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Ultraviolet Light Inhibits Grey Crescent Formation on the Frog Egg

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Summary. Work by others has shown that ultraviolet (UV) irradiation of the vegetal half of the uncleaved frog egg causes defects in neural development. We find that the earliest effect of irradiation of *Rana pipiens* eggs is to prevent grey crescent formation, the first indication of dorso-ventral polarization of the egg. The UV effect on the grey crescent and on neural development shows similarities in timing, dose-responses, and reversal by cold. We suggest that the UV effect on neural morphogenesis may be caused by the inhibition of cortical-cytoplasmic movement involved in grey crescent formation.

Key words: Ultraviolet irradiation – Amphibian – Grey crescent – Embryology.

Introduction

In the development of most animals, materials in the egg are unequally distributed among cells during cleavage. These cytoplasmic localizations play an important role in determining the fate of the cells. A classic example of such a localization is the region of the grey crescent on the amphibian egg. The grey crescent marks the future dorsal side of the embryo. When gastrulation begins, the cells which invaginate first and which form the dorsal lip of the blastopore are those at the edge of the grey crescent. In an attempt to study cytoplasmic materials important in morphogenesis of the amphibian embryo, Grant and Wacaster (1972) and Malacinski et al. (1975, 1977) irradiated the egg before cleavage with ultraviolet light (UV). Irradiation of the vegetal half caused delays in gastrulation, abnormal dorsal lips, and defects in neural morphogenesis. We have confirmed these results and have shown further that UV prevents the formation of the grey crescent. This very early effect may be involved in the subsequent pattern of developmental abnormalities.

Materials and Methods

Sexually mature, northern *Rana pipiens* were obtained in the fall from Nasco, Fort Atkinson, Wisconsin and Cimon, Loretteville, Quebec, and stored in the cold. Standard procedures were used for induction of ovulation and for insemination (DiBerardino 1967). Fertilized eggs were dejellied by removing the outer layers with forceps and the inner jelly by gently swirling the eggs in 1.5% thioglycolate, pH 8.6 for 5 min. After washing in 10% Ringer's or 20% Steinberg's, the eggs were placed in container with a quartz slide mounted to the bottom and allowed to rotate so that the vegetal halves were down. They were UV-irradiated for varying times at 5 cm with a UVS-11 Mineralite (Ultra-Violet Products, Inc., San Gabriel, CA, USA) whose principal emission is at 254 mμ. Eggs were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4 to score grey crescent, and in Smith's fixative to score maternal streaks (Elinson 1975). Neural development was scored by the method of Malacinski et al. (1975).

Results and Discussion

UV Effect on the Grey Crescent

We found no difficulty in seeing grey crescent on eggs from most of the females. The grey crescent formed gradually between 1–2 h after insemination. Figure 1 shows the parallel between the height of the grey crescent and the length of the maternal streak, a surface pigment line associated with the site of second polar body formation (Elinson 1975). The similarity between these two measures is expected since the grey crescent forms by a movement of the whole

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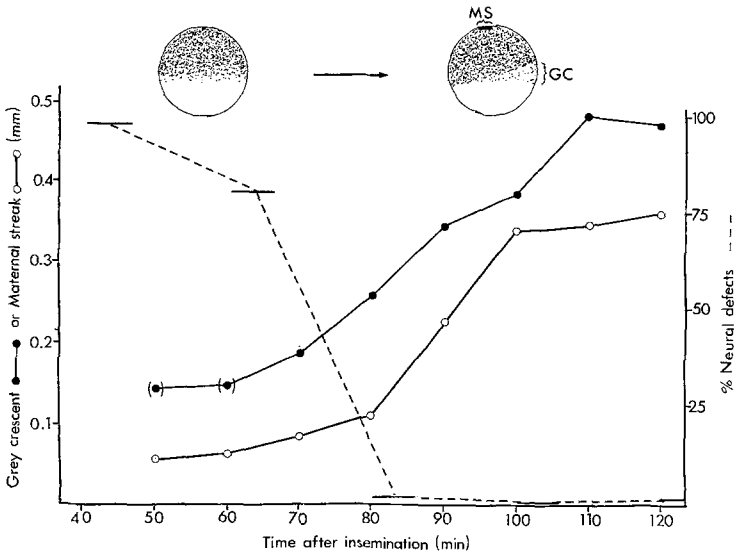

Fig. 1. Relationship between grey crescent formation and sensitivity of eggs to UV. The insets show an egg before and after grey crescent formation and indicate the measurements made. Each point on the graph is based on 7–15 eggs from one female. At 50 and 60 min, there was no grey crescent and so, the width of the symmetrical ring of indistinct pigmentation near the equator was measured. These points are enclosed by parentheses. After 60 min the height of the grey crescent (*GC*) and the length of the maternal streak (*MS*) increased parallel to each other. Also included is the sum of results from 6 experiments on the effect of UV in causing neural defects. Eggs were irradiated vegetally for 6 min as indicated by the bars, and the embryos were scored for defects at stage 17–18. The temperature was 22° ± 0.5° C, and first cleavage started at 130–140 min

cortex relative to the cytoplasm (Ancel and Vintemberger 1948; Elinson 1975; Elinson and Manes 1978).

When the vegetal half was UV-irradiated within 60 min after insemination, grey crescent formation was inhibited in a dose-dependent manner. We scored eggs qualitatively for grey crescents and expressed the results as a "grey crescent index". Crescents were scored as follows: 0 for a clearly visible grey crescent, 1 for narrow, faint grey crescent, and 2 for no visible grey crescent. The grey crescent index is the average score for a group of eggs. With longer periods of UV-irradiation, increasing numbers of eggs had no grey crescent, and the index approached 2.0. The effect on the grey crescent which we have found is the most immediate consequence of UV irradiation of these eggs that has been reported. The lack of a grey crescent suggests that the initial dorso-ventral polarization of the embryo which occurs at this time (Nieuwkoop 1977) may be altered.

