FOCUS: 50 YEARS OF DNA REPAIR: THE YALE SYMPOSIUM
REPORTS

DNA Damage Tolerance and a Web of Connections with DNA Repair at Yale

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This short article summarizes some of the research carried out recently by my laboratory colleagues on the function of DNA polymerase zeta (polζ†) in mammalian cells. Some personal background is also described, relevant to research associations with Yale University and its continuing influence. Polζ is involved in the bypass of many DNA lesions by translesion DNA synthesis and is responsible for the majority of DNA damage-induced point mutagenesis in mammalian cells (including human cells), as well as in yeast. We also found that the absence of this enzyme leads to gross chromosomal instability in mammalian cells and increased spontaneous tumorigenesis in mice. Recently, we discovered a further unexpectedly critical role for polζ: it plays an essential role in allowing continued rapid proliferation of cells and tissues. These observations and others indicate that polζ engages frequently during DNA replication to bypass and tolerate DNA lesions or unusual DNA structures that are barriers for the normal DNA replication machinery.

INTRODUCTION: FIRST CONTACTS WITH DNA REPAIR AND MUTAGENESIS AT YALE

Nearing completion of research in my PhD studies, I attended the May 1981 meeting of the Radiation Research Society in Minneapolis. There I met Franklin Hutchinson (known to friends and colleagues as “Hutch”), a Professor from Yale’s Department of Molecular Biophysics and Biochemistry [1]. Hutch had just embarked on a project to determine mutation spectra by direct DNA sequencing and was excited about his renaissance as a molecular biologist. His excellent postdoctoral fellow Tom Skopek (trained in Thilly’s lab at MIT) had set up the system and methods. The timing seemed perfect to join this exciting venture.

Arriving from California to frozen winter storms in January 1982, I was welcomed at the home of Hutch and his spouse
Edie. My wife Enid and I then rented the third floor of a house from Hutch’s secretary. The DNA repair researcher Erling Seeberg later told me that he had the same address during his time in New Haven [2]. A bonus of the Sherman Avenue location was “Dinky’s” jazz club across the street at the corner of Dixwell Avenue. It was friendly, very crowded, and smoke-filled. (In 2013, this location was occupied by a paternity testing service.)

Our small team was productive, with technician Judy Stein, Tom Skopek (for a year), and then postdoc Ken Tindall. However, the lab rooms were so dilapidated that Tom purchased paint shortly after arriving and refurbished the walls himself. After a few years, Yale had this done officially, though the contract painter (a graduate from Columbia University) was there for weeks, regaling us with tales of Mark Van Doren’s legendary English lectures. Fortunately, scientific interactions were rich because we shared a corridor of the Gibbs Laboratory building with Paul Howard-Flanders’ group. Steve West was in the Howard-Flanders group at this time.

**TARGETED AND NON-TARGETED MUTAGENESIS BY UV RADIATION**

In Hutch’s lab, Tom Skopek was sequencing mutations in lambda bacteriophage propagated in *E. coli* host cells [3]. By sequencing UV-irradiated phage, we established the preference for UV-induced mutations at dipyrimidine sequences [4]. Most frequently, mutations were at the 3’ C in a TC site. Mutations were infrequent at sites where TT cyclobutane pyrimidine dimers are formed, even though these are the major photoproducts formed by ultraviolet (UV) radiation [5]. This is because translesion DNA synthesis favors the incorporation of A residues opposite cyclobutane pyrimidine dimers [6].

Hutch also suggested that we work to understand “non-targeted UV mutagenesis” — mutations formed in unirradiated lambda phage when they are propagated in UV-irradiated host cells. It did not take long to realize that there were two kinds of non-targeted mutagenesis. One of these produced base change mutations and depended on induction of the *E. coli* SOS-system after relatively low doses of UV radiation. This type of mutagenesis depended on the UmUC gene [7,8], later shown to encode the catalytic subunit of the translesion synthesis DNA polymerase V in *E. coli* [9]. We focused on a second process that occurred only in host cells irradiated with high doses. Lambda phage replicating in these heavily irradiated *E. coli* cells suffered mainly frameshift mutations during rolling circle replication [10]. This spectrum was striking enough that we established it with only limited DNA sequencing. These mutations were umuC-independent, yet both irradiation and a functional SOS system were necessary. These mutations were soon found to depend on the dinB gene [11,12]. Later, the encoded DinB protein was shown to be the catalytic subunit of a distinct DNA polymerase IV in *E. coli* [9]. Indeed, induction of DinB protein increases the frequency of frameshift mutations under stress conditions in the *E. coli* bacterium [13,14].

