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REVIEW

DNA polymerase zeta (pol ζ) in higher eukaryotes

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Most current knowledge about DNA polymerase zeta (pol ζ) comes from studies of the enzyme in the budding yeast *Saccharomyces cerevisiae*, where pol ζ consists of a complex of the catalytic subunit Rev3 with Rev7, which associates with Rev1. Most spontaneous and induced mutagenesis in yeast is dependent on these gene products, and yeast pol ζ can mediate translesion DNA synthesis past some adducts in DNA templates. Study of the homologous gene products in higher eukaryotes is in a relatively early stage, but additional functions for the eukaryotic proteins are already apparent. Suppression of vertebrate REV3L function not only reduces induced point mutagenesis but also causes larger-scale genome instability by raising the frequency of spontaneous chromosome translocations. Disruption of Rev3L function is tolerated in *Drosophila*, *Arabidopsis*, and in vertebrate cell lines under some conditions, but is incompatible with mouse embryonic development. Functions for REV3L and REV7(MAD2B) in higher eukaryotes have been suggested not only in translesion DNA synthesis but also in some forms of homologous recombination, repair of interstrand DNA crosslinks, somatic hypermutation of immunoglobulin genes and cell-cycle control. This review discusses recent developments in these areas.

***Keywords*:** DNA repair, DNA polymerase, mouse, human, mutation, DNA damage
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Introduction

DNA damage does not create a mutation instantly. Rather, mutations are a consequence of the processing of DNA damage. For example, nucleotide misincorporation opposite a damaged base by a DNA polymerase during DNA replication or repair can generate mutations by base substitution, insertion or deletion. Depending on the type of DNA damage, other processes of misrepair can lead to chromosomal translocation or other genomic rearrangements. To maintain genome fidelity in the face of constant DNA damage by endogenous and environmental agents, cells have multiple means of DNA repair including nucleotide excision repair, base excision repair, mismatch repair and double-strand break repair mechanisms [1]. However, a fraction of DNA damage eludes these repair processes and normal checkpoints. These non-coding lesions can stall DNA replication, potentially causing collapse of a replication fork and a genotoxic double-strand break. To circumvent this problem, organisms have specialized DNA polymerases known as translesion synthesis polymerases, which bypass the damage by incorporating a nucleotide opposite a lesion rather than repairing it. DNA replication can continue, at the expense of potential mutations from misincorporation events.

This review focuses on the current status of molecular and cellular studies of one DNA polymerase in higher eukaryotes, DNA polymerase zeta (pol ζ). An understanding of how pol ζ functions is expected to provide insight into cellular tolerance of DNA damage as well as oncogenesis.

DNA pol ζ in *Saccharomyces cerevisiae*

Most present information about the biochemistry of pol ζ comes from studies using the budding yeast *S. cerevisiae* [1-4]. An early identification of genes associated with UV-induced mutagenesis came from studies of J Lemontt, working in R Mortimer's group [5, 6]. A screen for mutants conferring a "reversionless" phenotype (identifying cells with reduced UV radiation-induced mutagenesis) revealed a set of genes including *REV1* and *REV3*. An intensive investigation by C Lawrence's group [4, 7] of these mutant genes was undertaken, and the additional gene *REV7* was

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discovered [8]. A critical advance was the demonstration that *S. cerevisiae* DNA pol ζ consists of a core of two subunits: Rev3, the catalytic polymerase subunit, and Rev7, an accessory protein which enhanced the catalytic activity of the polymerase [9]. Rev3 is a member of the B-family DNA polymerase family, which includes pols α, δ and ε [10]. It lacks a 3′ to 5′ exonuclease activity, and has relatively low fidelity [11]. Pol ζ is not essential for viability or genomic DNA replication in yeast. However, REV3 is beneficial for survival when cells are exposed to DNA damage. Deletion of the *REV3* gene results in moderately increased sensitivity to UV radiation and some chemical DNA-damaging agents [7, 10, 12]. The most striking consequence of the absence of Rev3 in budding yeast is, however, the large reduction (by 90% or more) in the frequency of base pair substitution and frameshift mutations induced by UV radiation. Mutagenesis by γ-rays and methylmethane sulfonate is also lowered in *rev3* mutants [4, 10, 13], and the frequency of spontaneous point mutations is reduced by at least half [14-16]. Spontaneous mutation in *S. cerevisiae* is proportional to transcription level at some loci, and this transcription-associated mutagenesis is also Rev3-dependent [17]. These results indicate that the majority of mutagenic bypass events in budding yeast involve pol ζ. By allowing bypass of lesions that would otherwise lead to stalled or collapsed replication forks, pol ζ provides a survival advantage, but the price for such translesion DNA synthesis is the possibility of mutagenesis during bypass of the DNA damage.

