171 Probing the Dynamics of AIM-1/Aurora-B kinase in X-irradiated Living Cells during Mitosis and Cytokinesis
AIM-1 is a protein kinase having a passenger function and forms a complex with Inner Centromere Protein (INCENP). AIM-1 phosphorylates INCENP, and then, the activated complex does phosphorylate H3 histone at the initiation of mitosis. When X-irradiated, mammalian cells suffer G2-arrest. It has been known that there are at least two distinct pathways to generate this arrest in mammalian cells. The pathways are ATM/NBS/BRCA/Cdc25C-dependent and -independent, and the latter is involved in AIM-1 kinase suppression which generates dephosphorylation of H3 histon at Ser10. Here we examined the in vivo dynamics of AIM-1 in X-irradiated cells, using green fluorescent protein (GFP)-tagged AIM-1. The data showed that the dynamics was perturbed in X-irradiated mitotic cells and was similar to a behavior in cells expressing a kinase negative form, suggesting that impaired function of the AIM-1-INCENP complex led mitotic failure after X-irradiation.

172 Cyclin G1 associates with MDM2 and regulates accumulation and degradation of p53 protein
Cyclin G1 is a transcriptional target of p53 and is induced by DNA damage in a p53 dependent manner. Analysis of cyclin G1 disrupted mice demonstrated that cyclin G1 is involved in many of the functions regulated by p53. The results suggest that the main role of cyclin G1 is to mediate or regulate the function of p53. Western blot analysis revealed that the accumulation of p53 protein during the initial 24 h period following DNA damage is reduced in cyclin G1–/– cells compared to wild type cells. This decrease in p53 accumulation could be recovered by introducing a cDNA expressing cyclin G1. Cyclin G1 directly interacted with MDM2 and promoted the formation of the ARF/MDM2 complex within the initial 24 h period following DNA damage. Furthermore, 48 h after irradiation, accumulation of p53 protein was enhanced in cyclin G1–/– cells compared to wild-type cells. In contrast, in 48 h post-irradiated wild-type cells, the cyclin G1-MDM2 complex was found not to be associated with ARF but with the B’a subunit of protein phosphatase A. These results suggest that cyclin G1 stabilizes and promotes the degradation of p53 protein by associating respectively with MDM2 complexes containing ARF and PP2A.

173 Mechanisms of induction of apoptosis by co-treatment of X-rays with ECyd in Chinese Hamster V79 Cells
1-(3-C-ethynyl-beta-D-ribo-pentofuranosyl)cytosine (ECyd) is a newly developed anti-tumor drug targeting RNA synthesis. we have reported that the X-ray-induced apoptosis is enhanced by low doses of ECyd and ECyd sensitizes X-ray-induced cell death in clonogenic assay. In this study, we examined whether this mechanism of apoptosis was linked to abrogation of arrest of cell-cycle progress. V79 cells were synchronized by the double thymidine block, and then treated with X irradiation and/or 1 micro M ECyd. Cell-cycle was observed by using flow-cytometry. When synchronized cells were exposed to X-rays only, G2/M arrest occurred. In co-treatment of X-rays with ECyd, G2/M fraction decreased and subG1 fraction increased. Western blotting showed that X-rays increased the expression of cyclin B, phospho-cdc2 and wee1, whereas co-treatment with X-rays and ECyd decreased expression of these signaling molecules associated with G2/M arrest. These results indicated that ECyd sensitized X-ray-induced apoptosis through abrogation of cell-cycle arrest by downregulation of cdc2/cyclin B/wee1.