**MORE YALE CONNECTIONS IN THE UK**

After moving to England in 1985, I worked first in Tomas Lindahl’s laboratory at the Clare Hall Laboratories of the Imperial Cancer Research Fund (now Cancer Research UK). Within a few months, Steve West arrived to set up his own research lab, so we were again in the same building. Later, with Tomas’ support, I led my own group at Clare Hall. During this period, Phil Hanawalt (who was a graduate student at Yale) and Bill Summers (a professor at Yale) both visited Clare Hall for sabbatical periods with Lindahl. Phil’s colleague and spouse Graciela Spivak did experiments in my lab during this time. Earlier, Tomas Lindahl had spent a short time with Paul Howard-Flanders at Yale [15].

Our research at the ICRF was on the biochemistry of DNA nucleotide excision repair (NER) in mammalian cells. A key
early set of experiments was initiated by contact with Dean Rupp at Yale. His postdoctoral fellows Maureen Munn and Roger Kahn had synthesized double-stranded DNA plasmids containing a 2-(acetylamino)fluorene adducted to a specific guanine residue. Johan Hansson, a postdoctoral fellow in my laboratory, used these plasmids to demonstrate that NER DNA synthesis is localized to a short region encompassing the site of an adduct [16]. Work on repair DNA synthesis using site-specific lesions was carried on by David Szymkowski in my group [17-19]. Dave also had a Yale association: He had been a PhD student of Reginald Deering, who in turn had previously trained with both Ernest Pollard and Franklin Hutchinson [20]. My team and our collaborators identified many NER proteins and studied the biochemistry of their action. We reconstituted mammalian NER with purified proteins and determined the overall mechanism of DNA nucleotide excision repair in human cells [21-38].

As prostate cancer closed in on Hutch during his final years, he made a round of visits in Europe, presenting gifts of his wife’s art. He had a special fondness for the area around North London, having lived there during childhood. He had one of Edie’s etchings framed for us, and it hangs on the wall in our living room in Texas.

DNA POLYMERASE ZETA

More recently, first at the University of Pittsburgh and now at the University of Texas MD Anderson Cancer Center, we have investigated specialized DNA polymerases that help defend our cells against DNA damage. Mammalian genomes encode about 16 distinct DNA polymerases, each specializing in some aspect of DNA replication, DNA repair, recombination, or bypass of DNA damage [39-42]. Replicative DNA polymerases generally cannot proceed on damaged DNA templates [43,44]. When such a template is encountered, replication is stalled at least temporarily, and the lesion may be bypassed by invoking a process of translesion DNA synthesis (TLS) mediated by specialized DNA polymerases [39,45] or by switching to another undamaged DNA template. A perilous alternative is that the machinery at the DNA replication fork collapses. This can leave the DNA exposed to enzymes in the cell that cut the DNA and form a DNA double strand break. If TLS DNA lesion bypass is used at a stalled fork, replication can proceed. However, it can be mutagenic if the “wrong” base is inserted opposite a mis-instructional lesion in the DNA template [45,46].