The Rev1 protein has both a dCMP transferase activity that may function during DNA damage bypass [18, 19] and an important role as a scaffolding protein which associates with several translesion synthesis polymerases. Yeast pol ζ interacts with Rev1 as shown by co-immunoprecipitation [20, 21] and this association enhances the efficiency of extension from mismatched primer-templates and AP sites [19, 21]. Yeast DNA pol ζ also interacts functionally with PCNA and with the alternative "9-1-1" complex clamp [22,

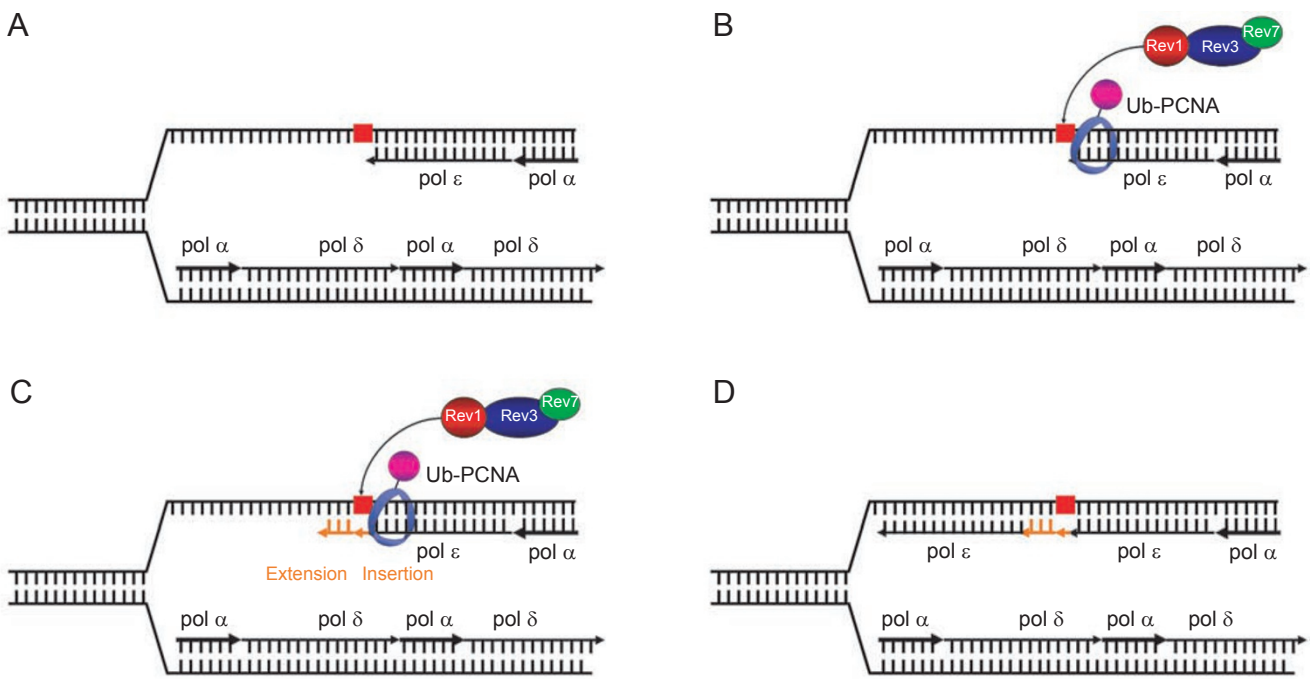

Figure 1 Model for lesion bypass by yeast Rev1-pol ζ at a stalled DNA replication fork. **(A-D)** The leading (top) and lagging (bottom) strand of a DNA replication fork during S-phase is represented. The red square signifies a non-coding DNA lesion. **(A)** DNA replication polymerases, pol δ and pol ε, are responsible for replicating genomic DNA. Genome replication occurs in the presence of DNA damage, but these DNA replication polymerases are often unable to bypass non-coding lesions and DNA replication is halted. **(B)** The cell responds to this stalled replication fork by activating the ubiquitin E2-E3 complex Rad6-Rad18, which mono-ubiquitinates PCNA (Ub-PCNA). Ub-PCNA presumably causes the dissociation of the DNA replication polymerases and the association of damage bypass polymerases. Pol ζ (Rev3-Rev7) associates with Rev1 and associates with Ub-PCNA through Rev1. **(C)** Pol ζ or possibly Rev1 inserts a nucleotide opposite this non-coding lesion and subsequently pol ζ extends several nucleotides from this (potentially misincorporated) nucleotide, bypassing the lesion. **(D)** By a yet undefined mechanism, the lesion bypass complex dissociates from the template, Ub-PCNA is removed and the normal DNA replication polymerases reassociate and continue genome replication.

23], via the Rev 7 subunit [23]. PCNA can stimulate DNA synthesis by pol ζ [22]. There is evidence that a Rev3-Rev7-Rev1 complex associates with monoubiquitinated-PCNA through ubiquitin-binding motifs in REV1, which helps to enable pol ζ or other REV1-interacting bypass polymerases to insert a base opposite damage and then extend from the resulting non-standard primer-template [22, 24] (see the review by Andersen *et al.* in this issue [25]). One current model for how yeast pol ζ and Rev1 may function at a stalled replication fork is shown in Figure 1.

The mammalian *REV3L, REV1* and *REV7* genes and gene products

