrinsic proofreading exonuclease activity but has a deoxyribose-5-phosphate (dRP) lyase activity [10]. The latter activity and the fact that pol λ can substitute for pol β in a reconstituted base excision repair reaction in vitro suggests that pol λ may participate in base excision repair in vivo [10].

This role may be facilitated by an amino-terminal BRCT domain believed to be involved in protein-protein interactions. Pol λ mRNA is expressed at high levels in testis [7–9], particularly in the nuclei of pachytene spermatocytes, suggesting that it may be involved in DNA transactions associated with germ cell development [7].

DNA pol μ, another recent addition to the pol X family, shares 41% sequence identity with deoxynucleotidyl transferase (TdT) [8, 11], the well-known template-independent mammalian DNA polymerase. Indeed, pol μ exhibits template-independent polymerization activity, but unlike TdT, it also has template-directed polymerase activity [11]. Similar to other members of the X family, pol μ is devoid of 3′ → 5′ exonuclease activity [11], but unlike pol β and pol λ, it does not have dRP lyase activity [10]. Pol μ is highly expressed in the germinal centers of peripheral lymphoid tissues [11]. This observation coupled with the fact that pol μ has low fidelity has led to the suggestion that it may participate in somatic hypermutation of immunoglobulin genes [11]. Given its similarity to TdT and its ability to perform synthesis at DNA ends and in short gaps, pol μ could be involved in mutagenic processing of double-strand breaks suggested to generate somatic hypermutation [12]. Alternatively, the presence of high levels of pol μ in germinal centers may reflect a role in repairing dsDNA breaks that are the consequence rather than the initiator of hypermutation processes [13]. The fact that low-level expression of pol μ mRNA is detected in almost all tissues [8, 11] raises the possibility that it may be involved in general repair of double-strand breaks.

The accurate segregation of chromosomes to daughter cells in mitosis requires that sister chromatids produced during replication remain physically attached by cohesin complexes until their separation in anaphase. The product of the yeast TRF4 gene, recently identified as a DNA polymerase (pol σ) with weak homology to other X family members [14], is one of the proteins required for establishing sister chromatid cohesion during S phase. A model wherein the establishment of cohesion is coupled to replication has been proposed [15]. It assumes that cohesin-bound sites on DNA could create a block for the replicative polymerase. A switch to pol σ would enable synthesis through these sites and after the passage of the replication fork, the Trf4/pol σ-dependent cohesion would be established. Trf4 mutants are hypersensitive to camptothecin, an antitumor drug that is believed to generate replication-coupled double-strand breaks. This suggests that Trf4/pol σ may also play a role in repair of camptothecin-mediated DNA damage [16]. Yeast cells have a homologue of TRF4, the TRF5 gene encoding a pol σ isoform. Thus, pol σ function in yeast is redundant and both Trf4 and Trf5 proteins need to be inactivated to stop DNA replication [15]. There are two homologues of TRF in human cells, hTRF4-1 and hTRF4-2, and the former encodes a DNA polymerase [15].

A SUPER family

Many recently identified DNA polymerases belong to the rapidly growing Y family whose members are found in all three kingdoms of life and can be subdivided into distinct groups based on phylogenetic analysis [17]. Four Y family members have been discovered in human cells: Rev1, pol η, pol ι, and pol κ (table 1). All four polymerases lack intrinsic 3′ → 5′ exonuclease activity [18–21] and each has remarkable properties. Rev1 is atypical in that it has a template-dependent deoxycytidyl transferase activity that preferentially incorporates dCMP opposite damaged bases or an abasic site [1, 22]. As mentioned above, Rev1 protein is required for mutagenesis induced by certain DNA-damaging agents, and at least part of this requirement is independent of its deoxycytidyl transferase activity. This may reflect Rev1-dependent protein-protein interactions needed for Rev3-dependent translesion synthesis [1, 23].

