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Polymerase η Recruits DHX9 Helicase to Promote Replication across Guanine Quadruplex Structures

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Abstract

DNA polymerase η (Pol η) catalyzes accurate bypass of ultraviolet light-induced cyclobutane pyrimidine dimers, and it also functions in several other related processes, including bypassing DNA with unusual structures. Here, we performed unbiased proteome-wide profiling of Pol η-interacting proteins by using two independent approaches, i.e., proximity labeling and affinity pull-down followed by LC-MS/MS analysis. We identified several helicases, including DHX9, as novel Pol η-interacting proteins. Additionally, ChIP-Seq analysis showed that Pol η is enriched at guanine quadruplex (G4) structure sites in chromatin. Moreover, Pol η promotes the recruitment of DHX9 to G4 structure loci in chromatin and facilitates DHX9-mediated unwinding of G4 structures. Deficiency in Pol η or DHX9 leads to attenuated replication across G4 regions in

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Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jacs.2c05312.

Detailed experimental procedures and supplementary data (PDF)

Table S1. Lists of candidate Pol eta-binding proteins identified from APEX labeling and affinity pull-down followed by LC-MS/MS analyses (XLSX)

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genomic DNA. Together, we unveiled the interaction between Pol η and DHX9 and demonstrated that the interaction promotes the replicative bypass of G4 structures in chromatin.

Human cells are exposed to various DNA-damaging agents,[1] and the resulting DNA lesions can block the progression of replication forks and induce mutations, thereby leading to cell transformation, senescence, and/or apoptosis.[2] To cope with unrepaired DNA lesions, cells are equipped with DNA damage tolerance mechanisms, one of which is translesion synthesis (TLS).[3]

DNA polymerase η (Pol η) displays accurate TLS over UV-induced DNA lesions, especially cyclobutane pyrimidine dimers.[4–6] Mutations in the *POLH* gene, which encodes Pol η, result in the variant form of xeroderma pigmentosum (XPV) syndrome in humans.[5,6] Pol η also assumes important functions in several other related processes, including bypassing a broad spectrum of other DNA lesions[7] and unusual DNA structures, such as fragile sites[8] and guanine quadruplexes (G4s).[9]

We reason that a comprehensive assessment about Pol η-interacting proteins at the global proteome scale may offer new insights into its functions. Recently, Ting et al.[10–13] developed an engineered ascorbate peroxidase (APEX)-based proximity labeling, in combination with LC-MS/MS analysis, to enable temporal and spatial profiling of interaction proteins of a target protein of interest in cultured cells. We fused APEX2 to the C-terminus of Pol η for the biotinylation of proximity proteins of Pol η, where APEX2 fused with a nuclear localization signal (NLS) served as a control (Figure S1). The LC-MS/MS results led to the identification of 279 proximity proteins of Pol η (Figure 1a and Table S1). Gene ontology (GO) and KEGG pathway analyses revealed the enrichment of DNA replication and DNA repair (Figure S2), which is in keeping with the known functions of Pol η and validates our method. Moreover, GO function analysis revealed the enrichment of DNA helicases.

The APEX labeling method entails ectopic overexpression of Pol η, which may introduce artifacts in identifying its interaction proteins. To profile the interactome of endogenous Pol η, we employed CRISPR-Cas9 to introduce three tandem repeats of Flag epitope tag to the C-terminus of endogenous Pol η protein in HEK293T cells (Figure S3).[14] After enrichment with anti-Flag beads and LC-MS/MS analysis, we identified 206 proteins with preferential binding toward Pol η (Figure 1b), among which 43 are overlapped with the proximity proteins identified from APEX labeling (Figure 1c), including several helicases, e.g., DHX9, DDX5, and DDX17.

Helicases assume functions in many steps of gene expression by unwinding double-stranded DNA/RNA and more complex nucleic acid structures.[15] For instance, DHX9 can unwind noncanonical DNA structures, including intramolecular triplex (H-DNA), DNA- and RNA-containing displacement loops (Dand R-loops), and G4.[16–18] Moreover, DDX5 was found to unfold DNA G4 and activate the transcription of the *MYC* gene in cancer cells.[19]

G4s are noncanonical secondary structures formed in guanine-rich sequences in DNA and RNA.[20] DNA G4s are involved in a number of critical cellular processes, including

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DNA replication, transcription, and maintenance of genome integrity.[20–22] Our quantitative proteomic data unveiled 2- and 4-fold enrichments of DDX5 and DHX9, respectively, in the Pol η pull-down samples relative to the control (Table S1). We also validated the interactions between Pol η and DDX5/DHX9 by immunoprecipitation followed with Western blot analyses (Figure 1d). Both DDX5 and DHX9 are capable of resolving DNA G4 structures,[16,19] and Pol η was found to be important in maintaining fork progression past naturally occurring non-B-form DNA structures, such as G4 DNA.[23] Thus, Pol η may function together with DNA helicases in promoting replication across G4 structure sites in DNA.