Polζ plays a major role in the bypass of many types of DNA damage in human and rodent cells, including (6-4) dipyrimidine photoproducts induced by ultraviolet (UV) radiation [47,48], lesions formed by chemical damaging agents such as the chemotherapeutic agent cisplatin and the carcinogen benzo[a]pyrene [48,49], as well as the frequent endogenously-formed abasic sites in DNA [48]. Polζ also appears to be involved in the bypass of unhooked DNA crosslinks during DNA interstrand crosslink repair [50-52].
The catalytic subunit of mammalian pol ζ is REV3L. Though REV3L has 3130 amino acids, only the C-terminal 25% contains the residues conserved in B-family DNA polymerases. A recognition is emerging that conserved domains in the remainder of REV3L interact with other proteins, and there are probably more such domains to be discovered (Figure 1). For example, human REV3L interacts with the POLD3 and POLD2 subunits of the replicative DNA polymerase polδ. An iron-sulfur cluster near the C-terminus of REV3L provides a docking site for POLD2 [53], and POLD3 associates with POLD2. In the yeast *S. cerevisiae*, the orthologous polδ subunits are designated pol31 and pol32 and they associate with both polδ and Rev3 via a similar (4Fe-4S) cluster that is conserved in B family DNA polymerases [54-56]. This connection is reasonable because both Rev3 and pol32 are necessary for TLS and mutagenesis [57-59]. The shared association with these subunits may provide a mechanism for polδ to switch places with polζ on some occasions when normal DNA replication is stalled at a template DNA lesion. There is good evidence that this takes place in yeast. The increased mutagenesis in *S. cerevisiae* strains carrying reduced fidelity alleles of polδ is largely Rev3 dependent, indicating that polζ action is invoked when replication is sub-optimal [60].

In mammals, polζ also appears to be employed when DNA replication forks stall in cells. DNA replication can stall frequently because some naturally occurring DNA sequences are inherently difficult to replicate, such as the “fragile-site” sequences found at multiple sites in mammalian genomes or sequences forming non-B DNA structures [61,62]. Replication of chromosomal fragile sites requires polζ in order to avoid chromosome breaks [63]. When summoned on such occasions of DNA replication fork stalling, polζ would have the opportunity to introduce mutations even into undamaged DNA.

**AN ESSENTIAL FUNCTION FOR POLζ IN MAMMALIAN CELLS**

The biological function of polζ in mammalian cells was challenging to study because disruption of its catalytic subunit (REV3L) is incompatible with mouse viability [64]. To overcome this problem, we generated mice and cells that could conditionally delete polζ. It was particularly interesting to know whether polζ status influences tumorigenesis. John Wittschieben derived the first animal model to study this subject [65], deleting *Rev3L* from tissues of adult mice using Cre recombinase expressed under control of the mouse mammary tumor virus (MMTV) promoter. The MMTV-driven Cre expression results in a “mosaic” pattern of deletion, with some cells in a tissue deleting the target gene and other cells remaining unaltered. This is a useful approach in cases where complete gene deletion causes lethality, because it allows a test of which cell types can tolerate deletion of the test gene. John found that hematopoietic cells did not tolerate loss of *Rev3L* but that epithelial tissues could survive deletion of *Rev3L* in at least some cells. We were unable to generate fibroblast cell lines from *Rev3L*-defective embryos, but did derive a cell line from an embryo that carried an additional mutation in the tumor suppressor *Tp53* gene. These *Rev3L−/− Tp53−/−* mouse embryonic cells had an increased frequency of “spontaneous” chromosomal translocations, as well as more micronuclei (chromosome fragments detached from the cell nucleus). This indicated that *Rev3L−/−* cells are genetically unstable [65].

