Rad17 and Rad9 are essential for DNA replication and S-phase DNA damage checkpoint controls in higher vertebrate cells.

Atsushi Hirano, Tomoyasu Kumano, Masahiko Kobayashi, Shuang-Lin Xiang, Hiroto Ono, Osamu Matsui, and Ken-ichi Yamamoto

Cell-cycle checkpoints are surveillance mechanisms that monitor the cell cycle and protect genome integrity by inducing cell-cycle arrest or programmed cell death (apoptosis) in response to DNA damage or DNA replication errors. Because several features of cell cycle checkpoints have been conserved throughout evolution, information about cell cycle checkpoints learned from yeast provides a framework for developing a further understanding of the checkpoint pathways in higher eukaryotes (1, 2). Genetic studies in the fission yeast *S. pombe* have identified four key checkpoint molecules (Rad1, Hus1, Rad9, and Rad17) and mammalian homologs of these molecules have been identified. Rad17 is a protein related to replication factor C (RFC) containing Walker A-type and B-type nucleotide binding sites, and associates with four RFC small subunits, forming a pentameric complex. Rad1, Rad9, and Hus1 are all structurally related to PCNA and form a trimeric hetero-complex. It is therefore postulated that the Rad17-RFC complex recognizes certain DNA damage structures and recruits the Rad1-Rad9-Hus1 complex to DNA lesions, in a manner similar to the loading of the PCNA trimeric homo-complex on DNA by the RFC1-5 complex. To study how Rad17-RFC and Rad9-Rad1-Hus1 complexes function in DNA damage response in higher vertebrate cells, we generated Rad17- and Rad9-deficient DT40 cells by targeted disruption. We found that, while $Rad17^{-/-}$ and $Rad9^{-/-}$ DT40 cells were mildly sensitive to X-ray radiation, these cells were highly sensitive to UV irradiation, DNA alkylating agent MMS, and DNA replication inhibitor hydroxyurea (HU). We then analyzed the cell-cycle distributions by flow cytometry after these genotoxic treatments. After UV irradiation or MMS treatment, the rates of DNA replication became much slower, although not blocked, in wild-type and $ATM^{-/-}$ cells. However, $Rad17^{-/-}$ and $Rad9^{-/-}$ cells were defective in this UV- and MMS-induced DNA replication slowing. We further found that, while wild-type and $ATM^{-/-}$ cells were blocked at early S-phase when treated with HU, $Rad17^{-/-}$ and $Rad9^{-/-}$ cells were defective in the DNA replication checkpoint control. In addition, after HU treatment, most of these $Rad17^{-/-}$ and $Rad9^{-/-}$ cells underwent apoptosis. These results indicate that Rad17 and Rad9 are essential for the S-phase DNA damage and DNA replication checkpoint controls.