3P260 イエロー蛋白質のM中間体から暗状態への回復過程の計算化学的研究
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Computational study of the recovery process from the M intermediate to the dark state in photoactive yellow protei
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Upon illumination of photoactive yellow protein (PYP), two major intermediates, L and M, are produced sequentially; the latter of which is a putative signaling state. In the final step of the photocycle, the original dark state is recovered from the M state. This process involves the protonation of chromophore's phenolic group and the cis-trans isomerization of its vinyl bond. In usual solution chemistry, the thermal isomerization of the C=C double bond is difficult to occur at room temperature. It is therefore likely that the above recovery process is caused by an unknown catalytic effect of the surrounding protein environment. In this study, to explore the recovery pathway, we carried out molecular dynamics (MD) and molecular orbital (MO) calculations for several possible models of the M intermediates, which were obtained from our previous multicanonical MD study for this intermediate. The MD simulation indicated that there is a correlated motion between the chromophore, Arg52 and Met100: the guanidium group of Arg52 approaches the above vinyl bond with a minimal distance of about 3 angstrom as the phenol OH of the chromophore is going away from the side chain of Met100. This suggests the possibility that the proton transfer may occur from the guanidium side chain to the vinyl bond, leading to the cleavage of the double bond. The MO calculation indicated that such a proton transfer easily occurs if the phenolic OH of the chromophore is dissociated. On the basis of these results, we propose a reaction scheme of the recovery step.

3P262 放射線感受性細胞を用いた放射線誘導性アポトーシス制御のクロストークの解析
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Analysis of cross-talk in radiation-induced apoptosis regulations using radiosensitive cells.
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To clarify network of radiation-induced apoptosis, analysis of cross-talk of the regulation was performed. Protein kinase cascades are known to regulate systems in radiation-induced apoptosis. In radiosensitive mouse thymic lymphomas, 3SBH5, Protein kinase C(PKC), a family of protein kinases, regulates the radiation-induced apoptosis. In this cell line, participants in the PKC-related radiation-induced apoptosis regulation were explored. In 3SBH5, after irradiation, a decreased protein was found by Western blot in immunoprecipitates with anti-PKC specific phospho-substrate antibody. The protein was analysed by LC-MS/MS, and Mascot analysis was tried for the identification. Some candidates were selected. Of them, HSC70 is reported to be related to PKC regulation and cell cycle regulation. Therefore, HSC70 is a candidate of the participants. XR223 is a radioresistant cell line, which is selected from 3SBH5. By comparative analysis by two-dimensional electrophoresis between the cell lines, a protein, which increased in 3SBH5, but decreased in XR223 after irradiation was detected. Furthermore, exploration of participants related to lipid mediator-related PKC cascades was performed. We demonstrated that alteration of PKC's regulation in 3SBH5 apparently renders the cells radiosensitive. Lipid mediator-related PKC's regulation might distinguish different radiosensitivity between the cell lines. Involvement of the proteins mentioned above in the radiation-induced apoptosis regulations will be discussed with other candidates.

3P261 放射光X線マイクロビームによるDNA損傷に対する細胞の応答
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3aD14 Cellular response to DNA damage induced by synchrotron X-ray microbeam
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Ionizing radiations efficiently induce DNA double-strand breaks (DSBs), which are one of the most lethal DNA damage. Nowadays, many DSB repair proteins have been known to form nuclear foci in the vicinity of DSBs. Imaging analysis of foci formation of DSB repair proteins, such as γ-H2AX, is one of the available methods to elucidate the mechanisms of DSB repair. In this study, we irradiated the targeted area of cell nuclei using synchrotron X-ray microbeam and observed the localization and phosphorylation of DNA repair and cell cycle checkpoint proteins by immunofluorescence. The co-localization of γ-H2AX and phosphorylated DNA-PKcs at Thr2609 was observed in the nuclei of HeLa cells irradiated with microbeam. In addition, localization of NBS1, 53BP1 and phosphorylated ATM at Ser1981 on the site of target irradiation could be observed predictably. Next we observed the localization of cell cycle checkpoint protein Chk1, Chk2 and SMC1. Phosphorylated Chk2 and SMC1, which contribute to the control of S/G2 checkpoint, are co-localized in the irradiated part of cell nuclei. On the other hand, phosphorylated Chk1 observed entire irradiated cell nuclei. Phosphorylated ATR at Ser428, which is one of the kinase of Chk1, also observed whole of irradiated cell nuclei. These results suggest that the response of DNA repair and cell cycle checkpoint proteins to DNA damage induced by synchrotron X-ray varies in the each process of DNA repair and/or genome surveillance

3P263 X線マイクロビームを用いたバystanダー細胞死の研究
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Bystander cell death in V79 cells irradiated with X-ray microbeams of different sizes
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Bystander effects are phenomena in which the biological effects appear in cells that have not been traversed by charged particles, but located nearby the cells actually traversed in low dose region. Mechanisms of the bystander effects are not yet clear, although the important role of intracellular and/or intercellular signaling is suggested. The microbeam cell irradiation system is a powerful tool to elucidate the mechanisms of the bystander effect, since we can observe individually cellular responses of both non-irradiated cells and irradiated cells. This study aims at elucidating the relationship between bystander cell death and intracellular energy deposited sites using a synchrotron X-ray microbeam irradiation system developed at the Photon Factory, KEK. We irradiated 5 cells (whole cells or only cell nuclei) with a 5.35 keV monochromatic X-ray microbeam in area of 6 mm by 6 mm, and survivals of non-irradiated cells were measured. In the case of nucleus-irradiation, survival fraction of bystander cell decreased down to 90% around 1 Gy and increased back to 96% in high dose region, whereas in the case of whole cell irradiation, it decreased monotonously to 92%. Dose-dependent enhancement of bystander cell death was observed only in nucleus-irradiated case. We speculate that the energy deposition in cytoplasm might reduce the bystander signal secretion through signal transduction system. The relationship between cell death, actually irradiated and bystander cells and intracellular energy-deposited sites will be discussed.