Repair of radiation damage

72 The role of TIP60 histone acetylase complex in radiation induced DNA damaged repair

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Recently we present evidence for novel roles of histone acetylases. The TIP60 histoneAcetylase purifies as a multimeric protein complex. This complex has a RuvB homologue which has a role in DNA recombinational repair in Ecoli. Ectopic expression of mutated TIP60 lacking histone acetylase activity results in cells with defective double-strand DNA break repair. Importantly, the resulting cells loose 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. However, the mechanism of TIP60 complex in DNA damaged repair remains unknown. On the other hand, it has been reported that Histone H2AX is phosphorylated after g-irradiation. Based on these evidences, we try to analyze t he relationship between acetylation of TIp60 complex and the phosphorylation of H2AX to clarify the role of TIP60 complex in DNA repair.

73 X-rays-induced phosphorylation of H2AX in mitotic cells

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H2AX, which is a H2A subfamily and a component of histone-core, is phosphorylated in response to ionizing radiation. Phosphorylation was detected immediately after irradiation, however, the biological significance of H2AX phosphorylation is not clear. We studied here the localization of phosphorylated H2AX at chromosomal breaks or its repaired sites in mitotic cells. Normal human diploid cells were irradiated with 4 Gy of X-rays, which make survival to 10%. Phosphorylated H2AX formed punctated foci, and the number of foci decreased thereafter. In metaphase cells, phosphorylated H2AX foci were detected on chromosome fragments as well as on chromosomes without detectable aberrations. Phosphorylated H2AX foci were also observed on chromosome bridge between dividing chromosomes in anaphase cells.These results indicate that H2AX is phosphorylated in response to DNA double strand breaks, but phosphorylated foci persists on chromosome whose breaks are repaired. It is suggested that phosphorylation of H2AX plays a role in chromatin reorganization, after DNA breaks are repaired.

74 Histone H2AX regulates the formation of NBS1 foci on DNA damage sites

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Once DNA damage is induced by irradiation or errors during DNA replication, the cells sense DNA damage, after then the damaged chromatin is remodeled and DNA damage is repaired. The recruitment of DNA repair-related factors to DNA damge sites is required to start DNA repair, but the mechanism of this recruitment is unclear. To clarify this mechanism we investigated the interaction between NBS1 and histone H2AX after DNA damage. Histone H2AX was phosphorylated and formed foci at early time after irradiation in both normal and NBS cells. NBS1 colocalized with phosphorylated H2AX on DNA damage sites and was immunoprecipitated with phosphorylated H2AX. These results suggests that the interaction between H2AX and NBS1 regulates the recuritment and focus formation of NBS1 complexes on DNA damage sites.