To test the above hypothesis, we asked if Pol η binds to specific DNA structure regions in cells by assessing its genome-wide occupancy with ChIP-Seq analysis. After peak calling against the control, we identified 2049 high-confidence Pol η-binding sites in two biological replicates (Figure S4). The Pol η peaks exhibit enrichment in promoters and 5′-UTR (Figure 2a, Figure S5), which is consistent with the distribution of G4 peaks.[24] Importantly, approximately 32% of Pol η peaks overlapped with G4 peaks (Figure 2b). Moreover, the Pol η ChIP-Seq signal was highly enriched and centered at endogenous G4 sites, and vice versa (Figure 2c,d). Integrative Genomics Viewer (IGV) plots also showed a high degree of co-localization between Pol η and BG4 peaks (Figure 2e).

On the basis of the above proteomic and ChIP-Seq data, we hypothesized that Pol η may promote replication across G4 structures by recruiting helicases to unwind these structures. To test this, we conducted a ChIP-qPCR assay to examine how the occupancy of DHX9 at G4 structure loci is modulated by Pol η with the use of patient-derived Pol η-deficient (XPV) human skin fibroblasts and the isogenic cells complemented with human Pol η (XPV+Pol η) (qPCR primers are listed in Table S2).[6] Based on the ChIP-Seq data, we selected four genes with both G4 and Pol η peaks in their promoters for DHX9 ChIP-qPCR analysis (Figure 3). Our results showed that the loss of Pol η confers diminished enrichment of DHX9 in G4 regions, but not in non-G4 regions of *PMS2*, *NEAT1*, and *JUN* genes (Figure 3e), suggesting a Pol η-dependent recruitment of DHX9 to these G4 structure sites. In this vein, DHX9 did not display enrichment in the G4 region of the *CLSPN* gene in Pol η-complemented cells, suggesting the lack of involvement of DHX9 in resolving the G4 structure at this site.

Our ChIP-qPCR with the use of BG4 antibody revealed that the knockdown of DHX9 resulted in increased enrichment of G4 structures in the promoter regions of *PMS2*, *NEAT1*, and *JUN* genes, but not that of the *CLSPN* gene, in XPV + Pol η cells; such increases, however, were abolished in XPV cells (Figure S6). This result demonstrated that the DHX9-mediated unwinding of G4 structures in the promoter regions of these genes requires Pol η.

On the basis of the above findings, we asked whether deficiency in Pol η or DHX9 may impede replication past G4 structure sites. To test this, we labeled newly synthesized DNA with 5-bromo-2′-deoxyuridine (BrdU) in XPV and XPV + Pol η cells, enriched the BrdU-labeled nascent DNA, and subjected the resulting DNA fragments to qPCR analysis. Our results unveiled that the amount of BrdU incorporation across G4 regions is lower in XPV cells than XPV + Pol η cells (Figure 3f), whereas no differences were observed in non-G4

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regions. In addition, genetic depletion of DHX9 leads to decreased BrdU incorporation in the promoters of *PMS2*, *NEAT1*, and *JUN* genes, but not that of the *CLSPN* gene, which is in line with the lack of DHX9 enrichment in the promoter of the *CLSPN* gene. Genetic depletion of DHX9 did not give rise to further diminutions in the amount of BrdU incorporation in Pol η-deficient cells, suggesting that DHX9 and Pol η function in the same pathway in promoting replication across G4 structure sites. Together, these results suggest that Pol η recruits DHX9 to unwind G4 structures prior to their replicative bypass.

