Biological Analysis of Irradiation Effects during the Embryonic Development of the Silkworm, *Bombyx Mori*, using the Detection of Somatic Mutation in Larval Stage

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The authors are making a plan to load the eggs of the silkworm, *Bombyx mori*, on International Space Station in order to investigate the effect for the organism of the cosmic radiations. Prior to this investigation, it is necessary to establish the method of the mutation detection in morphological and molecular level. In this study, to detect the mutation in the 5th instar larvae derived from the diapausing eggs which were irradiated X-ray or heavy particle beam, we used the strain of the silkworm with black-striped skin (*P*⁵). The albedo spot appeared in the black skin in the larval stage, when the egg of the heterozygote (*P*⁵/*p*) was irradiated with X-ray and heavy particle beam. This mutation occurred in superior gene (*P*⁵) which controlled the melanin synthesis in the epidermal cells of the integument. The relation between prevalence of this albedo spot and dose of radiation exposure was estimated. The somatic mutation rate increased dependently on dose and LET when the diapausing eggs were irradiated with carbon ion or neon ion, and about 30% of the mutation was caused by a neon ion with 2Gy and 150keV. However, the incidence lowered in 200KeV/µm. The somatic mutation level of the non-diapaused egg prepared by treatment with HCl 25 hours after oviposition, was higher than that of diapaused eggs. Somatic mutation rate in the larvae from the eggs kept at 25°C significantly lowered in comparison with those kept at 15°C for 15 days after oviposition. The accumulated content of sorbitol was 5.7 µ moles and 3.2 µ moles per 100 grains of former and the latter eggs, respectively, suggesting that the difference of somatic mutation between both lots may be resulted from the sorbitol content. According to the observation of the effects of 3-30 GY X-ray continuously irradiated to diapausing eggs for 1 week or 2 weeks, *P*⁵ gene mutation was shown to be caused clearly by a long term low level irradiation dependently on the irradiated total dose.

From these results, it was suggested that this method of the mutation detection using diapaused-eggs of the silkworm could be suitable for investigation of biological effects by cosmic lay irradiation.

Fig. 1  a) The eggs were kept at 25 °C for first 2.5 days after oviposition, and then transferred to 5 °C. Two months late, these eggs were irradiated with carbon ion beam (290MeV/u) b) The eggs were kept at 25 °C for first 15 days after oviposition, and 0 °C. These eggs were irradiated with neon ion beam (400MeV). These eggs of both lots were incubated at 25 °C for 12 days to resume embryogenesis, and the newly hatched larvae were reared through 5th instar. The mutation of the larva with white spot on the black integument was examined on the 3rd day of the 5th instar.

Fig. 2  The radio sensibility in the early development in silkworm egg from the viewpoint of somatic mutation. In Bombyx eggs, the blast genesis stops at about 24 hours from just after oviposition. These eggs enter into diapauses (dormancy). When these eggs are treated with hot HCl (sp.gr.1.075, 46 °C, 5min) around 24 hours after oviposition, embryogenesis continues for about 10 days (embryo developing eggs), and larvae hatched from these eggs. These eggs were irradiated with 1.0Gy of X-rays at an interval days after oviposition. The hatched larvae were reared, and the somatic mutation was detected in 5th instar.

Fig. 3  Effect of different temperatures on the incidence of somatic mutation on epidermal cells in Bombyx larvae which were hatched from X-ray (a) or ionic carbon irradiated eggs(b). a) The eggs were kept for first 7 days at 25 °C after oviposition, and some of these eggs transferred to 15 °C. The eggs were irradiated one or two times by X-rays at interval of 1 day b) The eggs were kept at 25 °C or 15 °C for 15 days after oviposition, and then irradiated by 0.5Gy (13keV) or 2.0Gy (80keV) of ionic carbon. The value were calculated by _2^2-test.

Fig. 4  Somatic mutation in epidermal cells of 5th instar larvae which were hatched from diapause eggs exposed to low-dose of X-rays. The eggs were kept at 25 °C for first 12 days after oviposition, and then transferred to 0 °C to maintain diapause state. On 42 days after oviposition, these eggs were irradiated continuously to 3, 7.5, 15, 22.5, 30µGy of X-rays for 7 or 14 days.