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Role of DNA polymerase κ in the maintenance of genomic stability

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Abbreviations: ATR, ataxia telangiectasia and Rad3-related; ATRIP, ATR interacting protein; BPDE, benzo[a]pyrene diol epoxide; NER, nucleotide excision repair; PCNA, proliferating cell nuclear antigen; PIP, PCNA-interacting peptides; RIR, Rev1-interacting region; RPA, replication protein A; TLS, translesion synthesis; UBZ, ubiquitin-binding zinc finger domain; XP, xeroderma pigmentosum

To ensure high cell viability and genomic stability, cells have evolved two major mechanisms to deal with the constant challenge of DNA replication fork arrest during S phase of the cell cycle: (1) induction of the ataxia telangiectasia and Rad3-related (ATR) replication checkpoint mechanism, and (2) activation of a pathway that bypasses DNA damage and DNA with abnormal structure and is mediated by translesion synthesis (TLS) Y-family DNA polymerases. This review focuses on how DNA polymerase kappa (Pol κ), one of the most highly conserved TLS DNA polymerases, is involved in each of these pathways and thereby coordinates them to choreograph the response to a stalled replication fork. We also describe how loss of Pol κ regulation, which occurs frequently in human cancers, affects genomic stability and contributes to cancer development.

Introduction

Maintenance of genome integrity and the ability to adapt to genotoxic stresses are two key elements that ensure both cell survival and the evolution of multicellular organisms. In human cells, accurate replication of genomic DNA in S phase requires the action of the most abundant and accurate "replicative" DNA polymerases: Pol α, δ, and ε. These are the major enzymes at replication forks and synthesize most of the 6 billion nucleotides that constitute the human genome. This is an intrinsically challenging task because progression of these replicative DNA polymerases is often slow or problematic at specific natural non-B DNA structures or at DNA that has been damaged by endogenous sources or environmental insults[1,2]. To preserve genomic integrity cells have evolved several mechanisms to deal with the constant challenge of replication stress. These include systems that can detect, tolerate, or repair damaged DNA, and replication checkpoints that sense stalled replication forks in S phase and direct the appropriate cellular responses. When either of these systems is defective, the resultant failure to stabilize and restart stalled forks leads to uncompleted DNA replication when the cells enter cell division and increased genetic instability.[1,2]

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The ataxia telangiectasia and Rad3-related (ATR) signaling pathway plays a crucial role in regulating the replication stress response to a large array of insults including DNA damaging agents and chemicals that cause replication arrest.[2] The ATR pathway involves the independent translocation of multicomponent protein complexes to the site of damage, where the ATR kinase is activated. ATR has many substrates, the main one being the protein kinase Chk1. Activation of ATR requires its localization at stalled forks with its partner ATRIP (ATR Interacting Protein) as well as the heterotrimeric 9–1-1 complex and the ATR activator TopBP1, which are recruited independently of ATR-ATRIP.[3,4]

Another important component of the cellular response to replication arrest or stalling as a result of DNA damage is induction of the DNA damage tolerance pathways. The human Y-family DNA polymerases (Pol η, Pol κ, Pol ι, Rev1, also termed "specialized" DNA polymerases) participate in these pathways by facilitating replicative bypass of DNA lesions. Y-family DNA polymerases promote damage tolerance in part through their ability to insert nucleotides opposite DNA lesions that cannot be bypassed by the replicative DNA polymerases, a process termed translesion synthesis (TLS).[5,6] Translesion DNA polymerases may function directly at the replication fork, or may fill in post-replication gaps containing lesions that are left behind by replication forks.[7] In some cases two specialized polymerases are required, one for insertion and one for extension.[8] These processes are facilitated by K164 monoubiquitylation of proliferating cell nuclear antigen (PCNA), which is dependent on the E2 ubiquitin-conjugating enzyme Rad6 and the E3 ubiquitin ligase Rad18.[9]

In addition to their capacity for performing translesion synthesis (as discussed below), some TLS DNA polymerases have recently been implicated in synthesis across non-B structured