Pol η is remarkable for its ability to bypass very efficiently a cis-syn thymine dimer in DNA while preferentially incorporating adenine opposite the covalently attached thymine residues of the dimer [24, 25]. Pol η is encoded by the XPV gene in humans, and XPV gene mutations that inactivate pol η enhance UV-induced mutagenesis [26, 27] and greatly increase the risk of sunlight-induced skin cancer [28, 29]. The current hypothesis to explain these phenotypes is that when a bulky UV photo-

product blocks elongation by the normal replicative polymerase (i.e., pol δ), pol η is delivered to the fork to conduct efficient translesion replication. Loss of efficient bypass due to inactivation of pol η leads to participation of another polymerase (e.g., pol ζ/Rev1), ultimately resulting in mutagenic bypass. Interestingly, pol η itself is one of the least accurate DNA polymerases studied to date [19, 30]. Its low fidelity may reflect low geometric selectivity resulting from a promiscuous active site that can accommodate lesions that distort the DNA helix. Recent X-ray crystal structural data on yeast pol η [31] are consistent with this idea. Any errors made during bypass by pol η, which has low processivity and performs limited chain elongation, may be subsequently proofread by an extrinsic exonuclease [32] or corrected by post-replication DNA mismatch repair [33]. In this way, intrinsically inaccurate DNA polymerases like pol η may participate in DNA transactions that are ultimately relatively accurate. Two recent studies [34, 35] also suggest that error-prone synthesis by pol η may contribute to somatic hypermutation of immunoglbulin genes, perhaps generating strand-specific mutations in WA (W = A or T) hotspot motifs during synthesis of the non-transcribed strand. Key to understanding the functions of pol η will be a better appreciation of the protein partnerships that deliver it for use in the appropriate DNA transactions.

Pol ι is a Y family enzyme that has both DNA polymerase and 5′-dRP lyase activities. Pol ι can substitute for pol β in a reconstituted base excision repair reaction in vitro [20, 36], suggesting that it may have a role in DNA repair. Clues to possible repair reactions in which pol ι might participate come from two unusual polymerization properties. Pol ι inserts T opposite template A with an efficiency that is much higher than for the other three correct base pairs. Thus, when dUTP is occasionally incorporated opposite template A during replication [37], pol ι may participate in repair by efficiently incorporating T after the uracil is removed in a base excision-repair reaction. Secondly, pol ι is the only polymerase known to violate Watson-Crick base-pairing rules by preferentially inserting G rather than A opposite template T [20, 38]. This property along with its ability to conduct a base excision repair reaction suggest that it may function to reinsert inadvertantly removed dGMP present opposite thymine arising from deamination of 5-methylcytosine. In this role, pol ι could prevent inadvertant misrepair and thus stabilize the genome against G-C to A-T transition mutations [36]. Pol ι has also been suggested to function in translesion synthesis [39–41] and in somatic hypermutation of immunoglobulin genes [42].

Human pol κ is a Y family member homologous to the bacterial DinB protein, *Escherichia coli* pol IV. Like pol IV (and pol η mentioned above), pol κ synthesizes DNA with low fidelity [43]. Clues to the low fidelity of DinB homologues come from recent structural studies of DinB polymerases from *Sulfolobus solfataricus* revealing that the active site is more open than those observed in more accurate polymerases in other families [44, 45]. In fact, the active site of *S. solfataricus* Dpo4 pol can accommodate two template nucleotides, one of which can be in a misaligned conformation [45]. These and several other unusual features may provide insights into the fidelity of Y family polymerases and the mechanism of translesion synthesis. Although the exact function of pol κ is unknown, a role in translesion replication is suggested by its ability to bypass certain bulky lesions in vitro, e.g., by preferentially incorporating cytosine opposite benzo[a] pyrene diol epoxide-N2-G adducts [46–48]. Consistent with this role is a recent study showing that expression of the mouse gene encoding pol κ is under the control of AhR, a crucial factor in activating benzo[a]pyrene into BPDE within mammalian cells [47]. A role for pol κ in spermatogenesis has also been suggested [18].

The discovery of many previously unknown eukaryotic DNA polymerases has generated a great deal of interest, as well as uncertainty, about the nature and number of different, specialized DNA transactions that may occur in eukaryotic cells. We are now faced with the challenge of understanding the roles and mechanisms of action of these polymerases. Sorting out where, when, how, and with whom they work, and what mechanisms prevent them from working at the wrong time, will be a long and exciting journey.