In a *p53* mutant background, these MMTV-Cre mice deleting *Rev3L* from some cells acquired thymic lymphomas even faster and with higher frequency than in *p53* mutant mice with intact *Rev3L* [66]. We considered some possible explanations for this result. It was conceivable that the high rate of hematopoietic cell death and the resulting systemic inflammation, coupled with a continued pressure for regeneration of cells, would create an environment favorable for induction and selective outgrowth of lymphomas. Alternatively, it was possible that the increased genetic instability in *Rev3L*-defective cells was responsible for the rapid emergence of tumors. The question could be resolved by testing the genetic status of...
Rev3L in the thymic lymphomas. If the first explanation was operative, lymphomas could harbor either intact Rev3L or disrupted Rev3L. If the second explanation was correct (increased genetic instability in Rev3L-defective cells favors tumors), then most of the lymphomas would be predicted to harbor disrupted Rev3L. The answer was that the majority of lymphomas were populated by Rev3L-null T cells [66]. Therefore, loss of polζ function is selectively favored for tumorigenesis in this setting. Loss of Rev3L can be tolerated if a cell can escape the checkpoints that normally restrict the proliferation of cells suffering ongoing DNA damage. Another striking finding was that the Rev3L-defective thymic lymphomas were frequently oligoclonal (that is, composed of two or more independent tumor cell populations). This result suggests that the frequency of genetic change leading to tumorigenesis in Rev3L-defective cells is very high compared to normal cells [65]. Indeed, in the MMTV-Cre mice that were conditionally deleting Rev3L, we found that mammary tumors also formed, with an increased frequency and months earlier than in control mice. This occurred in both Tp53+/+ and Tp53+/− (heterozygous) backgrounds, showing that the tumor suppressing activity of polζ is also relevant when p53 is functional. In the mammary gland tissue adjacent to these tumors in mice deleting Rev3L, there were also prominent preneoplastic changes such as atypical hyperplasia and mammary intraepithelial neoplasia. Moreover, there appeared to be multiple neoplastic foci in many of the mammary gland tumors, again suggesting tumors comprising several independent but confluent cell populations [66]. Thus, polζ is critical for limiting the incidence of lymphomas, mammary cancers and preneoplastic changes when deleted from tissues by MMTV-Cre in a mosaic fashion.

To directly track the consequences of very efficient polζ ablation, Sabine Lange joined my group and set up a system to conditionally delete Rev3L from mouse embryonic fibroblasts in culture. Sabine had been a PhD student with Karen Vasquez (who in turn did ground-breaking work at Yale on mechanisms of DNA structure-induced mutations [67-72]). Sabine made the surprising finding that polζ is needed in every cell cycle to maintain chromosome integrity and cell proliferation [73]. She established primary mouse fibroblasts in which Rev3L could be conditionally disrupted by Cre recombinase and where cells containing active Cre could be tracked and sorted. Even in a 2 percent oxygen atmosphere to minimize oxidative stress, Rev3L-deleted cells ceased growth within a few population doublings and rapidly became senescent or apoptotic. DNA damage accumulated quickly after Rev3L deletion, with 30 percent to 50 percent of cells harboring DNA double-strand breaks and chromatid aberrations after only one population doubling. These breaks were dependent on DNA replication. The DNA breaks were reduced somewhat by supplementing culture medium with the reactive oxygen species scavenger N-acetylcysteine, but this did not rescue the cell proliferation defect. This suggests that several classes of endogenously formed DNA lesions or sequences require Rev3L for tolerance or repair. Cells lacking damage-dependent checkpoint control (such as SV40 large T antigen-immortalized cells) can survive polζ deletion, but accumulate chromosome aberrations [73].

This major function for polζ in maintaining chromosome stability provides an explanation for the tumor-limiting properties of this enzyme. Slowly proliferating primary mouse tissues in vivo can tolerate loss of polζ, at the expense of accumulated chromosomal aberrations that can lead to tumorigenesis.

A CRITICAL ROLE FOR POL ZETA IN RESPONSES OF SKIN TO UV RADIATION

To extend these findings, we are determining the consequence of polζ deletion in mouse skin. At first it seemed somewhat paradoxical that polζ is required for embryonic development and for viability of hematopoietic cells, whereas skin epithelia
survive polζ deletion. We wanted to clarify whether there are tissue-specific roles for polζ. We also wanted to know how polζ influences tumorigenesis in the skin epithelium following UV irradiation. To answer these questions, another mouse strain was produced, conditionally deleting Rev3L with Cre produced in tissues expressing keratin 5 [74]. It proved possible to efficiently delete Rev3L from keratin 5-expressing epidermal tissues. The skin epithelium was not normal, however, with a lower density of cells, a disturbed hair cycle, and evidence of constantly accumulating DNA breaks. If mice were not challenged with UV radiation but simply left to age, they developed tumors in keratin 5-expressing epithelial tissues, especially in specialized sebaceous glands. This tumor formation is consistent with the chromosomal instability that accompanies the polζ defect [74].

