Mismatch repair in heteroduplex DNA intermediates of homologous recombination in Chinese hamster ovary cells.

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Abstract

Repair of single-base mismatches formed in recombination intermediates in vivo was investigated in Chinese hamster ovary cells. Extrachromosomal recombination was stimulated by double-strand breaks (DSBs) introduced into regions of shared homology in pairs of plasmid substrates heteroallelic at 11 phenotypically silent mutations. Recombination was expected to occur primarily by single-strand annealing, yielding predicted heteroduplex DNA (hDNA) regions with three to nine mismatches. Product spectra were consistent with hDNA only occurring between DSBs. Nicks were predicted on opposite strands flanking hDNA at positions corresponding to original DSB sites. Most products had continuous marker patterns, and observed conversion gradients closely matched predicted gradients for repair initiated at nicks, consistent with an efficient nick-directed, excision-based mismatch repair system. Discontinuous patterns, seen in approximately 10% of products, and deviations from predicted gradients provided evidence for less efficient mismatch-specific repair, including G-A-->G-C specific repair that may reflect processing by a homologue of Escherichia coli MutY. Mismatch repair was > 80% efficient, which is higher than seen previously with covalently closed, artificial hDNA substrates. Products were found in which all mismatches were repaired in a single tract initiated from one or the other nick. We also observed products resulting from two tracts of intermediate length initiated from two nicks.