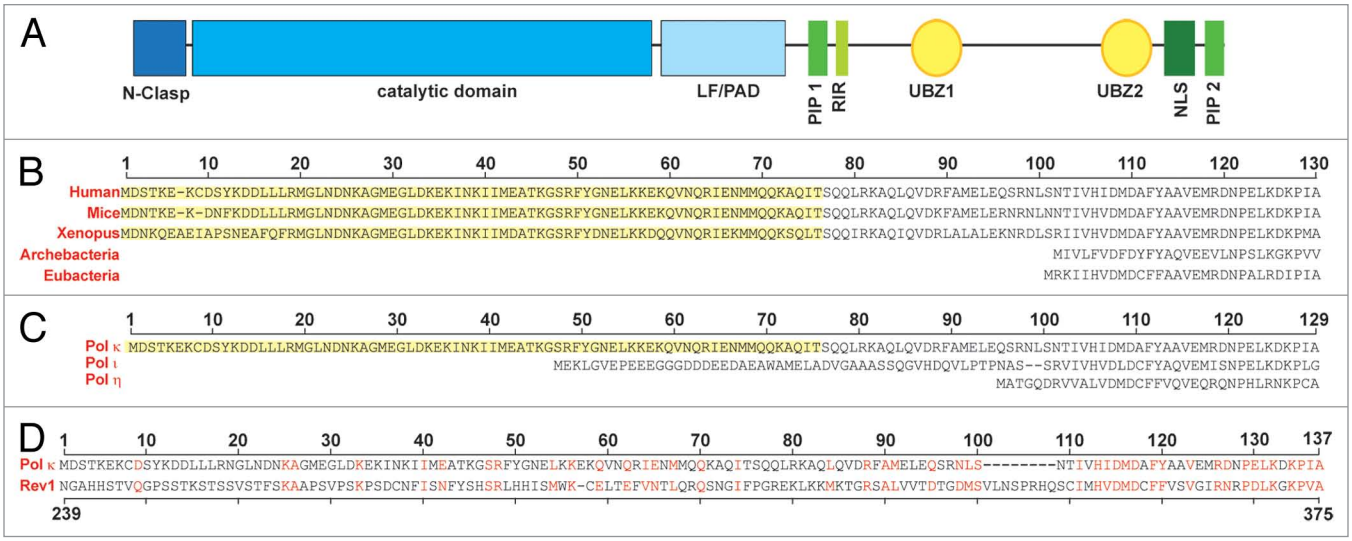

Figure 1. Structure of human Pol κ. (**A**) Human DNA Pol κ contains 870 amino acids (NP057302). Different domains that are important for Pol kappa functions in vivo are presented. *LF/PAD,* little finger/polymerase-associated domain; *PIP1* and *PIP2*, PCNA-interacting peptide boxes; *RIR,* Rev1-interacting region; *UBZ*, ubiquitin-binding zinc finger domain; *NLS*, nuclear localization signal. (**B**) Amino acid alignment of the N-terminal sequences of Pol κ from different organisms. Human NP_057302; Mouse NP_036178; *Xenopus* NP_001086552; Archebacteria: *Sulfolobus solfaraticus* Q97W02; Eubacteria: *Escherichia coli* Q47155. (**C**) Amino acid alignment of the N-terminal sequences of human Y-DNA polymerases. Pol κ NP_057302; Pol ι NP_009126; Pol η NP_006493. Source: http://multalin.toulouse.inra.fr/multalin/.[73] The N-Clasp domain is highlighted in yellow. (**D**) Amino acid alignment of the N-terminal sequence of human Pol κ (NP_057302) and partial sequence (aa 239–275) of Rev1 (AAH_37734).

DNA during replication.[10] Such DNA structures are formed within expanded microsatellites and are known to stall replication fork progression in vivo. Since a causal link between non-B DNA structure formation, replication fork stalling, and genome instability has been demonstrated in the etiology of diseases associated with microsatellite expansion,[11] this process contributes to the choreography of the cellular responses to fork stalling alongside the replication checkpoint and TLS. How the ATR signaling pathway, TLS, and synthesis across non-B structured DNA are coordinated remains an open question. We have addressed this issue by focusing on Pol κ, which is one of the most highly conserved TLS DNA polymerases and best known for its ability to replicate past several bulky adducts in DNA in vitro,[12] although its importance in replication checkpoint efficiency and microsatellite stability has recently been demonstrated. This review will focus on these critical roles for Pol κ and will also describe how permanent or transient loss of Pol κ regulation, which frequently occurs in human cancers, affects genomic stability and contributes to cancer development.