Working with expert collaborators on skin cancer, we were astonished to find that deletion of Rev3L in skin led to the highest sensitivity to UV radiation of any known mouse model [74]. UV-irradiated mice with polζ-defects in the epidermis failed to mount skin-regenerative responses and did not develop skin tumors after chronic UV irradiation. Further investigation revealed that a major reason for this is the limited proliferative potential of polζ-deficient skin epithelial cells. Isolated polζ-deleted keratinocytes exhibit a major proliferation defect and harbor chromosomal gaps and breaks.

Consequently, there appear to be two reasons for the pronounced sensitivity of polζ-deleting epithelia to UV radiation. First,
polζ is involved in tolerance of lesions in DNA by allowing bypass replication over them. Second, polζ is needed when rapid cellular proliferation is required, even in undamaged cells. We proposed a “threshold of tolerance” model incorporating DNA damage and proliferation rates [74]. Above a critical threshold, loss of polζ function inhibits cell growth. Below the threshold, loss of polζ can be dangerous because it promotes cancer in deleting tissues (Figure 2). A prediction of this model is that cellular stress responses may be abnormal in polζ-deficient cells.

CONCLUSIONS AND OUTLOOK

DNA polymerase ζ is exceptionally significant in the defense of mammalian cells against DNA damage. It is important for bypass of DNA lesions by translesion DNA synthesis and is responsible for the majority of DNA damage-induced point mutagenesis in mammalian cells and yeast [39]. Further, in recent research, we have learned that polζ is needed in each chromosomal replication cycle, at least in rapidly proliferating cells [73,74]. Without this DNA polymerase, DNA double-strand breaks form with ensuing chromosomal rearrangements [65,66,75]. Spontaneous tumorigenesis in mouse models increases in frequency and rate, favoring cells that circumvent DNA damage checkpoints. A likely scenario is that in normal cells, the replicative DNA synthesis machinery sometimes stalls at endogenously occurring lesions or difficult-to-replicate DNA sequences and that polζ is recruited as part of the system for bypass and/or reactivating stalled replication forks. Such switching of DNA polymerases is facilitated by the shared subunit structure with the replicative DNA polα, as described in the introduction to this article. Without polζ, there is an increased load of DNA double strand breaks following replication fork collapse. One important challenge is to discover the most important types of DNA damage or DNA structures that rely on polζ for resolution. Another critical question is whether the DNA polymerase activity provides the only important function of polζ, or whether other functions and interactions of the enzyme are critical. Although polζ function is clearly important in suppressing tumorigenic events, it remains to be seen whether alterations in polζ will be found specifically in human tumors. Because polζ is critical for normal cell growth, there is a strong selection for its preservation, and it may be inactivated only infrequently or under very special circumstances in human tumors. Some answers to these and other future questions will no doubt be solved via further interactions with our many Yale-connected colleagues in the DNA repair field.

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REFERENCES

1. Krasin F, Person S. Franklin Hutchinson III (1920-1997). Radiat Res. 1998;149(4):405-6.
2. Seeberg E, Rupp WD. Effect of mutations in lig and polA on UV-induced strand cutting in a uvrC strain of Escherichia coli. Basic Life Sci. 1975;5B:439-41.
3. Skopek TR, Hutchinson F. DNA base sequence changes induced by bromouracil mutagenesis of lambda phage. J Mol Biol. 1982;159(1):19-33.
4. Wood RD, Skopek TR, Hutchinson F. Changes in DNA base sequence induced by targeted mutagenesis of lambda phage by ultraviolet light. J Mol Biol. 1984;173:273-91.
5. Hutchinson F, Yamamoto K, Stein J, Wood RD. Effect of photoreactivation on mutagenesis of lambda DNA by ultraviolet light. J Mol Biol. 1988;202:593-601.
6. Strauss BS. The “A” rule revisited: polymerases as determinants of mutational specificity. DNA Repair (Amst). 2002;1(2):125-35.
7. Kato T, Shinoura Y. Isolation and characterization of mutants of Escherichia coli deficient in induction of mutations by ultraviolet light. Mol Gen Genet. 1977;156(2):121-31.
8. Walker GC. Mutagenesis and inducible responses to DNA damage in E. coli. Microbiol Rev. 1984;84:60-93.
9. Friedberg EC, Walker GC, Siede W, Wood RD, Schultz RA, Ellenberger T. DNA repair and mutagenesis. 2nd edition. Washington, DC: ASM Press; 2006.
10. Wood RD, Hutchinson F. Non-targeted mutagenesis of unirradiated lambda phage in Escherichia coli host cells irradiated with ultraviolet light. J Mol Biol. 1984;173(3):293-305.
11. Brotcorne-Lannoye A, Maenhaut-Michel G. Role of RecA protein in untargeted UV mutagenesis of bacteriophage lambda: evidence for the requirement for the dinB gene. Proc Natl Acad Sci USA. 1986;83(11):3904-8.

