Zvi Livneh

Sheera Adar
Gali Arad
Sharon Avkin
Shay Covo
Dalia Elinger
Moshe Goldsmith
Lior Izhar
Ayelet Maor-Shoshani
Tamar Paz-Elizur

Molecular mechanisms of DNA repair and mutagenesis: From the origins of cancer to the basis of evolution

Department of Biological Chemistry

Tel. 972 8 934 3203 **Fax.** 972 8 934 4169
E-mail: zvi.livneh@weizmann.ac.il

Genomic DNA is constantly damaged by external agents such as sunlight and by intracellular agents. In order to enable proper function of DNA replication and gene expression, all organisms utilize DNA repair mechanisms. Failure to repair DNA can cause severe biological consequences, including cancer, immunodeficiency, premature aging, and neurodegeneration. DNA lesions that have escaped repair are tolerated by translesion replication, also termed bypass synthesis or error-prone DNA repair. This reaction is carried out by a novel class of specialized DNA polymerases, discovered in 1999 in our and in other laboratories. In addition to their ability to replicate across DNA lesions, these polymerases are characterized by a high propensity to form mutations. The research in our laboratory focuses on the mechanism of translesion replication with emphasis on the novel DNA polymerases, and on error-free DNA repair mechanisms and their involvement in cancer.

A novel family of highly mutagenic DNA polymerases, specialized for replicating across DNA lesions

Mutagenesis by DNA damaging agents in E. coli is regulated by the SOS stress response. The in vitro reconstitution of SOS mutagenesis with purified components in our lab led us to the discovery of a novel DNA polymerase, termed DNA polymerase V, which has a remarkable ability to replicate across lesions that block other DNA polymerases. Pol V was shown to be highly mutagenic when replicating undamaged DNA, with a particularly high level of transversions. Biochemical and electron microscopy analysis has shown that the assembly of a RecA-DNA filament is essential for the activity of pol V. Analysis of MucB, a homolog of pol V, has shown that it is also a DNA polymerase, termed DNA polymerase RI, specialized for lesion bypass. Pol RI is encoded by the native conjugative plasmid R46, which has a broad host range specificity, and carries multiple antibiotics resistance genes. This raises the possibility that mutagenesis caused by pol RI plays a role in the spreading of antibiotics resistance among bacterial pathogens.

Mammalian translesion replication

Four homologues of UmuC were discovered in humans. Translesion replication by these, and by other DNA polymerases, is likely to play an important role in cancer. Mammalian

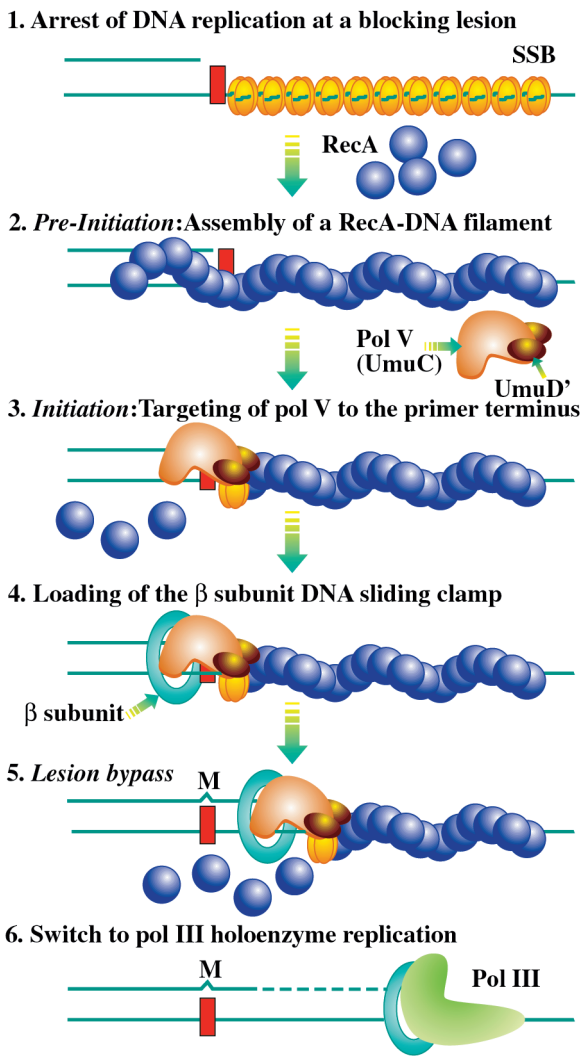

Fig. 1 *Model of SOS mutagenesis: translesion replication by DNA polymerase V. The two DNA strands are presented as green lines, and the replication-blocking lesion is shown as a red rectangle.*

translesion replication is studied using two approaches: In vitro analysis with purified mammalian DNA polymerases, and in vivo analysis, using cells in culture. A quantitative assay for translesion replication in cultured cells was developed. The assay is based on the transient transfection of cultured cells with a gapped plasmid, carrying a site-specific lesion in the gap region. Using this method it was found that translesion replication through a synthetic abasic site in several cell lines was high, up to 92%. Somewhat surprisingly, this effective lesion bypass required a replicative DNA polymerase, but did not requires the bypass-specific DNA polymerase eta, as shown with cell lines from xeroderma pigmentosum variant patients.

DNA repair as a risk factor in sporadic human cancer

DNA repair has emerged in recent years as a critical factor in cancer pathogenesis, as a growing number of cancer predisposition syndromes were shown to be caused by mutations in genes involved in DNA repair and the regulation of genome stability. These include the XP genes, DNA mismatch repair genes, the breast cancer BRCA1 and BRCA2 genes, and p53, a master regulator of cellular responses to DNA damage. However, it is not clear whether inter-individual variations in DNA repair among healthy people affect their cancer susceptibility. We are testing this hypothesis by examining whether a reduced capacity to repair a particular type of DNA damage, 8-oxoguanine, is a risk factor in sporadic human cancer.

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