At least two transcripts of greater than 10 kb have been identified for human *REV3L*. One form encodes a 3 130 amino acid protein [26, 27] and the other a 3 052 amino acid protein arising from alternative splicing and translation from a more 3′ initiation codon [28]. The predicted size of the larger protein is 353 kDa. REV3L is widely expressed in many different tissues and cell lines [27, 29-31]. There is a hairpin loop in the 5′ untranslated region immediately preceding the Kozak consensus sequence. It has been proposed as a possible regulatory element [27], but no functional effect of the hairpin loop has yet emerged [30]. Upstream of the *REV3L* promoter there is a response element to which p53 binds, as identified by chromatin immunoprecipitation and CpG island microarray hybridization [32]. *REV3L* transcription is inducible by treatment with the DNA-damaging agent adriamycin [32] and by *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine [33], but not by hypoxic conditions [32]. Murine *Rev3L* was first identified as a gene induced by treatment of primary cultured cerebral cortical cells with the seizure-inducing agent pentylentetrazol [34].

Mammalian REV3L is twice the size of yeast Rev3 (173 kDa). Much of this difference is due to one exon, exon 13, which encodes 1388 amino acid residues (Figure 2). Outside of this large exon, yeast Rev3 and mammalian REV3L proteins share three regions of sequence similarity: an N-terminal region, a region involved in Rev7 binding in the yeast protein and the DNA polymerase domain. As in yeast Rev3, there are six conserved B-family DNA polymerase motifs and two zinc-finger motifs in the C-terminal region of mammalian REV3L. The mammalian polymerase domain shares ~39% sequence identity with the yeast polymerase domain [26, 27]. The N-terminal domains share ~36% identity and the Rev7 binding region ~29% sequence homology [26, 35]. Several motifs in the N-terminal region of yeast Rev3 and human REV3L are also found in the B-family DNA pol δ [27]. However, no formal demonstration of REV3L DNA polymerase activity has been reported. Expression of REV3L is technically challenging because of the large size of the protein. In mammalian cells, endogenous REV3L protein is apparently expressed at a very low level and has not been detected with an antibody. However, several groups have expressed the smaller human and mouse REV1 and REV7 proteins [36-40].