DNA Polymerase Kappa

POLK gene expression

Pol κ was identified 15 years ago as the product of the human *POLK* gene and a homolog of *dinB* of *Escherichia coli*.[13,14] Pol κ has been largely conserved throughout evolution, and is present in the yeast *Schizosaccharomyces pombe* but is not found in *Saccharomyces cerevisiae* or in the fly *Drosophila melanogaster*. The protein is called PolIV in the eubacteria *E. coli*, Dpo4 in the archeabacteria *Sufolobus solfataricus,* and Pol κ in mammalian cells. The human *POLK* gene maps to the chromosomal 5q13.1 locus, where it is spread over 87.5 kb, and encodes a protein of 870 amino acids (aa). Pol κ is highly expressed in the testis and adrenal cortex.[13,15] Its TATA-less promoter contains elements involved in the activation and repression of Pol κ expression. Among the different transcription factor-binding sites,[16,17] Sp1 and cyclic AMP-response element binding protein (CREB) have been reported to activate the promoter of Pol κ.[16] In human cells, Pol κ expression does not seem to be modulated in response to UV (UV) light[15] but its expression is enhanced in response to polycyclic aromatic hydrocarbons such as benzo[a]pyrene or its active metabolite, benzo[a]pyrene diol epoxide (BPDE).[17,18] More studies are required to better understand how Pol κ expression is regulated during the cell cycle or by DNA damaging agents.

Structure, domains, and partners of Pol κ

Y-family DNA polymerases share several conserved motifs that are all in the catalytic domain and comprise fingers, palm, and thumb subdomains.[19] The "little finger" or polymerase-associated domain (LF/PAD) is required for correct binding to DNA, and the linker between the catalytic domain and the LF/PAD seems to perform a major function in polymerase activity and specificity (**Fig. 1A**).[20] In general Y-DNA polymerases show greater flexibility in accommodating nucleotides at their active site than replicative polymerases, which results in a greater propensity for performing TLS of DNA damage by incorporation of nucleotides opposite the lesions.[6] Moreover, the lack of 3′–5′ proofreading activity facilitates TLS synthesis but increases inaccuracy when replicating undamaged DNA.[6] The efficiency and fidelity of replication performed across a lesion depend on the specific polymerase that is recruited. Some functional divergences among polymerases might be related to structural differences.

For instance, Pol ι, which uses Hoogsteen base pairing instead of Watson-Crick interactions, is the best candidate for replicating through adducts of purines whereas Pol κ, through its N-terminal extension, is best adapted for efficient elongation of mispaired bases or accurate bypassing of a BPDE adduct on the N2 of guanine.[21,22] Indeed, the 75 amino acids located upstream of the catalytic domain of Pol κ constitute the N-clasp domain essential for DNA polymerase activity that confers the capacity to surround the DNA and locks the polymerase around the 3′ end of the primer-template junction[22] (**Fig. 1A**). The N-clasp of Pol κ is not shared by its prokaryotic homologs (**Fig. 1B**) or by Pol η and Pol ι (**Fig. 1C**), but we found that Rev1 contains a putative N-Clasp region upstream of its catalytic domain (**Fig. 1D**). In addition to this domain, Pol κ contains two PCNA-interacting peptides (PIP1 and PIP2) called the PIP-box, two ubiquitin-binding zinc finger domains (UBZ) and a Rev1-interacting region (RIR) that can promote its recruitment to chromatin (**Fig. 1A**). Indeed the Pol κ PIP2 box, which is located at its C-terminus (aa 858–870), is required for accumulation of the polymerase into foci after UV treatment.[23] The PIP1 box of Pol κ was recently identified immediately downstream of the catalytic core (aa 526–533).[19,24] The two PIP boxes seem to function redundantly to promote translesion synthesis of thymine glycol lesions as Pol κ can use PIP1 or PIP2 to interact with PCNA.[24] Neither of the PIP boxes of Pol κ completely match the canonical sequence contained in the p66 subunit of Pol δ that confers strong interaction of Pol δ with PCNA, possibly explaining why the interaction between PCNA and Pol κ is weak.[25] Therefore, the interaction of Pol κ with PCNA through its PIP boxes might not be the most critical determinant of its relocation to sites of DNA damage. Importantly, the two UBZ domains provide a molecular basis for the association between Pol κ and monoubiquitylated PCNA.[18,23,26] Thus, depletion of RAD18 or mutated K164-PCNA abrogates the interaction between PCNA and Pol κ after BPDE treatment.[27] Moreover, modification of the UBZ domains impedes the nuclear localization of Pol κ after UV or hydroxyurea (HU) treatment.[18,23] In addition, Pol κ interacts with the C-terminal domain of Rev1 through the RIR (aa 560–575), which is also found in Pol η and Pol ι. Although there is no consensus sequence defining the RIR, two consecutive phenylalanine residues are conserved. Interaction with Rev1 has been proposed to facilitate the exchange between TLS polymerases when more than one TLS Pol is required to perform TLS.[5] Moreover, Pol κ has been found in a complex with Pol δ, XRCC1,[28] Rad18,[27] and the PCNA-like heterotrimer Rad9/Hus1/Rad1 called 9–1-1,[29,30] but the domains of Pol κ that mediate these interactions are still unknown. The regulation of Pol κ through post-translational modification is also not well understood. Although Pol κ can be monoubiquitylated the functional significance of this modification remains unknown.[18] However, by analogy to Pol η, we can speculate that Pol κ ubiquitylation could occur on the lysine within the PIR domain (the domain covering the bipartite NLS and the PIP box, aa 840–870) and regulate recruitment of Pol κ to chromatin.[31] In addition, results of a genome-wide RNAi screen suggest that small ubiquitin-like modifier (SUMO)-mediated regulation of Pol κ can protect *Caenorhabditis elegans* against methyl methane sulphonate-induced injuries.[32]

