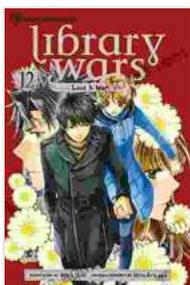


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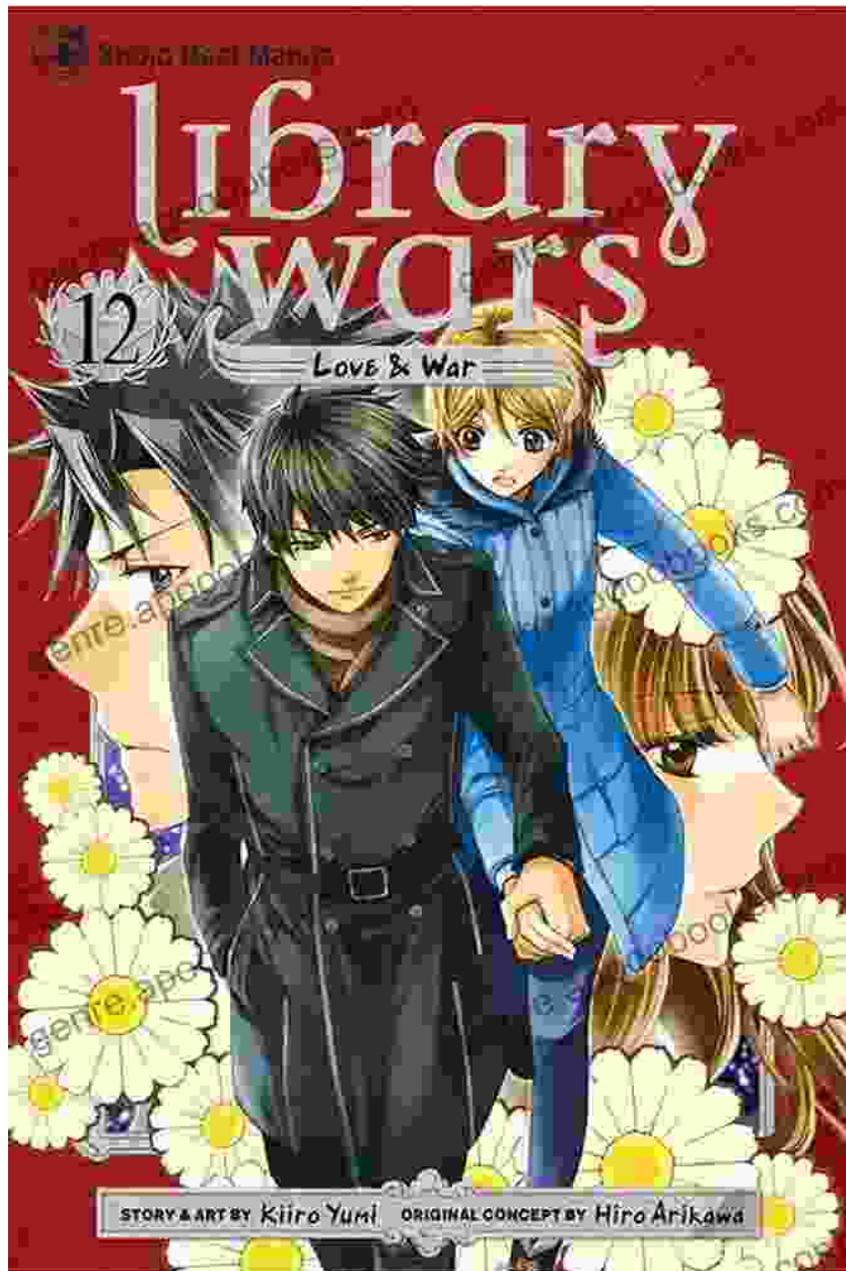
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Proc. Natl. Acad. Sci. U.S.A.
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Genetics

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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Contributed by P. Leder, M. Hsiao, and R. Losick, The Jane Goodall Institute, New Haven, Connecticut, and the Department of Biology, University of California, San Diego, La Jolla, California 92092

ABSTRACT DNA synthesis in *Escherichia coli* is inhibited transiently after UV irradiation. Inherent replication 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 *dnaII* (*polB*) gene, is inhibited as part of the global SOS response to DNA damage; thus we show that pol II plays a pivotal role in re-initiating DNA replication in cells exposed to UV irradiation. There is a 50-min delay in replication restart in mutant cells 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 *hmuD* (*hmuD*⁻). Because of the presence of pol II, a global replication-restart event is observed in a "quick-start" temperature-sensitive pol III mutant using 40°C as permissive temperature. However, complete recovery of DNA synthesis requires the concerted action of both pol II and pol III. Our data demonstrate that pol II and *hmuD* act in independent pathways of replication restart, thereby providing a phenotype for pol II in the repair of UV-damaged DNA.