Recently, several studies showed that TLS polymerases have functions beyond their canonical roles in bypassing damaged DNA, where these polymerases are capable of bypassing undamaged structured DNA in vitro and in vivo.[25,26] Additionally, Pol η is capable of inserting ribonucleotides opposite template DNA,[27] and it enables transcriptional bypass of minor-groove N^2-alkyl-dG lesions.[28] Moreover, biochemical assay showed that Pol η displays a 6-fold preference toward binding to G4 DNA than non-G4 DNA, and Pol η also exhibits a higher replication efficiency and fidelity than replicative polymerase (polymerase ε) when copying G4 DNA.[9] Furthermore, genetic depletion of Pol η renders U2OS cells sensitive to telomestatin, a G4-binding ligand.[23] Cellular assays also revealed the function of Pol η in promoting replication across common fragile sites,[8] and genetic ablation of Pol η in mice led to senescence in adipose tissues.[29]

By employing two separate approaches, relying on proximity labeling and affinity pull-down in combination with LC-MS/MS analysis, we explored systematically the interaction proteome of Pol η. Our proteomic data revealed pronounced enrichment of several helicases as interaction partners of Pol η. Additionally, the Pol η ChIP-Seq signal was highly enriched and centered at endogenous G4 sites. Moreover, our DHX9 and BG4 ChIP-qPCR results showed that the co-localization of DHX9 with G4 structure sites in gene promoters and unwinding of G4 structures at these sites require Pol η. Hence, our results support a model where G4 structures in template DNA stall replicative polymerases, which leads to the recruitment of Pol η. The latter further recruits DHX9 to unwind G4 structures, thereby enabling their replicative bypass (Figure 4). This model is in line with the $3' \rightarrow 5'$ unwinding activity of DHX9.[30] While Pol η was previously shown to support the replicative bypass of G4 structures in vitro,[9] our work demonstrated this function of Pol η in cells and revealed its relevance at the genome-wide scale. Our study also unveiled that this cellular function of Pol η involves the DHX9 helicase.

The mechanism through which DHX9 is recruited to Pol η is unclear and warrants further investigation. Along this line, DHX9 was identified as a DNA replication protein interacting with PCNA and WRN helicase, where the latter interaction stimulates WRN's activity in unwinding Okazaki fragment-like nucleic acid structures.[31,32] Pol η is recruited to stalled DNA replication forks through its interaction with monoubiquitylated PCNA.[33] Interestingly, Pol η can also be ubiquitylated on its C-terminus by the E3 ubiquitin ligase PIRH2, which inhibits its interaction with PCNA.[34,35] In addition, phosphorylation of Pol η at Ser601 and Ser687 assumes crucial roles in facilitating TLS.[36,37] It will be important to examine, in the future, whether a similar post-translational mechanism contributes to the recruitment of DHX9 to Pol η and the replicative bypass of G4 structures in DNA. It will also be important to investigate whether this function of Pol η can be extended to other TLS

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polymerases, whether the Pol η–DHX9 interaction also contributes to replicative bypass of other structured DNA and R-loops, and how other interaction partners of Pol η identified from the present study modulate the functions of this versatile TLS polymerase.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

■ ACKNOWLEDGMENTS

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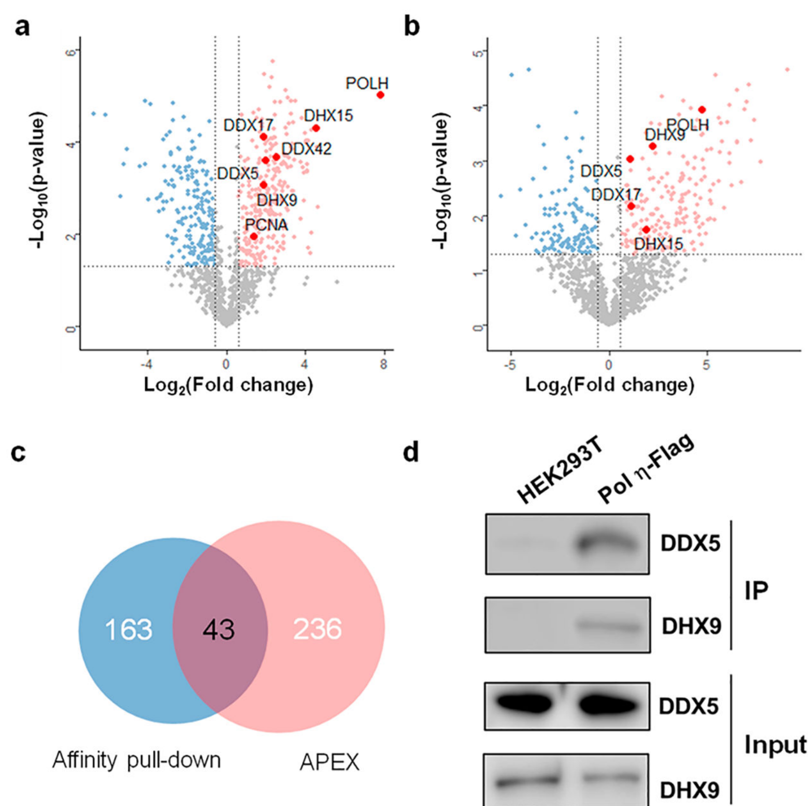