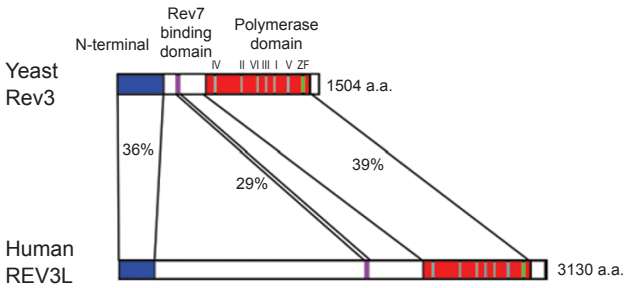

Figure 2 Diagram of yeast Rev3 and human REV3L. The human protein is twice the size of its yeast homolog. Related regions are similarly colored and percent identity is indicated. Three regions between the yeast and human protein share significant sequence identity, as indicated. Zinc finger: ZF.

A few studies have examined interactions between REV3L and other proteins. A fragment of human REV3L (amino acid residues 1776-2044) interacts with full-length REV7 as shown by yeast two-hybrid assays and by co-immunoprecipitation of polypeptides transiently expressed in HeLa cells [37]. Yeast two-hybrid assays show that a region of human REV7 (amino acids 21-155) interacts with human REV3L and REV1 [37]. However, an interaction between human REV7 and full-length human REV3L has not yet been demonstrated experimentally.

Human *REV1* cDNA encodes a 1 251 amino acid protein with a molecular weight of 138 kDa (Figure 3). Like yeast Rev1, human REV1 has a dCMP transferase activity that preferentially inserts a C across from a template G [36, 41]. Furthermore, human REV1 can insert C opposite an AP site or a uracil [36]. Another human REV1 variant, possibly generated by alternative splicing, codes for a 1 250 amino acid protein. Site-directed mutagenesis of D569 and E570 to alanine within the catalytic domain of this form of human REV1 inactivates the dCMP transferase activity [42]. When examined in yeast and chicken REV1, the dCMP transferase activity is not critical for damage tolerance activity [43, 44]. Other structural domains are known to be important. Three major regions of interest on REV1 are the BRCT domain, two ubiquitin-binding domains and its extreme C-terminal domain (Figure 3). The original yeast *rev1-1* point mutation leads to a G193R change in yeast Rev1. A homologous G76R change was made in the mouse protein. Mice containing this change are viable and fertile [45]. Embryonic stem cells harboring this mutation have an

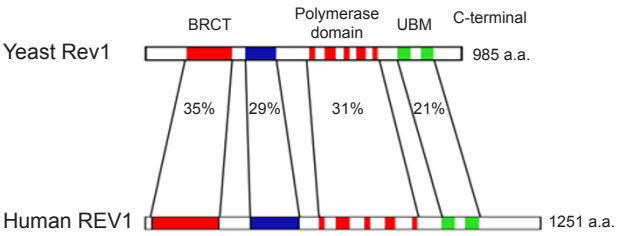

Figure 3 Diagram of yeast Rev1 and human REV1. Four regions between the yeast and human protein share high sequence identity, the BRCT (Brca1 C-terminus) domain, an internally conserved region, the polymerase domain and the ubiquitin binding motif (UBM). Related regions are similarly colored and percent identity is indicated.

increased sensitivity to UV radiation and a prolonged S and G2/M phase. Upon damage, these cells had an increased number of chromatid aberrations without an increase in the number of sister chromatid exchanges [45]. Mice have also been constructed with both a mutation in the dCMP transferase active site and a C-terminal deletion in REV1. These are inviable on the C57BL/6 background but are viable on a 129/OLA strain background, with moderate growth retardation in the first months of life [46].