1 Lawrence C. W. and Maher V. M. (2001) Eukaryotic mutagenesis and translesion replication dependent on DNA polymerase ζ and Rev1 protein. Biochem. Soc. Trans. **29:** 187–191

2 Murakumo Y., Ogura Y., Ishii H., Numata S., Ichihara M., Croce C. M. et al. (2001) Interactions in the error-prone postreplication repair proteins hREV1, hREV3, and hREV7. J. Biol. Chem. **276:** 35644–35651

3 Masuda Y., Takahashi M., Tsunekuni N., Minami T., Sumii M., Miyagawa K. et al. (2001) Deoxycytidyl transferase activity of the human REV1 protein is closely associated with the conserved polymerase domain. J. Biol. Chem. **276:** 15051–15058

4 Bemark M., Khamlichi A. A., Davies S. L. and Neuberger M. S. (2000) Disruption of mouse polymerase ζ (Rev3) leads to embryonic lethality and impairs blastocyst development in vitro. Curr. Biol. **10:** 1213–1216

5 Esposito G., Godindagger I., Klein U., Yaspo M. L., Cumano A. and Rajewsky K. (2000) Disruption of the Rev3l-encoded catalytic subunit of polymerase ζ in mice results in early embryonic lethality. Curr. Biol. **10:** 1221–1224

6 Wittschieben J., Shivji M. K., Lalani E., Jacobs M. A., Marini F., Gearhart P. J. et al. (2000) Disruption of the developmentally regulated Rev3l gene causes embryonic lethality. Curr. Biol. **10:** 1217–1220

7 Garcia-Diaz M., Dominguez O., Lopez-Fernandez L. A., Lera L. T. de, Saniger M. L., Ruiz J. F. et al. (2000) DNA polymerase lambda (Pol λ), a novel eukaryotic DNA polymerase with a potential role in meiosis. J. Mol. Biol. **301:** 851–867

8 Aoufouchi S., Flatter E., Dahan A., Faili A., Bertocci B., Storck S., et al. (2000) Two novel human and mouse DNA polymerases of the polX family. Nucleic Acids Res. **28:** 3684–3693

9 Nagasawa K., Kitamura K., Yasui A., Nimura Y., Ikeda K., Hirai M. et al. (2000) Identification and characterization of hu-