Misregulation of Pol K, Genomic Instability, and Cancer

Upregulation of Pol kappa in cancers and consequences for genome stability

Large-scale studies of DNA replication and the expression of DNA repair factors in patients with different types of cancers have revealed frequent alterations in the expression pattern of genes encoding the TLS DNA polymerases. This is notably the case for *POLK*, which has been found to be upregulated in lung tumors compared with paired adjacent nontumorous tissues[33] and has been observed by Serial Analysis of Gene Expression (SAGE)[34] to be upregulated in human ovarian and prostate cancers (http://www2.ncbi.nlm.nih.gov/SAGE/, the Cancer Genome Anatomy Project at NCBI). Moreover, Pol κ overexpression was found to be associated with advanced disease stages and shorter survival in patients with glioma and was identified as an independent prognostic factor for this disease.[35] The molecular basis of the relationship between permanent or transient upregulation of Pol κ and cancer progression could be mutator effects. The high fidelity of human DNA replication, approximately one error per 10^9–10^{11} nucleotides polymerized, is the result of a sequential multistep process involving the mismatch repair proteins and the replicative DNA polymerases and their associated proofreading activities. More than 10 years ago we hypothesized that this highly accurate process might be modified after upregulation of an error-prone DNA polymerase, leading to untargeted mutagenesis along the undamaged regions of the genome and contributing to the acquisition of a mutator phenotype that, combined with defects in cell cycle control or other genome stability pathways, could be a driver of cancer incidence and/or the acceleration of tumor progression.[36,37] This was confirmed in human cells, in which we found that overexpression of Pol κ confers a mutator phenotype.[38] Remarkably, when expressed ectopically Pol κ becomes part of the replication machinery in the absence of external stress,[38] supporting the idea that spontaneous mutagenesis could occur in S phase at the DNA replication fork during elongation of the nascent DNA chain. Alternatively, enhanced expression of a TLS DNA polymerase could affect the DNA replication program, thus inducing replicative stress. Recent analyses of precancerous lesions in human cells indicate that cancer development is associated with DNA replication stress, leading to DNA double-strand breaks, chromosome instability, and selective pressure for p53 mutations.[39] For the past several years many laboratories have attempted to decipher the causes of this replicative stress and relate these events to defined genotypic and phenotypic manifestations. It has been proposed that enhanced expression of Pol κ induces genomic instability by redirecting Pol κ to replication forks and by interfering with the normal process of DNA replication,[26,40] supporting the novel concept of a cancer-related modified replisome.[37] Indeed, altered fork progression and increased origin firing to compensate for the

reduced elongation rate have been demonstrated in cells expressing ectopic Pol κ. This mechanism may explain why excess Pol κ induces DNA breaks and micronuclei formation, increases the frequency of homologous recombination, and stimulates DNA exchanges as well as aneuploidy,[26,41] and corroborates findings in Pol κ-overexpressing non-small cell lung carcinomas, the majority of which exhibit loss of heterozygosity.[41] Therefore, excess Pol κ might not only directly induce a mutator phenotype, but might also modify important replication parameters that in turn trigger chromosome instability.