The BRCT domain of REV1 (amino acids 1-151) is important for nuclear localization. However, a larger fragment corresponding to the C-terminal half of the protein (residues 730 - 1 251) could translocate into the nucleus, suggesting other means to facilitate nuclear localization of REV1 protein [47].The BRCT domain of REV1 interacts with both PCNA and ubiquitinated (Ub) PCNA. A discrete point mutation (G76R) or complete deletion of the BRCT domain abolished the PCNA-REV1 interaction as measured by immunoprecipitation of recombinant proteins expressed in MRC5 cells [48]. Both the point mutant and the deletion mutant prevent REV1 from forming constitutive nuclear foci detectable by immunofluorescence of eGFP-tagged proteins in undamaged cells. Upon UV irradiation, the mutant proteins form fewer damage-induced nuclear foci in cells than does wild-type REV1 [48]. The damage-induced foci formed in the absence of a functional BRCT domain may be mediated by the ubiquitin-binding domains (UBM1: 933-962 and UBM2: 1 011-1 040). These domains both bind ubiquitin and enhance the association of REV1 with Ub-PCNA. Successive truncation fragments delineated a C-terminal portion of REV1 (amino acids 826-1 036) required for nuclear localization and focus formation [49]. This region includes the UBM regions. Deletion or mutation of the UBMs significantly diminished the amount of damaged-induced nuclear foci formed while a double BRCT-UBM knockout completely abolished the ability of REV1 to form nuclear foci [50]. Independent observations, however, show that human REV1 lacking its BRCT domain can be localized to the nucleus and form foci in unirradiated cells [49]. The BRCT domain of chicken REV1 is not required for DNA damage tolerance [51].

In summary, the ubiquitin-binding motifs of REV1 and possibly also the BRCT domain are required to bind PCNA, which in turn localizes REV1 to DNA primer termini. When DNA is damaged, ubiquitinated PCNA provides an enhanced platform for REV1 binding, allowing formation of nuclear foci at sites of DNA replication stalled at lesions, or at post-replication gaps.

Human *REV7* cDNA encodes a 211 amino acid protein with a predicted molecular weight of 24 kDa. Human REV7 has 23% sequence identity and 53% similarity to *S. cerevisiae* Rev7. The HUGO-gene nomenclature approved name for REV7 is MAD2B; in the remainder of this review we refer to it as REV7. Human REV7 has a 23% sequence identity and 54% similarity to human MAD2, a cell-cycle checkpoint protein associated with the mitotic spindle [38, 52]. Similar to its yeast counterpart, human REV7 interacts with CDC20, MAD2 and possibly REV3L. Thus, the function of REV7 seems to extend considerably beyond a putative function as a component of pol ζ. Another indication of additional functions for REV7 not shared by REV3L may be inferred by comparing the expression patterns for *Rev3L* and *Rev7*. Significant *Rev3L* mRNA expression in the mouse brain (http://www.brainatlas.org/aba/) is largely confined to the hippocampus and dentate gyrus, whereas *Rev7* expression is more widely distributed throughout the brain.

In response to DNA damage and cell stress, REV7 was reported to act as an adaptor by binding the transcription factor, ELK1, and a phosphorylated form of the MAP kinase, JNK. JNK phosphorylates ELK1, leading to the activation of genes such as *EGR1* [53]. The damage tolerance protein REV7 may thereby participate in response to DNA damage at the transcriptional level. Several independent studies suggest that REV7/MAD2B can inhibit the anaphase promoting complex through interactions with CDC20 and CDH1 [54-56]. However, overexpression of REV7/MAD2B did not lead to cell-cycle arrest [38]. Knockdown of human *REV7/MAD2B* with siRNA decreased cellular plating efficiency, but had no other obvious effect on cell proliferation, mitotic index, or cell-cycle parameters [57].

Using yeast two-hybrid assays and/or transient transfection, there have been several independent demonstrations of an interaction between REV7 and REV1. The mammalian REV1-REV7 interaction involves the C-terminal region (amino acids 1 130-1 251) of REV1 [37, 39, 40]. This C-terminal region of mammalian REV1 is important for inter-

acting with many other translesion synthesis polymerases. By yeast two-hybrid and transient co-transfection, mouse and human pols ι, η and κ interacted with the C-terminus of REV1 [40, 44, 47]. This supports the theory that REV1 may act as a scaffolding protein, facilitating the interchange between different DNA translesion polymerases at a site of damage. There has been no reported interaction between mammalian REV3L and REV1 fragments or full-length proteins, although such an interaction has been demonstrated in yeast [20, 21].

