94 The dynamics of histone acetylase complex on DNA damaged repair

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Recently we present the evidence for novel roles of histone acetylases. The TIP60 histone acetylase is purified as a multimeric protein complex. This complex has a RuvB homologue which has a role in DNA recombinational repair in E. coli. Ectopic expression of mutated TIP60 lacking histone acetylase activity results in cells with defective double-strand DNA break repair. Importantly, the resulting cells lose their apoptotic competence, suggesting a defect in the cells' ability to signal the existence of DNA damage to the apoptotic machinery. These results indicate that the histone acetylase TIP60-containing complex plays a role in DNA repair and apoptosis. We present here that how the histone acetylase TIP60-containing complex is involved in DNA damaged repair. Recently, we purified the TIP60 complex after DNA damage and revealed that TIP60 complex come to have a g-H2AX after DNA damage. Interestingly we also cleared that histone acetylase of TIP60 complex is required for the phosphorylation of H2AX.

95 Analysis of Damaged Base Recognition by DNA Polymerases using Atomic Force Microscopy

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DNA replication stalls at lesions such as pyrimidine dimers in a template strand. DNA polymerases from hyperthermophilic archaea recognize the presence of template uracil and stall DNA synthesis. To analyze the recognition mechanism, the binding mode of DNA polymerase B1 of *Sulfolobus solfataricus* (Pol B1) to uracil-containing DNA was examined by gel-shift assay and atomic force microscopy (AFM). Pol B1 tightly and specifically bound to uracil-DNA and retarded the mobility of DNA on agarose gel. When primer/template DNA was incubated with Pol B1 plus dNTPs, the presence of template uracil significantly inhibited the formation of double-stranded DNA. In the AFM image, single-stranded and double-stranded DNAs were observed as sphere and linear forms, respectively. Omission of dNTPs and primer from the reaction completely blocked the formation of double-stranded DNA and inhibited the formation of intermediates where Pol B1 appeared to bind to uracil-DNA. These results suggest that Pol B1 more efficiently recognizes template uracil by processive sliding on template DNA rather by random diffusion-mediated mechanism.

96 The cooperative role of DNA-PK and ATM protein in control of G2-M transition check point mechanism.

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When cells were exposed to ionizing irradiation, the cells were arrested and do not enter in mitosis. Otherwise, cells carrying unrepaired or misrepaired DNA damages might be lethal or prone to cancer. Recently accumulating evidences have been supported that DNA-PK and ATM play an important role in this mechanism, but it is still unclear how these proteins works cooperate. Using the ATM mutated cells AT5BISV and LY 294002, a specific inhibitor of DNA-PK, we investigated the role of DNA-PK and ATM in radiohypersensitivity and control of cell cycle check point following irradiation. Colony Surviving assay showed that 50 uM of LY294002 made AT5 cells 2 order hypersensitive to irradiation. AT5 cells in G2/M phase were accumulated of 60% of total, but more than 10% cells were escaped from G2 and reached in mitosis. The mitotic chromosomes of AT5 were quite badly damaged, whilst those of control cells were almost repaired. These findings suggested that DNA-PK and ATM cooperatively control DNA repair machinery and cell cycle check point regulation, particularly G2-M transition control.

97 A Role of WRN Protein in the Recovery from DNA Replication Arrest

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Werner syndrome (WS) is a disorder with multiple features of premature aging. The responsible WRN gene encodes a protein possessing DNA helicase and exonuclease. To examine the differential function of helicase and exonuclease of WRN protein, we investigated cell cycle progression after treatment with hydroxyurea (HU) in a WS cell line (WS780) and a human cell line (293delta231) defected in a WRN exonuclease activity. The results indicated that WS780 cells were highly sensitive to HU treatment, showing a more delayed recovery from the DNA replication (S) arrest than a control GM638 cell line. In contrast, the WRN exonuclease defective cell line (293delta231) showed no difference in the recovery from the S arrest as compared with a control 293 cell line. The present results suggest that WRN protein play a role in the recovery from the S arrest although WRN exonuclease is not involved in this process.