Downregulation of Pol kappa in cancer

Downregulation of *POLK* has frequently been observed in lung, stomach, colorectal, and breast cancers.[42-46] This may be at least partially due to reduced expression of CREB and Sp1, two activators whose binding elements are present in the promoter region of the *POLK* gene.[16] Several lines of evidence suggest that loss of expression of Pol κ in these pathologies contributes to genetic instability and cancer progression. First, depletion of Pol κ in unstressed cells leads to increased formation of γ-H2AX foci in S phase, which is indicative of the presence of stalled or collapsed forks. This was observed in both xeroderma pigmentosum A (XPA) cells (which are defective in nucleotide excision repair [NER]) and under low oxygen conditions, therefore it cannot be attributed to a defect in NER or to oxidative stress.[30] In addition, the basal level of γ-H2AX is higher in Pol κ-deficient mouse embryonic fibroblast cells than in their wild type counterparts.[47] Pol κ depletion also leads to the recruitment of replication protein A (RPA) onto chromatin, indicating the presence of ssDNA, and increases the formation of 53BP1 nuclear bodies in G1 phase,[30] a hallmark of regions that were under-replicated in the previous S phase.[48,49] Collectively, these observations support the idea that Pol κ-defective cells exhibit a DNA replication program defect and could explain the spontaneous genetic alterations observed in mice deficient in Pol κ [50].

In summary, both over- and under-expression of Pol κ lead to genetic instability in human cells, but most likely with a different mechanistic basis. It appears that excess Pol κ interferes with fork progression and triggers both a mutator phenotype and chromosome instability, whereas the phenotypes caused by Pol κ downregulation could represent the in vivo functions of Pol κ that are presented below.

In Vivo Functions of Pol Kappa that Prevent Genomic Instability

DNA damage-dependent functions of Pol κ

During translesion synthesis, the insertion and extension steps can be performed by a single or two different TLS polymerases.[8] Based on structural analysis and primer extension experiments, Pol κ was proposed to be a better extender than inserter.[21,51] However in vivo experiments have shown that Pol κ is able to insert nucleotides and perform error-free bypassing of lesions such as thymine glycol or BPDE adducts in collaboration with the extender Pol ζ.[52,53] The dual action of Pol κ/Pol ζ is also necessary for translesion synthesis across cisplatin adducts in vivo[53] although these are blocking lesions in vitro.[14,54] Pol κ has also been implicated in protection against oxidative stress in human cells both in vitro and in vivo since it accomplishes TLS of lesions such as 8-oxo-7,8-dihydro-2×-deoxyguanosine (8-oxo-G) or thymine glycol and prevents the accumulation of DNA breaks after H_2O_2 treatment.[47,51,52] In addition, Pol κ is able to bypass abasic sites and N-2-acetylaminofluorene (AAF) adducts in vitro[54-56] and can perform TLS across DNA monoalkylation damage in vivo.[57] Whether TLS catalyzed by Pol κ takes place at the fork or during the gap filling process is yet to be clarified.[58]

Pol κ does not support TLS across UV lesions in vitro or in vivo[14] but depletion of Pol κ in human XPV cells, mouse embryonic stem cells, or chicken DT40 cells increases their sensitivity to UV irradiation.[12,57,59] Importantly, Pol κ has been shown to be involved in the NER pathway. This DNA repair function of Pol κ may explain its protective role against UV irradiation.[28,60] Moreover, Pol κ has been implicated in the repair of interstrand cross links.[61] Therefore the DNA damage-related functions of Pol κ are not restricted to TLS.

DNA replication checkpoint function of Pol κ

Induction of the ATR replication checkpoint pathway is a fine-tuned mechanism that allows a rapid slowing of S-phase progression in response to replication fork stalling caused by exogenous DNA damage or replication inhibitors. ATR and the protein kinase Chk1, its main effector, also ensure normal replication fork progression through endogenous barriers during unperturbed S phase, explaining why ATR and Chk1 are both essential proteins in mammalian cells.[62] The DNA polymerase blockage produced by exogenous and endogenous fork barriers causes the polymerases to uncouple from DNA helicases, which then proceed ahead. The resulting long stretches of ssDNA that become covered by RPA allow the recruitment of ATRIP, which is required for recruitment of ATR to the stalled fork. TopBP1, claspin, and the checkpoint clamp Rad9-Rad1-Hus1 (the 9–1-1 complex) are also necessary for full activation of the ATR kinase.[3,4]

