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Library Wars: Love & War, Vol. 12 by Kiiro Yumi

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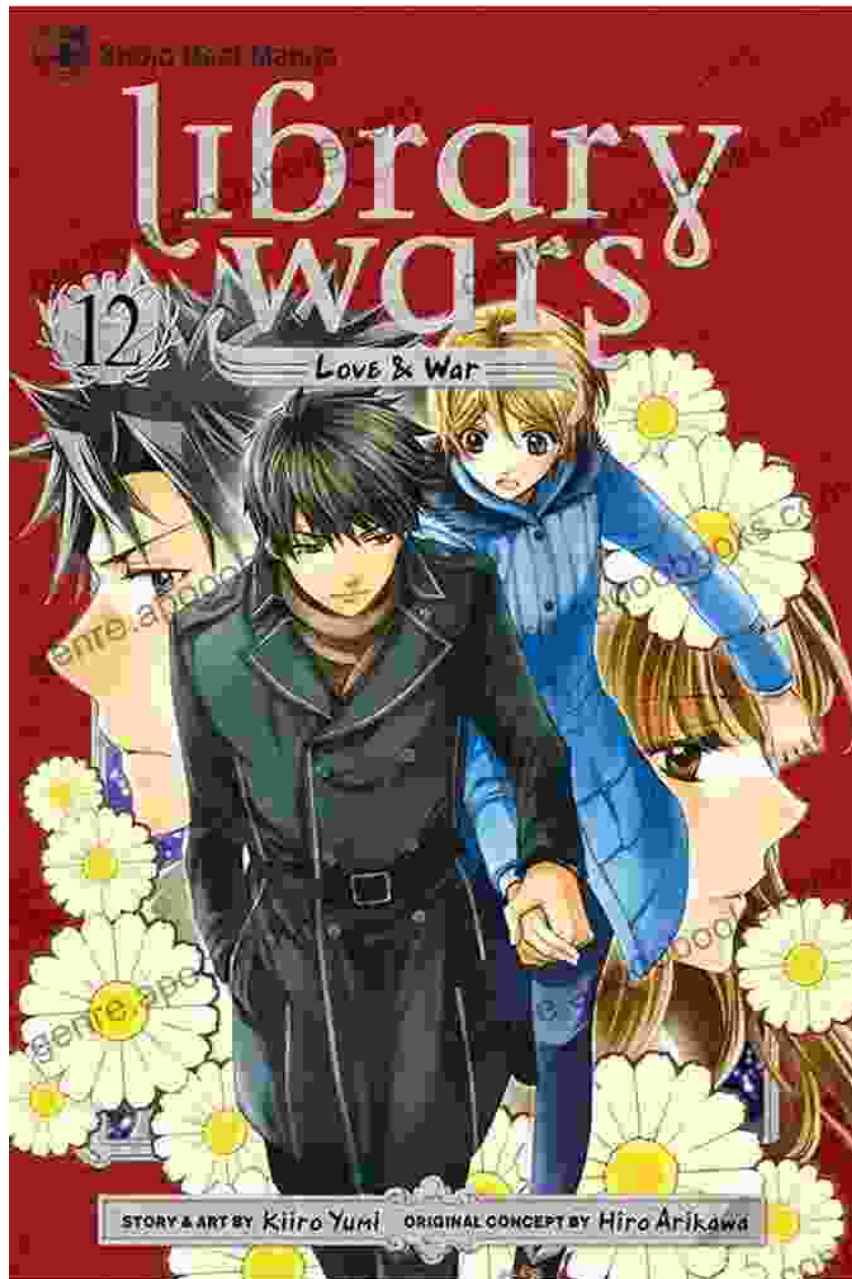
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Delve into the Heart-Pounding Finale

Volume 12 transports readers to the war-torn world of "Library Wars," where the Library Defense Force faces off against the oppressive Media Control Bureau. At the heart of this conflict is Iku Kasahara, a passionate librarian who dreams of a world where books are cherished. As the battle

intensifies, Iku finds herself torn between her love for the dashing Tatewaki Kusakabe and her unwavering commitment to protecting the written word.



Follow Iku Kasahara's captivating journey in Volume 12.

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This concluding volume delves deeper into the enigmatic Third Division, a covert unit tasked with unconventional missions. Led by the enigmatic Lieutenant Colonel Shibazaki, the Third Division is involved in a clandestine operation that could shift the balance of power in the war. As Iku and Tatewaki navigate the complexities of this operation, they uncover secrets and face unexpected challenges that test their resolve.

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Genetics

A phenotype for enigmatic DNA polymerase II: A pivotal role for pol II in replication restart in UV-irradiated *Escherichia coli*

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ABSTRACT DNA synthesis in *Escherichia coli* is inhibited transiently after UV irradiation. Inhibitory response reactivation or “replication restart” occurs shortly thereafter, allowing cells to complete replication of damaged genomes. At the present time, the molecular mechanism underlying replication restart is not understood. DNA polymerase II (pol II), encoded by the *dnaE* (*polB*) gene, is believed as part of the global SOS response to DNA damage; thus we show that pol II plays a pivotal role in reinitiating DNA replication in cells exposed to UV irradiation. There is a 30-min delay in replication restart in a mutant with a lacking pol II. Although replication restart appears normal in *delta-DNA* strains containing pol II, the restart process is delayed for >90 min in cells lacking both pol II and *E. coli* C. Because of the presence of pol II, a global replication-restart event is observed in a “quick-start” temperature-sensitive pol III mutant using *phi*80 phage as template. However, complete recovery of DNA synthesis requires the concerted action of both pol II and pol III. Our data demonstrate that pol II and *E. coli* C are in independent pathways of replication restart, thereby providing a phenotype for pol II in the repair of UV-damaged DNA.