DNA polymerase II (pol II), encoded by the *dnaII* (*polB*) gene of *Escherichia coli*, is regulated at the transcriptional level by the LexA repressor (1,2) and is induced in *hmuD* from \sim 20-min SOS response per damaged cell (3). Although pol II has also been reported to have intracellular functions other than those of a replisome (4,5), it has remained an enigma, in contrast to polymerases I and III, which carry out clearly defined roles in DNA repair and replication, respectively (6).

Recent studies reveal that pol II may be involved in repairing DNA damaged by UV irradiation (7) or oxidation (8). In hypoxic cells, transient arrest of the advance of host-chick induction (9) and in the repair of *hmuD* mutation-damaged (10). It has been shown that pol II catalyzes apical DNA synthesis (11,12) and synthesizes chromosomal DNA in a pol III mutant strain (*hmuD*⁻) (13) and (14). Pol II pol II is essential for protein synthesis in vivo in the presence of a plating clump and clamp-loading γ -complex. A cloning vector for pol II is replication and transmissible (15).

After UV irradiation, a replication fork may become stalled when encountering a DNA template lesion (16). In this report, we report that pol II plays a pivotal role in replication restart (17). A temperature-sensitive reactivation (18–22), a process referred to as "replication restart" of DNA synthesis on UV-damaged DNA, allows lesion bypass or occurs in an *hmuD*⁻ pathway. Our data provide a well-defined role for pol II in re-initiating UV-damaged chromosomal DNA synthesis.

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MATERIALS AND METHODS

Bacterial Strains and Growth Conditions. The *Escherichia coli* 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 generated *polB* (10) and *hmuD* (23) mutant strains 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 abnormal size morphology consistent with the acquisition of additional chromosomal mutations in *polA* (25). In most instances, we refer to the *delta-DNA* allele as *delta-DNA* (26) or *delta-DNA* (27) or *delta-DNA* (28) or *delta-DNA* (29) or *delta-DNA* (30) or *delta-DNA* (31) or *delta-DNA* (32) or *delta-DNA* (33) or *delta-DNA* (34) or *delta-DNA* (35) or *delta-DNA* (36) or *delta-DNA* (37) or *delta-DNA* (38) or *delta-DNA* (39) or *delta-DNA* (40) or *delta-DNA* (41) or *delta-DNA* (42) or *delta-DNA* (43) or *delta-DNA* (44) or *delta-DNA* (45) or *delta-DNA* (46) or *delta-DNA* (47) or *delta-DNA* (48) or *delta-DNA* (49) or *delta-DNA* (50) or *delta-DNA* (51) or *delta-DNA* (52) or *delta-DNA* (53) or *delta-DNA* (54) or *delta-DNA* (55) or *delta-DNA* (56) or *delta-DNA* (57) or *delta-DNA* (58) or *delta-DNA* (59) or *delta-DNA* (60) or *delta-DNA* (61) or *delta-DNA* (62) or *delta-DNA* (63) or *delta-DNA* (64) or *delta-DNA* (65) or *delta-DNA* (66) or *delta-DNA* (67) or *delta-DNA* (68) or *delta-DNA* (69) or *delta-DNA* (70) or *delta-DNA* (71) or *delta-DNA* (72) or 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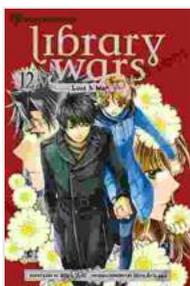
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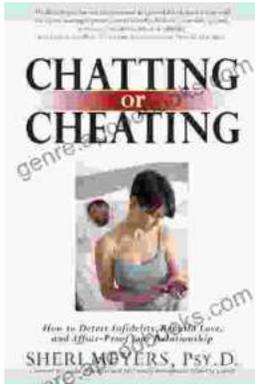
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