Regulation of RecA-dependent homologous recombination by 3'-5' exonucleases and the UvrD helicase in Escherichia coli K-12

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Abstract

Homologous recombination is generally considered a major mechanism by which cells repair many types of DNA lesions and damaged replication forks. However, if this process is left unchecked, cells often show a hyper-recombination (hyper-rec) phenotype, and are susceptible to large deletions, duplications, or inversions of important genetic information.^ This dissertation describes two projects aimed at examining molecular mechanisms by which cells regulate homologous recombination. The first shows several 3'-5' exonucleases prevent RecA-GFP loading by destroying potential substrates. It is shown that two genetic pathways exist: one consisting of ExoIII and another comprised of ExoVII, ExoIX, ExoX, and ExoXI. ExoI acts upstream of both of these pathways. Although *xthA* cells have an increase in DSBs and *recB*-dependent loading of RecA-GFP, they are viable with a *recB* mutation and do not display a large increase in SOS expression. The increase in RecA-GFP is also independent of base excision repair (BER). These experiments uncovered that DNA in a population of wild type cells undergoes DSBs and is often repaired in a RecA-independent manner after processing by ExoI and ExoIII. ^ The second project shows the helicase, UvrD limits the number and intensities of RecA-GFP foci. This activity is due to the ability of UvrD to remove RecA from DNA where it is loaded in a RecF pathway-dependent manner. This activity requires ATP binding by UvrD, suggesting that helicase/translocase activity is important for RecA-removal. The hyper-helicase mutation, *uvrD303* confers UV sensitivity to cells. Epistasis analyses showed *uvrD303* is defective in the *recA* pathway of UV repair and not in nucleotide excision repair (NER). Surprisingly, UvrD303 does not directly remove RecA after UV, as new RecA-GFP foci appear like in wild type cells. UvrD303 does, however, slightly inhibit SOS induction, and constitutively activating the SOS response restores UV resistance to these cells in a way that is independent of *recA* overexpression. Furthermore, *uvrD303* was capable of suppressing the constitutive SOS phenotype of *recA730.* These experiments suggested that UvrD303 antagonizes the ability of RecA filaments to induce the SOS response, rendering cells UV sensitive.^

Subject Area

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