man DNA polymerase β2, a DNA polymerase β-related enzyme. J. Biol. Chem. **275:** 31233–31238
10 Garcia-Diaz M., Bebenek K., Kunkel T. A. and Blanco L. (2001) Identification of an intrinsic 5′-deoxyribose-5-phosphate lyase activity in human DNA polymerase λ. J. Biol. Chem. **276:** 34659–34663
11 Dominguez O., Ruiz J. F., Lain De Lera T., Garcia-Diaz M., Gonzalez M. A., Kirchhoff T. et al. (2000) DNA polymerase μ, homologous to TdT, could act as a DNA mutator in eukaryotic cells. EMBO J. **19:** 1731–1742
12 Ruiz J. F., Dominguez O., Lain De Lera T., Garcia-Diaz M., Bernard A. and Blanco L. (2001) DNA polymerase μ, a candidate hypermutase? Phil. Trans. R. Soc. Lond. B **356:** 1–11
13 Rogozin I. B., Kunkel T. A. and Pavlov Y. I. (2002) Double-strand breaks in DNA during somatic hypermutaion of immunoglobuline genes: cause or consequece? Trends Immunol. **23:** 12–13
14 Wang Z., Castano I. B., De Las Penas A., Adams C. and Christman M. F. (2000) Pol κ: a DNA polymerase required for sister chromatid cohesion. Science **289:** 774–779
15 Carson D. R. and Christman M. F. (2001) Evidence that replication fork components catalyze establishment of cohesion between sister chromatids. Proc. Natl. Acad. Sci. USA **98:** 8270–8275.
16 Walowsky C., Fitzhugh D. J., Castano I. B., Ju J. Y., Levin N. A. and Christman M. F. (1999) The topoisomerase-related function gene TRF4 affects cellular sensitivity to the antitumor agent camptothecin. J. Biol. Chem. **274:** 7302–7308
17 Ohmori H., Friedberg E. C., Fuchs R. P., Goodman M. F., Hanaoka F., Hinkle D. et al. (2001) The Y-family of DNA polymerases. Mol. Cell **8:** 7–8
18 Gerlach V. L., Feaver W. J., Fischhaber P. L., Richardson J. A., Aravind L., Konin E. V. et al. (2000) Human DNA polymease κ: a novel DNA polymerase of unknown biological function encoded by the *DINB1* gene. In: Cold Spring Harbor Symp. Quant. Biol. **65:** 41–49
19 Matsuda T., Bebenek K., Masutani C., Hanaoka F. and Kunkel T. A. (2000) Low fidelity DNA synthesis by human DNA polymerase-η. Nature **404:** 1011–1013
20 Tissier A., McDonald J. P., Frank E. G. and Woodgate R. (2000) Pol ι, a remarkably error-prone human DNA polymerase. Genes Dev. **14:** 1642–1650
21 Gerlach V. L., Feaver W. J., Fischhaber P. L. and Friedberg E. C. (2001) Purification and characterization of polkappa, a DNA polymerase encoded by the human DINB1 gene. J. Biol. Chem. **276:** 92–98
22 Gibbs P. E., Wang X. D., Li Z., McManus T. P., McGregor W. G., Lawrence C. W. et al. (2000) The function of the human homolog of *Saccharomyces cerevisiae* REV1 is required for mutagenesis induced by UV light. Proc. Natl. Acad. Sci. USA **97:** 4186–4191
23 Nelson J. R., Gibbs P. E., Nowicka A. M., Hinkle D. C. and Lawrence C. W. (2000) Evidence for a second function for *Saccharomyces cerevisiae* Rev1p. Mol. Microbiol. **37:** 549–554
24 Johnson R. E., Prakash S. and Prakash L. (1999) Efficient bypass of a thymine-thymine dimer by yeast DNA polymerase, Pol η. Science **283:** 1001–1004
25 Masutani C., Araki M., Yamada A., Kusumoto R., Nogimori T., Maekawa T. et al. (1999) Xeroderma pigmentosum variant (XP-V) correcting protein from HeLa cells has a thymine dimer bypass DNA polymerase activity. EMBO J. **18:** 3491–3501
26 Wang Y. C., Maher V. M., Mitchell D. L. and McCormick J. J. (1993) Evidence from mutation spectra that the UV hypermutability of xeroderma pigmentosum variant cells reflects abnormal, error-prone replication on a template containing photoproducts. Mol. Cell. Biol. **13:** 4276–4283
27 Waters H. L., Seetharam S., Seidman M. M. and Kraemer K. H. (1993) Ultraviolet hypermutability of a shuttle vector propagated in xeroderma pigmentosum variant cells. J. Invest. Dermatol. **101:** 744–748
28 Johnson R. E., Kondratick C. M., Prakash S. and Prakash L. (1999) hRAD30 mutations in the variant form of xeroderma pigmentosum. Science **285:** 263–265