After UV irradiation, *E. coli* cells are inhibited by the absence of both the ϕ 80 gene of *Escherichia coli* as regulated at the transcriptional level by the *lexA* repressor (1–4) and is induced to 7-fold from 10 min to 20 min per damaged cell (5). Although pol III has the major role in DNA synthesis (6), pol II also has been shown to functionally substitute for pol III (7, 8), which carry out clearly defined roles in DNA repair and replication, respectively (9).

Recent studies reveal that pol II may be involved in repairing DNA damaged by UV irradiation (9) or mutation (10). In hypothesis, after lesion is seen at the absence of host check induction (11) and in the repair of *delta-dnaE*-*delta* DNA (12), it has been shown that pol II catalyzes apical DNA synthesis (13, 14) and synthesizes chromosomal DNA by a pol III intermediate (15, 16, 17). Pol II also is involved in catalytic protein synthesis in vivo in the presence of phishing clamp and clamp-loading complex, resulting in only for pol II in replication and repair (18, 19).

After UV irradiation, a replication fork may become stalled when encountering a DNA triple-helix lesion (20). In this report, we report that pol II plays a pivotal role in replication restart (21). A temperature-sensitive reactivation (22), a process involving “reactivation” of DNA synthesis on UV-damaged DNA starts before an event in the reinitiation pathway. Our data provide a well defined role for pol II in reinitiating UV-damaged chromosomal DNA synthesis.

The authors thank the members of the Goldberg laboratory for their generous gifts of *phi*80 phage. This work was supported by the National Institute of Health (NIH) Grant GM 47714 (M.F.G.) and the National Science Foundation Grant IBN 9506024 (M.F.G.).

MATERIALS AND METHODS

Bacterial Strains and Growth Conditions. The *E. coli* K-12 strains used in this study are listed in Table 1. To avoid complications arising from the use of non-optimal strains, we derived the previously described *polB* (10) and *dnaE* (23) null mutants into the commonly used K-12 laboratory strain AB1157, by standard methods of P1 transduction (24). The presence of the *delta-DNA* allele was detected on LB agar plates containing 20 μ g/ml spectinomycin and was confirmed by PCR analysis (see below). Use of higher concentrations of spectinomycin were avoided because they resulted in colonies with aberrant cell morphology consistent with the acquisition of additional chromosomal mutations in *polA* (25). In these sections, we refer to the *delta-DNA* *polB* allele as *delta-DNA* (*delta*-DNA) or simply *delta*-DNA instead of LB agar plates containing 20 μ g/ml chloramphenicol, and *delta*-DNA strain was confirmed by PCR analysis (see below). Again, cell morphology was used to the *delta-DNA* allele as *delta*-DNA. The temperature-sensitive *polB* allele was identified from the appropriate *delta*-DNA selection for the check-point mutation (*polB* temperature-sensitive) and verified in a *delta*-DNA background. All media and supplements were prepared as described (24), with 1% thymine (0.05%) and supplemented with thymine (2 μ g/ml), 0.1 M MgSO₄, and minimal amino acids (20 μ g/ml, 100 μ M α -ketoglutarate). Antibiotic concentrations were as follows: spectinomycin (20 μ g/ml), streptomycin (10 μ g/ml), chloramphenicol (20 μ g/ml), and tetracycline (15 μ g/ml).

Colony PCR Assay to Test for *Delta*-DNA and *Delta*-DNA Genotypes. Although both *delta*-DNA (*polB*) and *delta*-DNA (*polB*) null mutants alleles were constructed by using standard gene insertion techniques and can be simply selected directly by using the appropriate antibiotic resistance, the presence of each allele was confirmed by colony PCR. In both cases primers were designed to amplify to the insert and flanking sequence. A PCR product is obtained, therefore, only if the insert is in the correct chromosomal location. The primers used to detect the *delta*-DNA allele were 5'-TCTGTGCTGACCTGTCGAAGCA-3' for the *delta*-DNA primer and 5'-CCGACGGGATCAATCAGAAGGTG-3' for the *delta*-DNA primer. The primer pair used to detect the *delta*-DNA allele were 5'-GGTTCAGGTGAGCACAAGATAGAG-3' and 5'-TTTAAAGGATGATCAAGCAGTCAAGT-3' for the *delta*-DNA primer. We did not require the absence of pol II and *E. coli* C in *delta*-DNA and *delta*-DNA strains, respectively, by

the presence of pol II in strains with *delta*-DNA plasmids (26, 27, 28). The authors thank Dr. Jonny M. Aldrich for his generous gifts of *phi*80 phage. This work was supported by the National Institute of Health (NIH) Grant GM 47714 (M.F.G.) and the National Science Foundation Grant IBN 9506024 (M.F.G.).

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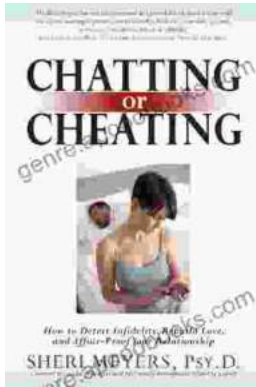
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