12. Kim SR, Maenhaut-Michel G, Yamada M, Yamamoto Y, Matsui K, Sofuni T, et al. Multiple pathways for SOS-induced mutagenesis in Escherichia coli: an overexpression of dinB/dinP results in strongly enhancing mutagenesis in the absence of any exogenous treatment to damage DNA. Proc Natl Acad Sci USA. 1997;94(25):13792-7.

13. McKenzie GJ, Lee PL, Lombardo MJ, Hastings PJ, Rosenberg SM. SOS mutator DNA polymerase IV functions in adaptive mutation and not adaptive amplification. Mol Cell. 2001;7(3):371-9.

14. Foster PL. Adaptive mutation in Escherichia coli. Cold Spring Harb Symp Quant Biol. 2000;65:21-9.

15. Ljungquist S, Lindahl T, Howard-Flanders P. Methyl methane sulfonate-sensitive mutant of Escherichia coli deficient in an endonuclease specific for apurinic sites in deoxyribonucleic acid. J Bacteriol. 1976;126(2):646-53.

16. Hansson J, Munn M, Rupp WD, Kahn R, Wood RD. Localization of DNA repair synthesis by human cell extracts to a short region at the site of a lesion. J Biol Chem. 1989;264(36):21788-92.

17. Szymkowski DE, Hajibagheri MA, Wood RD. Electron microscopy of DNA excision repair patches produced by human cell extracts. J Mol Biol. 1993;231(2):251-60.

18. Szymkowski DE, Lawrence CW, Wood RD. Repair by human cell extracts of single (6-4) and cyclobutane thymine-thymine photo-products in DNA. Proc Natl Acad Sci USA. 1993;90:9823-7.

19. Szymkowski DE, Yarema KJ, Essigmann JE, Lippard SJ, Wood RD. An intrastrand d(GpG)-platinum complex in duplex M13 DNA is refractory to repair by human cell extracts. Proc Natl Acad Sci USA. 1992;89:10772-6.

20. Deering RA, Hutchinson F, Schambra PE. Biological effects of accelerated heavy ions. Aerosp Med. 1961;32:915-20.

21. Wood RD, Aboussekhra A, Biggerstaff M, Jones CJ, O’Donovan A, Shivji MKK, et al. Nucleotide excision repair of DNA by mammalian cell extracts and purified proteins. In: Stillman B, Alberts B, editors. Cold Spring Harbor Symposia on Quantitative Biology. Cold Spring Harbor: Cold Spring Harbor Press; 1993. p. 625-32.

22. Coverley D, Kenny MK, Munn M, Rupp WD, Lane DP, Wood RD. Requirement for the replication protein SSB in human DNA excision repair. Nature. 1991;349:538-41.

23. Araújo SJ, Tirode F, Coin F, Pospiech H, Syväoja JE, Stucki M, et al. Nucleotide excision repair of DNA with recombinant human proteins: definition of the minimal set of factors, active forms of TFIH and modulation by CAF. Genes Dev. 2000;14:349-59.