Consequences of REV3L, REV1 and REV7 reduction in human cell lines

Various approaches have been taken to lower or eliminate *REV* gene function by genetic knockout, anti-sense suppression and small hairpin RNA (shRNA) methods. Human foreskin fibroblasts expressing REV3L antisense RNA have a diminished frequency of mutations induced by UV radiation or benzo[*a*]pyrene diolepoxide [58]. There was no significant difference between the anti-sense and the parental lines in sensitivity to killing with either damaging agent. Fibroblasts generated from adult mice expressing antisense RNA to Rev3L also had overall decrease in UV radiation-induced mutagenesis at the *Hprt* gene [59]. There was also no observed difference in sensitivity to UV radiation between the knockdown cell line and the parental controls. Diminished mutagenesis without a significant change in cell survival was similarly observed for a foreskin fibroblast cell line expressing *REV1* antisense RNA and treated with UV radiation [60]. The greater relative effect on mutagenesis compared to survival is analogous to the situation in yeast, indicating alternative pathways to increase survival. Nevertheless, antisense and RNAi knockdown of mammalian *REV3L* apparently does not completely ablate REV3L function, because complete ablation of the gene function prevents normal mouse development and inhibits growth of many cells, as described below.

Cells expressing *REV3L* anti-sense RNA were hypersensitive to killing by cisplatin and had diminished mutagenesis compared to the parental lines [61]. Knockdown of *REV1* in ovarian cancer cell line 2008 and of *REV3L* in the colorectal tumor cell line HCT116 using shRNA also was associated with more sensitivity to cisplatin as measured by clonogenic assays, compared to the parental lines. Similarly, these mRNA suppressed cells had diminished spontaneous and cisplatin-induced mutagenesis in the *HPRT* gene [62, 63]. Further, overexpression of REV1 in ovarian tumor lines led to some resistance to cisplatin and increased mutagenic frequency compared to parental lines [64]. Among DNA repair gene knockouts in chicken DT40 cells, *rev3* mutants are the most sensitive to cisplatin of any single mutant examined [65]. Vertebrate REV3 and REV1 apparently play a more important role in cellular tolerance of some DNA-damaging agents than others.

Role of pol ζ in interstrand crosslink repair and recombination

The human fibroblast cells stably expressing *REV3L* or *REV1* anti-sense constructs both demonstrated diminished spontaneous and damage-induced extrachromosomal homologous recombination using a two plasmid reporter system [61]. A nasopharyngeal carcinoma cell line expressing shRNA to suppress REV7 was found to have an increased sensitivity to cisplatin and gamma radiation, and a decrease in cisplatin-induced mutagenesis [57]. Increased chromosomal aberrations, but diminished sister chromatid exchange were observed in response to DNA damage. The increased sensitivity to cisplatin and the diminished homologous recombination in human *REV1*, *REV3L* and *REV7* knockdown cell lines suggests that all three human REV proteins are involved in some post-replication DNA recombination pathway.

Contrasting results have been obtained with chicken DT40 cells carrying disruptions of *REV1*, *REV7* or *REV3L*. Single disruption mutants of these genes were reported to have increased frequencies of sister chromatid exchange, both spontaneously and in response to 4-nitroquinolone-1-oxide and UV radiation [65, 66]. An independent study using the same REV3L-disrupted cell line found that sister chromatid exchanges were still induced normally by 4-nitroquinolone-1-oxide, but were not induced by treatment with mitomycin C or cisplatin [67]. Results in the DT40 cell line may not be directly comparable to the results in the human cell lines, as the DT40 line mediates an exceptionally high level of homologous recombination activity.

REV3L plays a role in at least one pathway of recombination-independent repair of interstrand DNA crosslinks. In one study, a host cell reactivation assay using plasmids containing single psoralen or mitomycin C interstrand crosslinks was used. *Rev3L–/–* murine embryonic fibroblasts (MEFs) and DT40 cells carrying disruptions of *REV3L* and *REV1* showed defective reactivation of such plasmids [68]. A suggested role for REV3L in this process is in synthesis across an unhooked crosslink produced by processing of one side of the crosslink by nucleotide excision repair [69]. A similar role may apply to budding yeast Rev3. An allele of *REV3* was isolated as the *PSO1* mutant, sensitive to photoactivated psoralen [70]. *REV3* mutant cells are sensitive to crosslinking agents in stationary phase or G1, but are not particularly sensitive to such agents while in the exponential growth phase [71].