We recently found that Pol κ is required for checkpoint activation after replication stress induced by replication inhibitors. Indeed, in both Pol κ-deficient cells and in Pol κ-depleted *Xenopus* extract, phosphorylation of Chk1 on Ser345 was considerably reduced after treatment with two replication inhibitors, hydroxyurea and aphidicolin, and after UV exposure. Interestingly, the latter observation could explain the hypersensitivity of Pol κ-deficient cells to UV irradiation. The contribution of Pol κ to efficient checkpoint activation seems to rely on its synthesis of short DNA intermediates, thereby generating 5′-ended primer-template junctions that constitute likely binding sites of the 9–1-1 complex.[30] This corroborates the observation that DinB of *S. pombe* performs error-prone DNA synthesis at semi-disabled forks in a manner dependent upon the checkpoint clamp loader protein Rad17.[29] Therefore, in addition to its functions in ensuring genomic stability against exogenous DNA damage, Pol κ also has evolutionarily conserved broader roles in response to replication stress. Further studies will be required to determine the molecular basis of Pol κ recruitment at stalled forks. One possibility is that this recruitment could be favored by unmasking interaction motifs on PCNA after

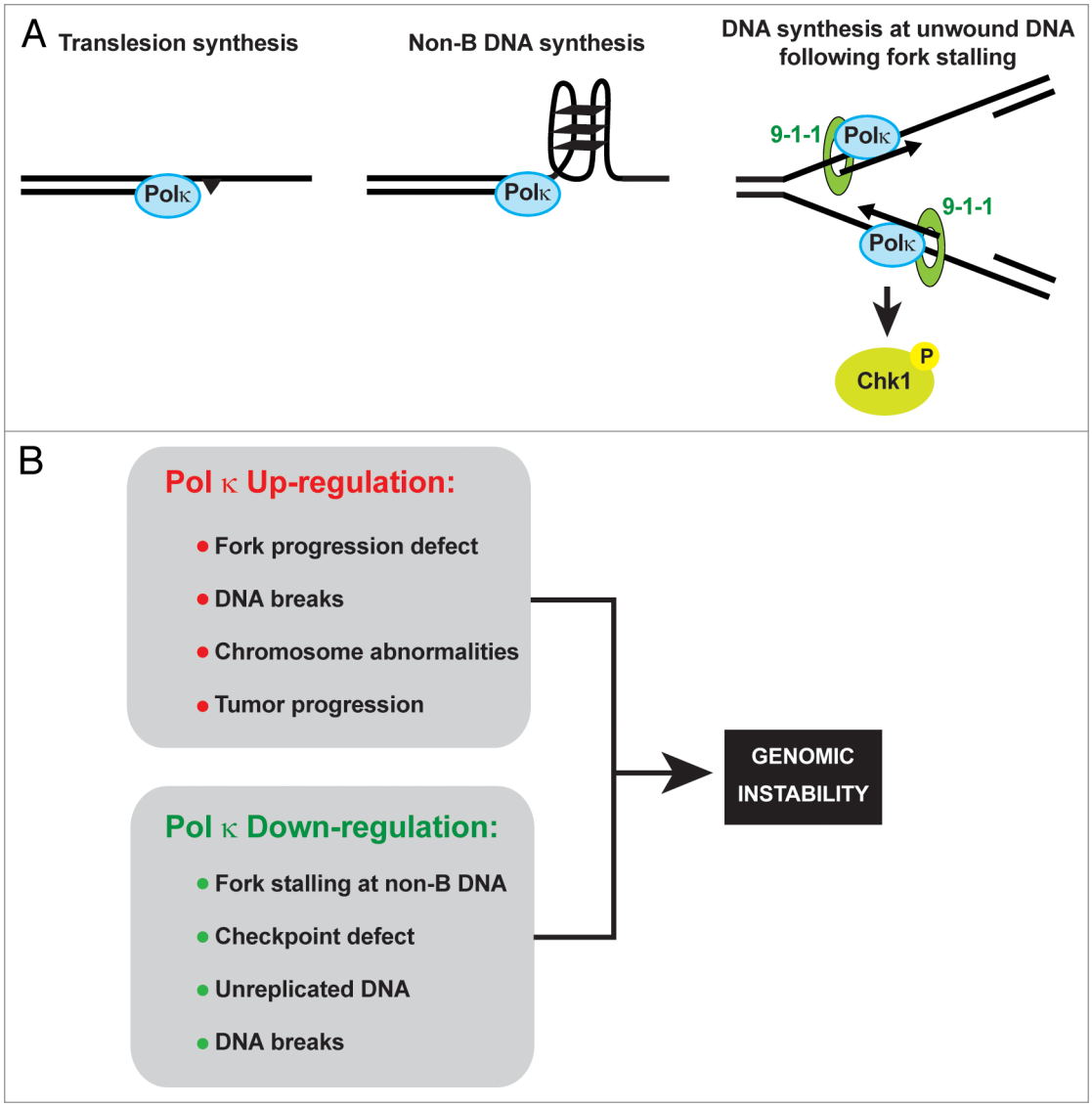

Figure 2. Diverse functions of Pol κ and the effect of its misexpression on genomic stability. (**A**) Functions of Pol κ in human cells: translesion synthesis, non-B DNA replication, and synthesis of small DNA segments during activation of the DNA replication checkpoint. (**B**) Over- or under-expression of Pol κ, both of which are often found in cancers, triggers multiple manifestations of genetic instability in human cells.

Cdt1 degradation as recently demonstrated for Pol κ accumulation into nuclear foci after DNA damage.[63] Whether the Pol κ domains required for TLS are similar to those involved in its checkpoint function is another question that needs to be addressed.

Pol κ performs DNA synthesis through non-B DNA

In addition to the canonical right-handed double helix, DNA molecules can adopt several other non-B DNA structures. These secondary conformations are readily formed in the genome at specific DNA repetitive sequences, and present a distinctive challenge to the progression of DNA replication forks. By impeding normal DNA synthesis, cruciforms, hairpins, H-DNA, Z-DNA, and G4 DNA considerably affect genome stability and in some instances play a causal role in disease development.[64] Together with previously-discovered dedicated DNA helicases, Pol κ, but also Pol η, recently emerged as a new actor that can synthesize DNA through these distorted structures.