SPERM IRRADIATION AND INDUCTION OF GYNOGENETIC EMBRYOS

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Quantitative studies of the effect of sperm irradiation (UV or gamma-rays) on the embryonic development of *Oryzias latipes* are reported.

1. Ultraviolet light (UV) irradiation

Sexually mature females of the orange-red variety of *O. latipes* were sacrificed and ripe eggs were recovered in an isotonic balanced salt solution. The testes from mature male fish were isolated in the same manner and macerated, then the sperm suspension was irradiated with UV (253.7nm) and used to inseminate the eggs.

Dose-survival relationship

The rates of survival with respect to UV dose, namely a dose-survival relationship curve, was obtained at several developmental stages from fertilization to hatching. Fertilization took place normally with UV-irradiated sperm in all the dose ranges from 0 to 1000J·m^{-2}. As development advanced (Fig. 1, showing the curve at stage 19 or stage of optic bud formation as a typical example), we observed a dose-dependent decrease in survival rate at low doses (0-27 J·m^{-2}). However, as the UV dose increased beyond this value up to 1000J·m^{-2}, a better survival rate was obtained (commonly referred to as "Hertwig effect").

Photoreactivation phenomenon

Illumination with visible light was performed using fertilized eggs prior to cleavage (10-70 min after insemination). The results (indicated in Fig. 1 by a dashed line) showed the existence of photoreactivation (PR), demonstrating that pyrimidine dimers are probably produced in sperm DNA by UV-irradiation and are mainly responsible for Hertwig effect.

The effective period for visible light treatment to repair UV damage was examined. Only illumination of single-cell embryos during the early phase (up to around 20-30min after insemination at 25°C) was effective for PR, whereas illumination thereafter was not.

Genetic analysis

Sperm of the wild-type were irradiated at several UV doses and used for insemination of the eggs of orange-red females. The rates of appearance of "black embryos" (i.e., embryos carrying melanophores on the yolk sac, showing the dominant gene *B*) were examined. At low doses (8.0 and 13.3J·m^{-2}) in darkness, almost all the embryos exhibited melanophores. At high doses of 100-500J·m^{-2}, only few embryos possessed melanophores, implying a failure in syngamy ; the embryos were developing with only one set of chromosomes. At an intermediate dose, both types of embryos (*b* and *Bb*) emerged.

Post-irradiation treatment with visible light (10-70min after insemination) changed the UV dosage that was effective in causing the Hertwig effect and black embryos appeared even with a dose of 100J·m^{-2}.

The lethality of embryos which exhibited no melanophores on their yolk sacs at stage 19 (presumably, gynogenetic haploids) was examined. The number of viable embryos gradually decreased until stage 27, then there was a sudden mass extinction at stage 29. This implies that *O. latipes* embryos with the haploid chromosomes can not develop further than stage 27.

Analyses of chromosome numbers.

The number of chromosomes in cells of embryos at stage 26 was determined by means

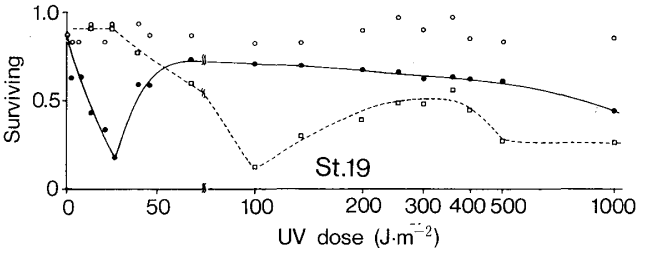

Fig. 1. Dose-survival curve. (-●-) UV+dark, (-□-) UV +visible light, (○) non-irradiated control.

of the usual squash method. The results are in agreement with the genetic analysis above, showing that embryos irradiated with dose which gave the Hertwig effect had a haploid number of chromosomes.

2. Gamma-ray irradiation

Gamma-ray irradiation of the sperm of the fish *Oryzias latipes* was also performed in the same manner, and the Hertwig effect was observed with a dose-dependent decrease in survival rate at low doses (0-12.5kR), but with a better survival rate at higher doses (50-150 kR). As in the UV experiments, the dose-survival relationship curve, genetic analyses and chromosome numbers were determined. The results were similar to those obtained with UV-irradiation.