29 Masutani C., Kusumoto R., Yamada A., Dohmae N., Yokoi M., Yuasa M. et al. (1999) The XPV (xeroderma pigmentosum variant) gene encodes human DNA polymerase η. Nature **399:** 700–704
30 Matsuda T., Bebenek K., Masutani C., Rogozin I. B., Hanaoka F. and Kunkel T. A. (2001) Error rate and specificity of human and murine DNA polymerase η. J. Mol. Biol. **312:** 335–346
31 Trincao J., Johnson R. E., Escalante C. R., Prakash S., Prakash L. and Aggarwal A. K. (2001) Structure of the catalytic core of *S. cerevisiae* DNA polymerase η: implications for translesion DNA synthesis. Mol. Cell **8:** 417–426
32 Bebenek K., Matsuda T., Masutani C., Hanaoka F. and Kunkel T. A. (2001) Proofreading of DNA polymerase η-dependent replication errors. J. Biol. Chem. **276:** 2317–2320
33 Pavlov Y. I., Nguyen D. and Kunkel T. A. (2001) Mutator effects of overproducing DNA polymerase η (Rad30) and its catalytically inactive variant in yeast. Mutat. Res. **478:** 129–139
34 Rogozin I. B., Pavlov Y. I., Bebenek K., Matsuda T. and Kunkel T. A. (2001) Somatic mutation hotspots correlate with DNA polymerase η error spectrum. Nature Immunol. **2:** 530–536
35 Zeng X., Winter D. B., Kasmer C., Kreamer K. H., Lehmann A. R. and Gearhart P. J. (2001) DNA polymerase η is an A-T mutator in somatic hypermutation of immunoglobulin variable genes. Nature Immunol. **2:** 537–541
36 Bebenek K., Tissier A., Frank E. G., McDonald J. P., Prasad R., Wilson S. H. et al. (2001) 5′-Deoxyribose phosphate lyase activity of human DNA polymerase ι in vitro. Science **291:** 2156–2159
37 Nilsen H., Rosewell I., Robins P., Skjelbred C. F., Andersen S., Slupphaug G. et al. (2000) Uracil-DNA glycosylase (UNG)-deficient mice reveal a primary role of the enzyme during DNA replication. Mol. Cell **5:** 1059–1065
38 Zhang Y., Yuan F., Wu X. and Wang Z. (2000) Preferential incorporation of G opposite template T by the low-fidelity human DNA polymerase ι. Mol. Cell. Biol. **20:** 7099–7108
39 Johnson R. E., Washington M. T., Haracska L., Prakash S. and Prakash L. (2000) Eukaryotic polymerases ι and ζ act sequentially to bypass DNA lesions. Nature **406:** 1015–1019
40 Zhang Y., Yuan F., Wu X., Taylor J. S. and Wang Z. (2001) Response of human DNA polymerase ι to DNA lesions. Nucleic Acids Res. **29:** 928–935
41 Tissier A., Frank E. G., McDonald J. P., Iwai S., Hanaoka F. and Woodgate R. (2000) Misinsertion and bypass of thymine-thymine dimers by human DNA polymerase ι. EMBO J. **19:** 5259–5266
42 Goodman M. F. and Tippin B. (2000) The expanding polymerase universe. Nature Rev. Mol. Cell Biol. **1:** 101–109
43 Ohashi E., Bebenek K., Matsuda T., Feaver W. J., Gerlach V. L., Friedberg E. C. et al. (2000) Fidelity and processivity of DNA synthesis by DNA polymerase κ, the product of the human DINB1 gene. J. Biol. Chem. **275:** 39678–39684
44 Zhou B., Pata J. D. and Steitz T. A. (2001) Crystal structure of a Dinb lesion bypass DNA polymerase catalytic fragment reveals a classic polymerase catalytic domain. Mol. Cell **8:** 427–437
45 Ling H., Boudsocq F., Woodgate R. and Yang W. (2001) Crystal structure of a Y-family DNA polymerase in action: a mechanism for error-prone and lesion-bypass replication. Cell **107:** 91–102
46 Zhang Y., Yuan F., Wu X., Wang M., Rechkoblit O., Taylor J. S. et al. (2000) Error-free and error-prone lesion bypass by human DNA polymerase κ in vitro. Nucleic Acids Res. **28:** 4138–4146
47 Ogi T., Mimura J., Hikida M., Fujimoto H., Fujii-Kuriyama Y. and Ohmori H. (2001) Expession of human and mouse genes encoding polκ: testis-specific developmental regulation and AhR-dependent inducible transcription. Genes Cells **6:** 1–12
48 Ohashi E., Ogi T., Kusumoto R., Iwai S., Masutani C., Hanaoka F. et al. (2000) Error-prone bypass of certain DNA lesions by the human DNA polymerase κ. Genes Dev. **14:** 1589–1594