Video Article

Visualization of UV-Induced Replication Intermediates in Escherichia coli Using Two-Dimensional Agarose-Gel Analysis

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Abstract

Inaccurate replication in the presence of DNA damage is responsible for the majority of cellular rearrangements and mutagenesis that are observed in all cell types and is widely believed to be directly associated with the development of cancer in humans. DNA damage, such as that induced by UV irradiation, severely impairs the ability of replication to copy the genomic template. Significant advances have been made to identify the gene products that are required when replication encounters DNA lesions in the template. However, the cellular mechanisms by which these lesions are processed during replication in vivo has remained a critical but challenging problem to address experimentally. Using Escherichia coli as a model system, we describe a procedure in which two-dimensional agarose-gel analysis can be used to identify the structural intermediates that arise on replicating plasmids in vivo following UV-induced DNA damage. The procedure has been used to demonstrate that replication forks blocked by UV-induced damage undergo a transient reversal that is stabilized by RecA and several genes associated with the RecF pathway. The technique demonstrates that these replication intermediates are maintained until a time that correlates with when the damage is repaired by nucleotide excision repair and replication resumes.

Disclosures

No conflicts of interest declared.