Figure 1.

APEX labeling and affinity pull-down for revealing the Pol *η* interactome. (a, b) Volcano plots displaying enriched proteins (highlighted in red) for Pol *η*-APEX versus the control (NLS-APEX) obtained from APEX labeling and LC-MS/MS analysis (a) or anti-Flag pull-down using CRISPR-engineered Pol *η*-Flag cells versus parental HEK293T cells (b). Proteins with *a* > 1.5-fold signal over control and a *p*-value < 0.05 are considered enriched. (c) Venn diagram illustrating the candidate Pol *η*-interaction proteins obtained from the two proteomic approaches. (d) Western blot results showing Flag pull-down using lysates of Pol *η*-Flag cells and parental HEK293T cells.

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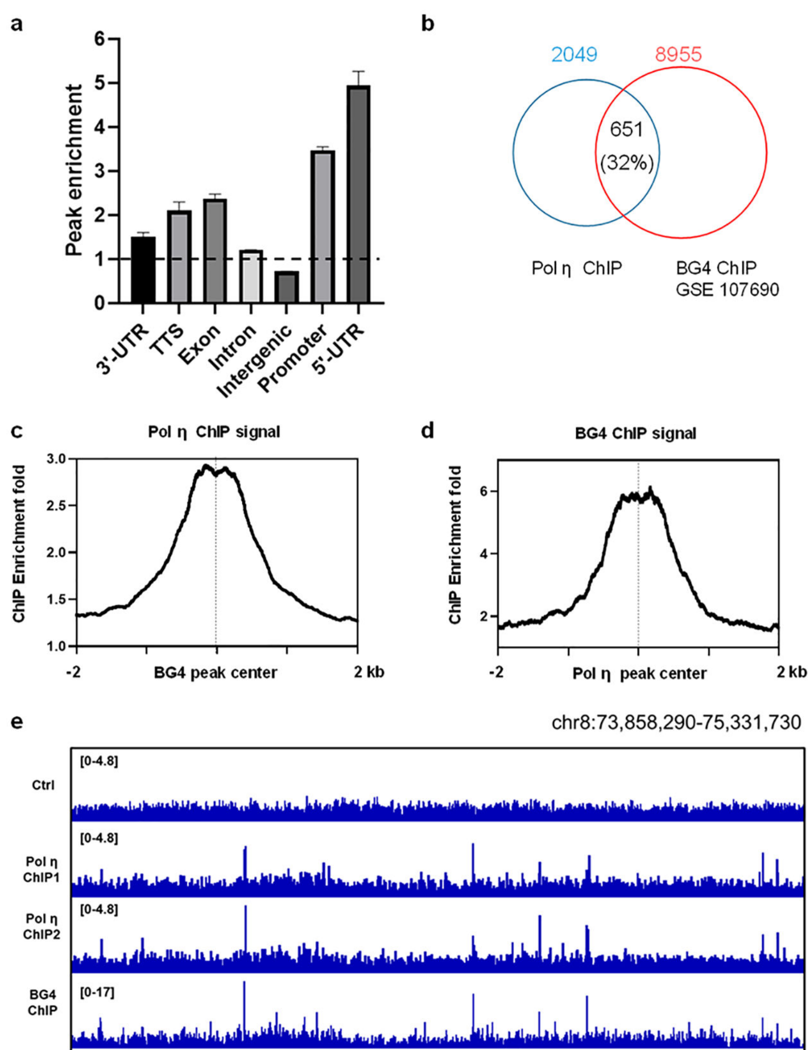

Figure 2.