24. Evans E, Fellows J, Coffer A, Wood RD. Open complex formation around a lesion during nucleotide excision repair provides a structure for cleavage by human XPG protein. EMBO J. 1997;16:625-38.

25. Evans E, Moggs JG, Hwang JR, Egly J-M, Wood RD. Mechanism of open complex and dual incision formation by human nucleotide excision repair factors. EMBO J. 1997;16:6559-73.

26. Moggs JG, Yarema KJ, Essigmann JM, Wood RD. Analysis of incision sites produced by human cell extracts and purified proteins during nucleotide excision repair of a 1,3-intrastrand d(GpTpG)-cisplatin adduct. J Biol Chem. 1996;271:777-86.

27. Constantinou A, Gunz D, Evans E, Lalle P, Bates PA, Wood RD, et al. Conserved residues of human XPG protein important for nuclease activity and function in nucleotide excision repair. J Biol Chem. 1999;274:5637-48.

28. Clarkson SG. The XPG Story. Biochimie. 2003;85:1113-21.

29. Aboussekhra A, Biggerstaff M, Shivji MK, Viljo PA, Moncollin V, Podust VN, et al. Mammalian DNA nucleotide excision repair reconstituted with purified protein components. Cell. 1995;80(6):859-68.

30. Ariza RR, Keyse SM, Moggs JG, Wood RD. Reversible protein phosphorylation modulates nucleotide excision repair of damaged DNA by human cell extracts. Nucleic Acids Res. 1996;24(3):433-40.

31. Batty DP, Otrin VR, Levine AS, Wood RD. Stable binding of human XPC-hHR23B complex to irradiated DNA confers strong discrimination for damaged sites. J Mol Biol. 2000;302(2):275-90.

32. Shivji MKK, Kenny MK, Wood RD. Proliferating cell nuclear antigen is required for DNA excision repair. Cell. 1992;69(2):367-74.

33. Sijbers AM, de Laat WL, Ariza RR, Biggerstaff M, Wei Y-F, Moggs JG, et al. Xeroderma pigmentosum group F caused by a defect in a structure-specific DNA repair endonuclease. Cell. 1996;86:811-22.

34. O’Donovan A, Davies AA, Moggs JG, West SC, Wood RD. XPG endonuclease makes the 3’ incision in human DNA nucleotide excision repair. Nature. 1994;371(6496):432-5.

35. O’Donovan A, Wood RD. Identical defects in DNA repair in xeroderma pigmentosum group G and rodent ERCC group 5. Nature. 1993;363:185-8.

36. Rapic Otrin V, Kuraoka I, Nardo T, McLennigan M, Eker AP, Stefanini M, et al. Relationship of the xeroderma pigmentosum group E DNA repair defect to the chromatin and DNA binding proteins UV-DDB and replication protein A. Mol Cell Biol. 1998;18(6):3182-90.
37. Robins P, Jones CJ, Biggerstaff M, Lindahl T, Wood RD. Complementation of DNA repair in xeroderma pigmentosum group A cell extracts by a protein with affinity for damaged DNA. EMBO J. 1991;10(12):3913-21.

38. Tanaka K, Wood RD. Xeroderma pigmentosum and nucleotide excision repair of DNA. Trends Biochem Sci. 1994;218:83-6.

39. Lange SS, Takata K, Wood RD. DNA polymerases and cancer. Nat Rev Cancer. 2011;11(2):96-110.

40. Sweasy JB, Lauper JM, Eckert KA. DNA polymerases and human diseases. Radiat Res. 2006;166(S):693-714.

41. Eckert KA, Sweasy JB. DNA polymerases and their role in genomic stability. Environ Mol Mutagen. 2012;53(9):643-4.

42. Menezes MR, Sweasy JB. Mouse models of DNA polymerases. Environ Mol Mutagen. 2012;53(9):645-65.

43. McCulloch SD, Kunkel TA. The fidelity of DNA synthesis by eukaryotic replicative and translesion synthesis polymerases. Cell Res. 2008;18(1):148-61.

