University of South Carolina
Department of Biological Sciences

Doctoral Dissertation Defense

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"Mismatch tolerance during homologous recombination in mammalian cells"

Abstract:
Homology dependency of recombination was examined in cultured thymidine kinase-deficient mouse fibroblasts. Cells have chromosomal integration of DNA constructs harboring a herpes tk gene (the "recipient") and a closely linked truncated "donor" tk sequence. The recipient was rendered non-functional by insertion of the recognition site for endonuclease I-SceI, and the donor sequence could restore the function of recipient through spontaneous gene conversion or via recombinational repair provoked by a double-strand break (DSB) at the I-SceI site. Recombination events were recoverable by HAT selection for tk-positive clones. Three different donor sequences contained 16, 25, or 33 mismatches relative to the recipient, and these mismatches were clustered within these "homeologous" sequences surrounded by region of high homology. Previous work indicated that mammalian cells fastidiously avoid recombination between homeologous sequences, while our results revealed that when homeologous sequences are surrounded by high homology, mismatches are frequently included in gene conversion events. Knock-down of DNA mismatch repair provided evidence that incorporation of mismatches into gene conversion tracts involved repair of mismatched heteroduplex intermediates. Our results demonstrate that mismatch repair of multiple mispaired bases does not function to impede exchange between homeologous sequences. Moreover, gene conversion tracts from spontaneous recombination showed that either all or none of the mismatches were transferred from donor to recipient, suggesting that recombination must begin and end in high homology. But this requirement was somewhat relaxed for DSB-induced events. To address the relaxed homology requirement in DSB-induced events, further experiments with rearranged construct were attempted to collect and analyze recombinant clones solely from double Holliday junction resolution. Apart from the study on homology requirement of recombination, research works were also carried out to characterize the impacts of RecQ Helicases on DSB repair and the choice among different pathways with preliminary data obtained.

Seminar:
Tuesday – March 28, 2017
11:30 AM – 12:30 PM
Location: GSRC 110