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DNA polymerase δ, RFC and PCNA are required for repair synthesis of large looped heteroduplexes in *Saccharomyces cerevisiae*

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ABSTRACT

Small looped mispairs are corrected by DNA mismatch repair (MMR). In addition, a distinct process called large loop repair (LLR) corrects loops up to several hundred nucleotides in extracts of bacteria, yeast or human cells. Although LLR activity can be readily demonstrated, there has been little progress in identifying its protein components. This study identified some of the yeast proteins responsible for DNA repair synthesis during LLR. Polyclonal antisera to either Pol31 or Pol32 subunits of polymerase δ efficiently inhibited LLR in extracts by blocking repair just prior to gap filling. Gap filling was inhibited regardless of whether the loop was retained or removed. These experiments suggest polymerase δ is uniquely required in yeast extracts for LLR-associated synthesis. Similar results were obtained with antisera to the clamp loader proteins Rfc3 and Rfc4, and to PCNA, i.e. LLR was inhibited just prior to gap filling for both loop removal and loop retention. Thus PCNA and RFC seem to act in LLR only during repair synthesis, in contrast to their roles at both pre- and post-excision steps of MMR. These biochemical experiments support the idea that yeast polymerase δ, RFC and PCNA are required for large loop DNA repair synthesis.

INTRODUCTION

DNA looped heteroduplexes (loops) arise in *vivo* as synthetic errors within microsatellites (1–4), minisatellites (3), or between tandemly repeated sequences (5). Loops are mutagenic precursors to insertion or deletion mutations, depending on whether the newly replicated or parental strand, respectively, contains the loop. The ability to correct loops prior to a subsequent DNA synthetic event helps avoid mutations. When cells lack such correction activity, insertion and deletion mutants occur at increased frequencies (3,6–10). For example, it is thought that small insertions and deletions in important tumor suppressor genes contribute to hereditary nonpolyposis colon cancer when mismatch repair (MMR) is defective (11). Loops are also formed during recombination between alleles that differ in length, and the correction of these recombination intermediates contributes to gene conversion (12). Recombination studies in yeast and mammalian cells indicate that loops up to several kilobases undergo efficient repair (12–17).

The repair of loops is complex. Depending on loop size, they can be excellent substrates for MMR. Small loops, from 1 to ~8 nt, undergo very efficient MMR (3,18–22). However, repair efficiency by this pathway decreases to very low levels as the loop size reaches ~16 nt in eukaryotes (3,23). Thus, MMR function cannot explain repair of loops >16 nt, nor does MMR correct all molecules with loops between ~8 and 16 nt (19,22–26). Instead, several activities are reported to repair large DNA loops (13–16,23,25–33). These activities differ in efficiency, genetic requirements and response to secondary structure within the loop.

One system that we call large loop repair (LLR) corrects loops up to several hundred nucleotides in size. There is indirect but compelling evidence that LLR may be quite similar in mammalian cells, in yeast and in bacteria. First, robust LLR has been observed in several laboratories using different approaches, such as in *vivo* assays (26–28) and biochemical assays using cell-free extracts (22,25,26,29,32–34). Second, while loops of ~8–16 nt are moderately good substrates for LLR (19,22,23,25), larger loops up to several hundred nucleotides are corrected even more efficiently (25–29,32–34). The upper size limit for efficient repair has not been firmly established. Third, LLR is independent of MMR and nucleotide excision repair, as mutant cell lines lacking these repair pathways retain LLR activity (22,23,25,29,32). Fourth, the presence of a nick in loop-containing substrates stimulates repair on the nicked strand (26,29,32–35). However, some studies also report loop removal on the continuous strand of nicked molecules and in covalently closed substrates (25,26,32, 33,35). Either a nick is not absolutely required in all cases, or spurious nicking promotes LLR. Fifth, LLR can repair looped substrates to either of two products, depending on the location of the loop and a nick (25–27,29,32). In one instance, the looped strand is used as a template for DNA

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