[10,65,66] Depletion of Pol κ increases cell sensitivity toward telomestatin, a ligand that stabilizes G4 structures, and this hypersensitivity correlates with an increase in γ-H2AX foci formation.[66] Pol κ was also shown to efficiently function on DNA templates containing sequences able to adopt secondary structures within common fragile sites.[67] Moreover, mice deficient in the *POLK* gene manifest elevated levels of frameshift mutations in the germline at tandem repeat minisatellite loci.[50,68] Finally, depletion of Pol κ in the *dog-1 C. elegans* mutant (deficient in the Fancini anemia J [FANCJ] helicase) induces an increase in the frequency of small deletions in G/C tracts.[69] Collectively, these observations strongly suggest a role for Pol κ in the synthesis of non-B DNA, facilitating the completion of DNA replication at these particular DNA sequences. Pol κ may also promote microsatellite stability through its recruitment to stalled forks and the DNA synthesis activity associated with its checkpoint function, and may be particularly important for the

replication of repeats as it shows the capacity to realign slipped strands.[70] Moreover, the replication of these particular repetitive sequences is believed to locally affect the dNTP pool balance by depleting one or two nucleotides,[71] which may hinder polymerization by the replicative DNA polymerases Pol δ or Pol ε and favor their exonuclease activity over polymerization. Thus, Pol κ, which is devoid of exonuclease activity, is likely to be much more efficient than replicative DNA polymerases in the replication of large repeat sequences.

Conclusion

This review illustrates how Pol κ could play a critical role in ensuring high cell viability and genomic stability by contributing to and coordinating three major pathways that allow stalled replication forks to restart, namely TLS, the replication checkpoint, and non-B DNA synthesis (**Fig. 2A**). Although Pol κ has been shown to perform accurate microsatellite DNA synthesis in vitro,[72] it is generally believed to be an error-prone enzyme that may generate mutations when acting in these fork restart mechanisms. This might be part of the subtle equilibrium necessary for cell survival that balances accurate genomic DNA synthesis, which is critical for duplication of the genome before chromosomal partitioning during mitosis, with less stringent DNA synthesis. We also discuss how both over- and under-expression of Pol κ can lead to genetic instability in human cells (**Fig. 2B**), most likely with a different mechanistic basis in which excess Pol κ interferes with fork progression and triggers both a mutator phenotype and chromosome instability whereas the phenotypes due to Pol κ downregulation might reflect the physiologic function of Pol κ.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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