Induction of Localized Cuticle Defects by UV-Laser Irradiation of Early Embryos in *Bombyx mori* Linné

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The elimination of defined embryonic cells or cell groups in order to elucidate their prospective fates was among the earliest experimental methods of developmental biology. In the silkworm *Bombyx mori*, the establishment of fate maps has been achieved mainly by means of local cautery (Miya, 1953; Takami, 1964), however, because of its coarse egg shells, the method so far has yielded results of only limited value (see Kuwana and Takami, 1957 or Sakaguchi, 1978, for a review).

Here we show that a UV-laser microbeam (355 nm, Workstation ACAS470, Meridian) is useful for the inactivation of selected cells in the early *Bombyx* embryos. The technique is promising for the establishment of more accurate and detailed fate maps of *Bombyx mori*, as it permits damaging a defined area of an egg in a much more controlled fashion than with cautery.

Two nondiapause strains, *pnd* p^s and *pnd re ch*, were used throughout this study. Eggs were irradiated ventrolaterally at the cellular blastoderm stage (11–15 hr after the oviposition at 25 °C) for 0.2 mm square, *i. e.*, about 2% of the egg surface area (Fig. 1). After irradiation, eggs were kept at 25 °C for 12–13 days. Hatched larvae (first instar) were fixed in glycerol-acetic acid (1 : 4) for 3–4 hr at 60°C and then at room temperature for 24 hr or longer. Unhatched larvae were harvested before fixation by removing chorion with hypochlorite.

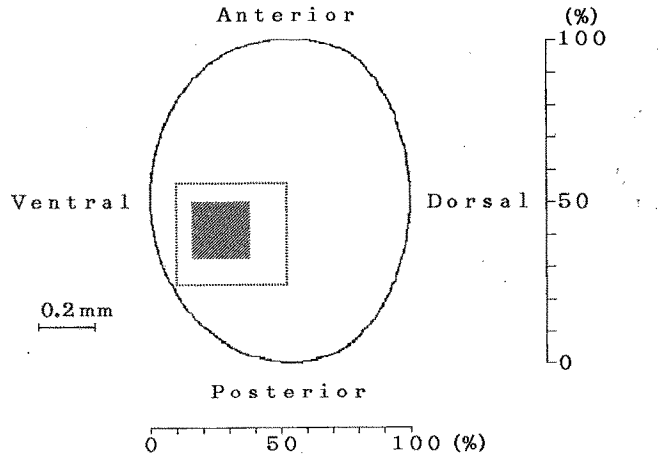

Fig. 1 Sites of irradiation. *Bombyx* eggs at the cellular blastoderm stage were irradiated with UV-laser ventrolaterally for 0.2 mm square (hatched area). The scale at the right indicates position along the anteroposterior egg axis in relation to the egg length (EL). Position along the egg circumference are indicated on the transverse scale, in relation to the egg width (EW). The square drawn by dotted lines refers to the limits of irradiation sites in this study; 10–52% EW and 25–56% EL.

Irradiation was carried out at various doses, from 0.001 to 40 J/cm^2, to find an appropriate condition for the larval defect induction. As shown in Table 1, even the strongest irradiation did not increase the embryo mortility. However, up to 80% of the larvae which developed from eggs irradiated at doses higher than 10 J/cm^2 showed defects in the integument, whereas none of those irradiated at doses lower than 1 J/cm^2 showed cuticle defects. The UV dose

found in this study to be necessary to induce defective *Bombyx* larvae, *i. e.*, 10J/cm^2, was about 50 times higher than that used for induction of cuticle defects in *Drosophila* (Lohs-Schardin *et al.*, 1979). The discrepancy may be ascribed to the employment of dechorionated eggs and of shorter wavelength (257 nm) in the *Drosophila* study.

Table 1 Induction of cuticle defects by UV (355 nm) irradiation of *Bombyx* embryos at the cellular blastoderm stage.

Dose (J/cm^2)	Number of eggs irradiated	Cuticle formation	Defective larvae
0	285	279 (89%)	0 (0%)
0.001～ 0.01	34	32 (94%)	0 (0%)
0.05 ～ 0.1	53	52 (98%)	0 (0%)
0.25 ～ 1	52	51 (98%)	0 (0%)
2.5 ～ 5	30	28 (93%)	1 (4%)
10 ～40	77	74 (96%)	61 (82%)

(1 J=1 W·sec)

The cuticle defects induced in this study included deletions, fusions, and partial duplications of thoracic appendages or abdominal legs. The location of the defects within the cuticlar pattern corresponded to the site of irradiation, indicating that the commitment of the cells to develop into the larvel integument occurs before the cellular blastoderm stage.

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