In the yeast *S. cerevisiae*, Rev3 has an intriguing but not

yet understood role in mutagenesis during recombination. Intrachromosomal recombination between inverted repeats on a chromosome as well as interchromosomal recombination can be stimulated by creating a double-strand break between the repeats with HO nuclease. Adjacent to the test gene, frameshift and base substitution mutations are found in association with the recombination event [72-74]. In cells lacking Rev3, double-strand break repair still occurs, but >75-80% of the associated mutations do not occur [72, 73]. Thus, it appears that Rev3 is enlisted in some way as a DNA polymerase during the recombination process, and that the action of Rev3 leads to mutations. By chromatin immunoprecipitation, yeast pol ζ and Rev1 were found associated with an HO double-strand break [20].

Consequences of REV3L disruption in higher organisms

Several independent studies have been made on the effects of *Rev3L* gene disruption in the mouse. The uniform result, disrupting several different regions of the gene and in different types of ES cells and strain backgrounds, is that disruption of mouse *Rev3L* results in lethality during development. *Rev3L* null embryos do not survive past 8.5-12.5 days after fertilization and those that do survive to mid-gestation are delayed in their development [75-79]. Heterozygous *Rev3L*+/– embryos and mice are developmentally normal. The level of p53 protein was increased, and increased apoptosis was observed in embryos lacking *Rev3L*, probably as a response to genotoxic stress [79]. However, disruption of *p53* is unable to rescue *Rev3L* null embryos [75, 79-81].

In order to observe the distribution of *REV3L* in different cell types, a *Rev3L* locus was modified to contain an IRES-*lacZ-Neo*^{R} cassette which enabled β-galactosidase staining. *Rev3L* expression is most pronounced in the somites and other mesodermally derived tissues early in development, and later more widespread through other regions of the developing embryo [75].

Attempts to generate mouse embryonic fibroblasts from the *Rev3L* null fetuses proved difficult even after elimination of *p53*. However, mitotically active MEF disrupted for both *Rev3L* and *p53* has been generated by two groups. In one case, *Rev3L* null, *p53* null MEFs had a slower growth rate compared to *Rev3L* wt, *p53* null MEFs, were hypersensitive to UV radiation and cisplatin, and accumulated in S and G2/M in response to DNA damage [82]. An independent cell line displayed moderate sensitivity to a variety of DNA-damaging agents (cisplatin, UV and gamma radiation and MMS). Most striking was the widespread spontaneous genome instability in these cells, with a pronounced increase in chromosome translocation events [81].

Mice expressing *Rev3L* anti-sense RNA have also been generated [83]. These mice were healthy with normal body weight and lifespan, and perhaps survived because of a low level of residual REV3L expression. There were fewer B-cells in bone marrow and spleen, and the affinity of antibodies following immunization was reduced. In the memory B-cells of mice expressing *Rev3L* antisense, there were fewer somatic mutations in the Ig V_H gene compared to parental controls, suggesting a decrease in overall somatic hypermutation [83]. In mice and in chicken DT40 B-cells, the dCMP transferase activity of Rev1 appears to be important for immunoglobulin diversification. Mutational analysis of immunoglobulin chains indicated that loss of the catalytic activity of Rev1 shifted nucleotide incorporation from C to A or T [46, 51].

The *Drosophila melanogaster* MUS205 protein closely resembles *S. cerevisiae* Rev3 by sequence homology, and mutation of *Mus205* led to increased UV and alkylating agent sensitivity but had no effect on the mutability of germs cells in flies exposed to 4-nitroquinoline-1-oxide, methylmethansulfonate or ionizing radiation [84]. Endogenous *D. melanogaster* (Dm) Rev3 was purified using a DmRev1 protein-affinity column. A protein of ~240 kDa was isolated, which was a processive DNA polymerase on a poly(dA)/oligo$(dT)_{10:1}$ template. This activity was unable to bypass cyclobutane pyrimidine dimers, (6-4) photoproducts or mismatched primer termini. A DmRev3 fragment (amino acids 858-1 217) could interact with DmRev7, but this interaction did not enhance DmRev3's catalytic activity [85]. DmRev7 also interacts with the Dm AP endonuclease Rrp1. Rrp1 preferred 3′-mismatched substrates and Rev3 could perform strand displacement on a template with a single-nucleotide gap. One possibility is that Rev7 functions by interacting with Rrp1 (and Fen1) in order to excise an AP site via base excision repair while Rev3 would then fill the excised gap [86]. Rrp1 also has DNA strand transferase activities [87, 88], and so an alternative possibility is that the Rev7-Rrp1 interaction is involved in some step of a homologous recombination pathway.