ChIP-Seq revealed enrichment of Pol η at G4 structure loci in chromatin. (a) Relative enrichment of Pol η peaks in different genomic elements. The dashed line represents an enrichment fold of 1.0. Error bars represent standard deviations from two biological replicates. (b) Venn diagram showing the overlap between Pol η ChIP-seq and BG4 ChIP-Seq peaks. (c) Average signal of Pol η ChIP-Seq against the center of the BG4 ChIP-Seq peaks and (d) vice versa. (e) IGV plots showing the comparison of Pol η occupancy and G4 structure loci in the indicated genomic region.

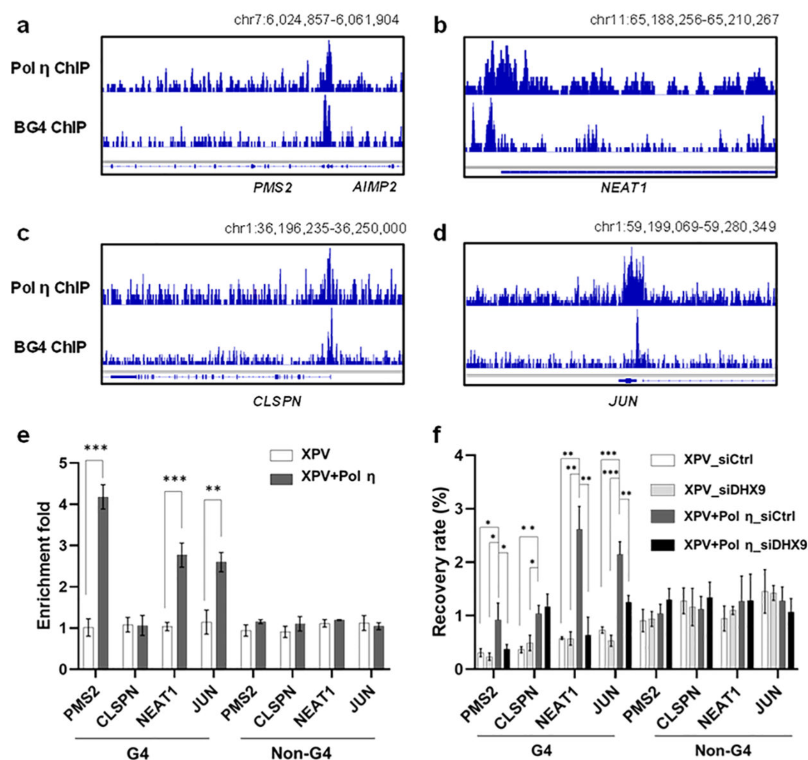

Figure 3.

Pol η recruits DHX9 to promote the replication across G4 structure sites in DNA. Representative IGV plots showing signal tracks for Pol η and BG4 ChIP-Seq results for *PMS2* (a), *NEAT1* (b), *CLSPN* (c), and *JUN* (d) genes in HEK293T cells. (e) ChIP-qPCR analyses at the G4/non-G4 loci using DHX9 antibody. (f) Amount of BrdU incorporation at the G4/non-G4 regions of the indicated genes with or without knocking down DHX9 in XPV and XPV + Pol η cell lines. The data represent mean ± SD from three independent experiments. The p values were calculated by using two-tailed, unpaired Student's t-test: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

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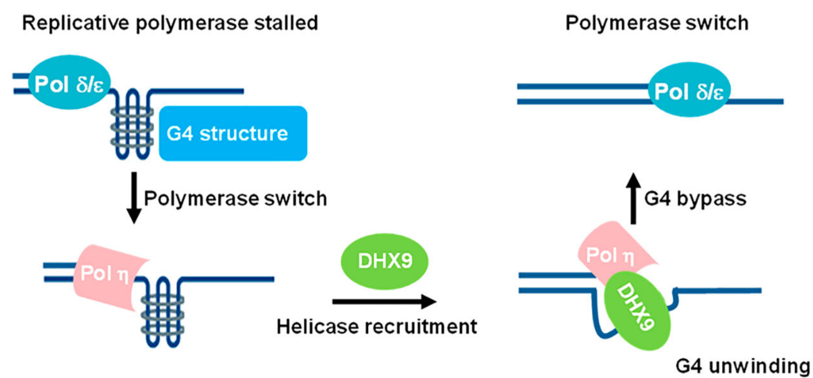

Figure 4.

Model of Pol η's function in promoting replicative bypass of G4 structures through DHX9.