44. Schmitt MW, Matsumoto Y, Loeb LA. High fidelity and lesion bypass capability of human DNA polymerase delta. Biochimie. 2009;91(9):1163-72.

45. Sharma S, Helchowski CM, Canman CE. The roles of DNA polymerase zeta and the Y family DNA polymerases in promoting or preventing genome instability. Mutat Res. 2013;743-744:97-110.

46. Shachar S, Ziv O, Avkin S, Adar S, Wittschien J, Reissner T, et al. Two-polymerase mechanisms dictate error-free and error-prone translesion DNA synthesis in mammals. EMBO J. 2009;28:383-93.

47. Yoon JH, Prakash L, Prakash S. Error-free replicative bypass of (6-4) photoproducts by DNA polymerase zeta in mouse and human cells. Genes Dev. 2010;24(2):123-8.

48. Shachar S, Ziv O, Avkin S, Adar S, Wittschien J, Reissner T, et al. Two-polymerase mechanisms dictate error-free and error-prone translesion DNA synthesis in mammals. EMBO J. 2009;28(4):383-93.

49. Jansen JG, Tsaalbi-Shytlik A, Hendriks GJ, Verspuy J, Gali H, Haracska L, et al. Mammalian polymerase zeta is essential for post-replication repair of UV-induced DNA lesions. DNA Repair (Amst). 2009;8(12):1444-51.

50. Räschle M, Knipsheer P, Enoiu M, Angelov MA, Marini F, Gearhart PJ, et al. Disruption of the developmentally regulated Rev3I gene causes embryonic lethality. Curr Biol. 2000;10(19):1217-20.
65. Wittschieben JP, Gollin SM, Reshmi SC, Wood RD. Loss of DNA polymerase zeta causes chromosomal instability in mammalian cells. Cancer Res. 2006;66(1):134-42.
66. Wittschieben JP, Patil V, Glushets V, Robinson LJ, Kusewitt DF, Wood RD. Loss of DNA polymerase zeta enhances spontaneous tumorigenesis. Cancer Res. 2010;70(7):2770-8.
67. Rogers FA, Vasquez KM, Egholm M, Glazer PM. Site-directed recombination via bifunctional PNA-DNA conjugates. Proc Natl Acad Sci USA. 2002;99(26):16695-700.
68. Vasquez KM, Dagle JM, Weeks DL, Glazer PM. Chromosome targeting at short polypurine sites by cationic triplex-forming oligonucleotides. J Biol Chem. 2001;276(42):38536-41.
69. Datta HJ, Chan PP, Vasquez KM, Gupta RC, Glazer PM. Triplex-induced recombination in human cell-free extracts. Dependence on XPA and HsRad51. J Biol Chem. 2001;276(21):18018-23.
70. Vasquez KM, Narayanan L, Glazer PM. Specific mutations induced by triplex-forming oligonucleotides in mice. Science. 2000;290(5491):530-3.
71. Vasquez KM, Wang G, Havre PA, Glazer PM. Chromosomal mutations induced by triplex-forming oligonucleotides in mammalian cells. Nucleic Acids Res. 1999;27(4):1176-81.
72. Vasquez KM, Christensen J, Li L, Finch RA, Glazer PM. Human XPA and RPA DNA repair proteins participate in specific recognition of triplex-induced helical distortions. Proc Natl Acad Sci USA. 2002;99(9):5848-53.
73. Lange SS, Wittschieben JP, Wood RD. DNA polymerase zeta is required for proliferation of normal mammalian cells. Nucleic Acids Res. 2012;40(10):4473-82.
74. Lange SS, Bedford E, Reh S, Wittschieben JP, Carbajal S, Kusewitt DF, et al. Dual role for mammalian DNA polymerase zeta in maintaining genome stability and proliferative responses. Proc Natl Acad Sci USA. 2013;110(8):E687-96.
75. Schenten D, Kracker S, Esposito G, Franco S, Klein U, Murphy M, et al. Pol zeta ablation in B cells impairs the germinal center reaction, class switch recombination, DNA break repair, and genome stability. J Exp Med. 2009;206(2):477-90.