The ortholog of REV3L in the model plant *Arabidopsis thaliana* has been disrupted in two independent studies. *Arabidopsis* carrying a mutation in *Rev3* are viable, but root growth is hypersensitive to ultraviolet-B radiation (313 nm), with stem cells in the root being particularly sensitive to inactivation by such radiation [89, 90]. *Arabidopsis* with the mutant Rev3 were also more sensitive than normal plants to mitomycin C and to ionizing radiation [89]. Disruption of the *Arabidopsis Rev1* and *Rev7* genes has also been performed [91]. The phenotype of the *Rev7* disruption is not identical to the *Rev3* disruption; for example, *Arabidopsis Rev7* mutants are not hypersensitive to ultraviolet-B radiation [91].

Concluding remarks

This review has focused on the current understanding of the REV3, REV7 and REV1 proteins in higher eukaryotes. The seminal findings in yeast have led many researchers to explore whether these REV genes and proteins possess similar functions in multi-cellular eukaryotes. Cells from all organisms utilize pol ζ function to tolerate different types of DNA damage, having a relatively greater need for pol ζ to bypass and/or repair damage caused by DNA crosslinking agents. DT40 *rev3* knockouts are particularly sensitive to the interstrand crosslinking agents melphalan, cisplatin, transplatin and mitomycin C [92]. Similar to yeast pol ζ and Rev1, anti-sense studies in human and mouse cells have determined that the mammalian enzymes introduce mutations during DNA synthesis bypass of damage caused by different DNA-damaging agents. Such a function has not yet been identified for the *Drosophila* homolog of Rev3, and DmRev3 and DmRev7 might function during base excision repair synthesis, rather than to misincorporate or extend nucleotides opposite damaged templates.

Removal or disruption of the *Rev* genes in mouse and chicken cells results in both chromosome and chromatid aberrations. These presumably result from the collapse of DNA replication forks stalled at sites of DNA damage. Most of the current evidence suggests that homologous recombination is reduced in *Rev*-deficient cells. Studies deleting these genes in non-transformed cells followed by immediate chromosome analysis will lead to an improved understanding of how Rev proteins function to maintain chromosome stability.

Investigation of yeast Rev3, Rev7 and Rev1 biochemistry have begun to explain how these three proteins interact and how they function during damage tolerance. The ability of mouse Rev7 and the Y-family pol η, ι and κ to interact with mouse Rev1 has suggested an important function of Rev1 as a scaffolding protein, presumably coordinating access of these translesion synthesis polymerases to DNA lesions. These four proteins all interact through the C-terminus of Rev1. Results in the DT40 system indicate that this region, and not the BRCT or dCMP transferase domains, is required for damage tolerance. It is the C-terminus of yeast Rev1 that also interacts with the polymerase domain of yeast Rev3 and it will be important to determine whether Rev3 in higher eukaryotes (REV3L) is also targeted to lesions by the same mechanism. The phenotypes of *Rev1* mouse mutants with either a targeted BRCT domain mutation or dCMP transferase/C-terminal truncation are considerably milder than all Rev3L knockouts. This suggests either that REV3L may be able to interact with PCNA in the absence of REV1 or that REV3L possesses an additional, essential function.

Studies of the higher eukaryotic REV enzymes have been hampered by the inability to express the very large mammalian Rev3 homologs or to detect the protein in cells. What is the function of the large non-conserved region of Rev3L? Do levels of Rev3L protein, which are presumably kept low through alternative splicing and translation controls, increase in response to DNA damage? While sequence analysis suggests that it is highly likely that REV3L is a functional DNA polymerase, the ability to incorporate and/or extend nucleotides opposite different types of damaged template is unexplored. Potential stimulation by REV7, and even whether full-length REV3L interacts with mammalian REV7 and REV1 remain important unanswered questions.

Acknowledgments

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