193 Analysis of X-Ray-Induced Mutation in Werner Syndrome Cells

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Werner syndome(WS) is a rare premature aging syndrome. Because the WS gene, *WRN,* is a member of the RecQ helicase family, it is assumed that WRNp functions as a caretaker of genomic integrity. Recently, it was reported that Ku86/70 complex interacts with WRNp. Therefore, we hypothesize that WRNp plays a role in the maintenance of repair fiedelity of DNA double-strand breaks. In this study, we examined X-ray-induced mutation frequency at *HPRT* locus in WS cells and analyzed the types of mutations by multiplex PCR. The results indicate that the mutation frequency in WS cells is similar to that in control cells and that the majority of mutants shows a deletion mutation, which is previously shown as one of the WS phenotypes. This suggests that the defect of WRNp is involved in the induction of large deletions initiated by DNA double-strand breaks.

194 **Retarded recovery of DNA replication in Bloom's syndrome fibroblasts following release from inhibition by hydroxyurea**

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Bloom's syndrome (BS) is a rare genetic disorder characterized by genomic instability. BLM gene that is defective in BS encodes a member of RecQ helicase family. Yeast cells bearing a mutation at SGS1, which encodes a helicase homologous to RecQ helicase, show an increased sensitivity to hydroxyurea (HU) in terms of the inhibition of cell growth. To examine the function of BLM helicase in human cells, BS and normal cells were treated with HU, and then cell growth and bromodeoxyuridine incorporation after release from the inhibition by HU were compared between the treated and untreated cells. The hypersensitivity of BS cells to HU for cell growth inhibition was clearer at low concentrations of HU and for a shorter exposure time. BS cells were intrinsically slower than normal cells in DNA replication rate throughout S phase. HU treatment retarded the progression of DNA replication in both BS and normal cells. However, normal cells recovered the progression of DNA replication immediately after release from HU inhibition, while BS fibroblasts did so far behind. Supposing that BS cells suffer from a leaky defect in BLM helicase, we would infer that the substrates (e.g., aberrant DNA structures) for BLM helicase are spontaneously generated and further increased by HU treatment during DNA replication.

195 End-joining Reaction of Double-strand Breaks in Plasmid DNA by the Nuclear Extract from Bloom Syndrome Cells

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Bloom syndrome (BS) is a rare autosomal recessive disorder characterized by severe pre- and postnatal growth deficiency, immunodeficiency, and a predisposition to a wide variety of neoplasms. The genomic instability is evidenced in BS somatic cells as a high incidence of chromosome aberrations and locus-specific mutations. Molecular analysis of spontaneous mutant clones with deficiency at the*HPRT* gene revealed a high frequency of large deletion mutations in BS cells. BS arises from a mutations in*BLM*, a gene encoding a protein with homology to the RecQ helicase family.

In order to clarify the role of the BLM helicase in illegitimate recombination, the end-joining reaction of double-strand breaks in DNA using*in vitro* system with nuclear extracts from BS cells was analyzed. Both the efficiency of end-joining and the frequency of mis-rejoining by the nuclear extract from a BS lymphoblastoid cell line was comparable to those by the normal cell extract. However, the size of deletions found in the mis-rejoined molecules by BS nuclear extract was much larger than that caused by normal extract. Sequencing analysis of mis-rejoined molecules also showed some characteristics of BS extract.

These results suggest an involvement of the BLM helicase in the genomic instability in BS cells caused in some part by illegitimate recombination.