We generally scored grey crescents at 90–120 min post-insemination, but the inhibition was not transient. UV-irradiated eggs scored at the eight-cell stage still had no crescents. By this time, the cleavage furrows would prevent the cytoplasmic reorganization that takes place during normal grey crescent formation. UV affects the formation rather than the appearance of the grey crescent since UV-irradiation after grey crescent formation had no effect on its morphology. UV-irradiation of the animal half within 60 min after insemination for 6–10 min did not affect the grey crescent, but these embryos were haploid probably due to the UV-destruction of the female pronucleus.

Since the formation of the grey crescent and the elongation of the maternal streak parallel each other, we wanted to see whether the maternal streak was affected when the grey crescent was inhibited. UV-treated eggs which had faint or no grey crescents (grey crescent index 1.5) showed a 43% reduction in the length of the maternal streak (average from five spawnings). The UV light should not be able to penetrate to the animal pole, and so, it is unlikely that the maternal streak is affected directly. Rather, since the grey crescent forms by a relative cortical-cytoplasmic rotation, the inhibition of rotation at one point should have an effect at other points as well.

The elongation of the maternal streak although reduced was never completely inhibited, even in groups of eggs with no grey crescents. The irradiated eggs, then, may have a weak dorso-ventral polarity rather than lacking this axis entirely. It would be interesting to see the effect of UV on the distribution of cytoplasmic components which also are redistributed during this time (Klag and Ubbels 1975; Palaček et al. 1978).

The targets affected by the UV have not been determined. Grant et al. (1976) speculated that UV might interfere with the action of microfilaments. Whether microfilaments are involved in grey crescent formation is not known although cytochalasin B did not inhibit grey crescent formation (Manes et al. 1978). On the other hand, colchicine prevented grey crescent formation (Manes et al. 1978) suggesting that microtubules are a possible target for UV.

UV Effect on Development

The UV-inhibition of grey crescent formation paralleled the action of UV in causing defects in neural

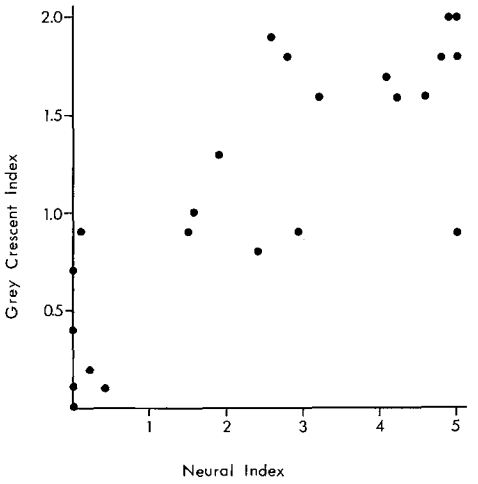

Fig. 2. Effect of UV irradiation on the grey crescent and on neural morphogenesis. For each point, 20–40 eggs from one of nine females were irradiated vegetally for 1–10 min. About half of the eggs were fixed and were scored for grey crescents while the rest were allowed to develop and were scored for neural development. Results are expressed as: *grey crescent index:* 0, no effect, grey crescent clear; 1, grey crescent faint but visible; 2, no visible grey crescent, and *neural index* (see Malacinski et al. 1975): 0, no effect; 1, 2, 3, degrees of microcephaly; 4, acephalic; 5, aneural. When the grey crescent index and neural index are compared (excluding cases where both are 0), the correlation coefficient is 0.84

morphogenesis. Animal half irradiation did not affect either, and vegetal half irradiation affected both. Although we have not determined the exact timing, neural morphogenesis became insensitive to UV early in the process of grey crescent formation (Fig. 1). Eggs irradiated after grey crescent formation developed normally. The relationship between the UV effect on the grey crescent and on neural morphogenesis was examined more closely using different UV doses, and a good correlation was found for these two effects (Fig. 2). We also found that cold treatment of UV-irradiated eggs which reversed the UV effect on neural morphogenesis (Malacinski et al. 1974), led to the formation of the grey crescent on those eggs. Eggs were irradiated vegetally for three minutes, placed at either 18° or 10° C, and examined for neural development and grey crescents. In a typical experiment, the 10° treatment reduced the neural index from 2.4 to 0.2 and the grey crescent index from 0.8 to 0.1. Similar results were found in three other experiments. We do not know why cold treatment has this effect. Perhaps since first cleavage is delayed, the egg has time to recover from the UV and form a grey crescent.

Based on these correlations, we favor the hypothesis that the defects in neural morphogenesis are the result of a failure in a proper egg polarization as evidenced by the lack of a grey crescent. Alternatively, UV may have multiple, independent effects on early development. Others have proposed that UV affects development by disrupting cell division (Grant and Youngdahl 1974) or by damaging cytoplasmic components involved in neural induction (Malacinski et al. 1975, 1978; Chung and Malacinski 1975). A way to decide between these hypotheses would be to examine other procedures for producing embryos without grey crescents and to see the effect on neural development. This has been done in the past by separating the embryo in half in the frontal plane during early cleavage. The half without the grey crescent has, in the hands of different investigators, either failed to gastrulate (Landström and Løvtrup 1974), gastrulated but failed to form an axis, i.e., a "Bauchstück" (Spemann 1928; Schmidt 1931), or gastrulated and neurulated (Dollander 1950). Although these differences in results should be resolved, we are impressed that the description of the "Bauchstück", an embryo which gastrulates but remains round and does not neurulate, is a good description of the UV-induced aneural syndrome.

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