動物学雑誌 Zoological Magazine 90: 290-294 (1981)

Activation of Eggs of the Frog, *Rana nigromaculata* Hallowell, after Localized UV-irradiation

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Received December 22, 1980

ABSTRACT To analyze the mode of transmission of the activating stimulus, the change in time course of the 2nd maturation division was examined in eggs of the frog, *Rana nigromaculata*, which were activated by insemination or by pricking after UV-irradiation at either the animal or the vegetal hemisphere. Irradiation of the animal (UAF) or the vegetal (UVF) hemisphere before insemination retarded the 2nd maturation division in comparison with that in normally fertilized eggs (CF). When the eggs were pricked at the irradiated surface (UAP and UVP, respectively), however, the 2nd maturation division progressed faster than that of CF. The comparison of the velocity of the 2nd maturation division between UAF and UVF, and also between UAP and UVP, showed that the cortical reaction in the vegetal hemisphere was affected by UV-irradiation more severely than that in the animal hemisphere. The results suggested that the quality of the granules or the constituent within the egg cortex might vary according to the regional difference in the egg surface. (*Zool. Mag. 90: 290-294, 1981*)

It is well known that continuous disintegration of cortical granules results from the transmission of activating stimulus in eggs of various kinds of animals including frogs (Motomura, 1952; Kemp and Istock, 1967), mammals (Austin, 1956), echinoderms (Sugiyama, 1956; Allen and Griffin, 1958), and fish (Yamamoto, 1956). Recently, it was reported in *Xenopus* eggs that one kind of cortical granules (GA) was distributed in the cortex of the animal hemisphere, while two kinds of cortical granules (GA and GV) were found in a multilayer condition at the vegetal hemisphere (Grey *et al.*, 1974). Sometimes intact granules remaining after activation at the vegetal cortex (Grey *et al.*, 1974; Kotani *et al.*, 1973) were also found. It is clear that the cortical reaction is transmitted through the overall surface and even at the vegetal surface, and that most cortical granules disintegrate after insemination or artificial activation. In spite of these accumulated findings concerning the cortical reaction, there still remains an important question as to whether the cortex at the vegetal hemisphere is the same as that at the animal hemisphere. In order to contribute to a solution of this and other problems, the following question was asked in the present experiment; does a difference in the velocity of cortical reaction exist between the cortex of the animal and the vegetal hemisphere?

Material and Methods

Adult frogs of *Rana nigromaculata* Hallowell were collected early in the breeding season and mature eggs were obtained through artificially induced ovulation by means of pituitary implantation (2 pieces/indiv.) and hormone injection (0.05 mg progesterone/indiv.). The insemination was done artificially by sperm suspension and the pricking was done with a clean glass needle. Eggs were reared in temperature regulated water (20±0.5°C) after insemination or pricking.

For irradiation, stripped eggs were arranged separately on a slide glass with either the animal or vegetal hemisphere upward. The control eggs were arranged in the same way and sham-irradiated. UV-irradiation was done with a high pressure mercury lamp (HU-2 type, Toshiba Co.). The irradiation time was 20 min at 21 cm distance from the lamp (under the energy level of 22.5 ergs/mm^2/sec). The time required for the completion of the second maturation division was chosen as a criterion for the velocity of cortical reaction. Eggs were fixed for 24 hr in Smith's fixative at 3, 6, 9, 15 and 20 min after insemination or pricking. Following dehydration, eggs were mounted into Tissue-mat, sectioned at 12 μm and stained with Feulgen reagent and light green.

To express the various combinations of experimental procedures, the following abbreviations are used in this paper: CF, normally inseminated eggs; UAF, eggs irradiated at the animal hemisphere and inseminated; UVF, eggs irradiated at the vegetal hemisphere and inseminated; CAP, eggs pricked at the animal hemisphere; CVP, eggs pricked at the vegetal hemisphere; UAP, eggs irradiated and pricked at the animal hemisphere; UVP, eggs irradiated and pricked at the vegetal hemisphere.

Results

Retardation in cleavage by UV-irradiation

The first cleavage was usually completed between 120-140 min after insemination at 20± 0.5°C in the control eggs. The eggs did not cleave synchronously even in a batch from the same female. UV-irradiation of the eggs at the animal or vegetal half induced a remarkable retardation at the beginning of the cleavage. The time required from the first appearance of the cleavaging egg to completion in all eggs was also prolonged to about twice that of the control eggs. In the control eggs, it required 5-7 min from the beginning of cleavage to completion for about half of the eggs in a batch, while it took 10-12 min in UAF and 13-15 in UVF (Fig. 1). Thus, a retardation of the cleavage process was evidenced after irradiation of the animal or vegetal half, the effect being more pronounced in the latter case. In about 80 per cent or more of the UVF eggs, the first cleavage furrow appeared crossing over the vegetal hemisphere without rotation

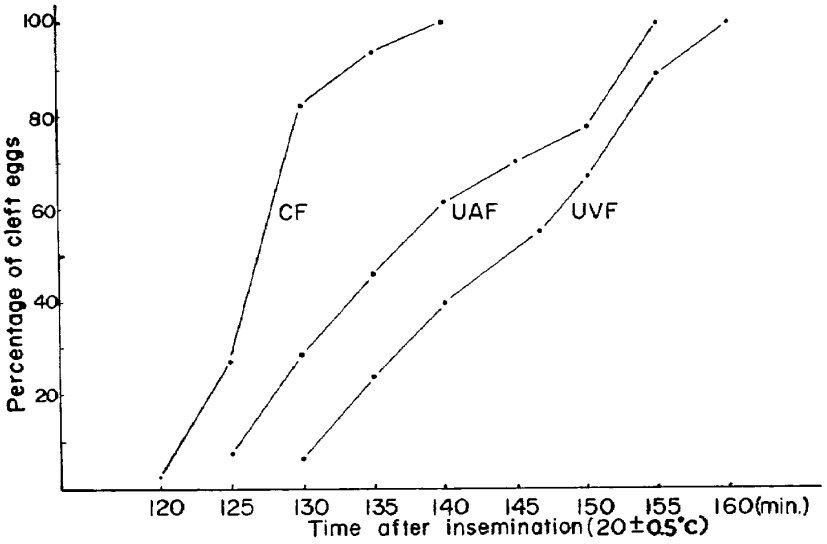

Fig. 1. Retardation in cleavage by UV-irradiation
CF: Eggs inseminated normally.
UAF: Eggs inseminated after irradiation of animal half.
UVF: Eggs inseminated after irradiation of vegetal half.

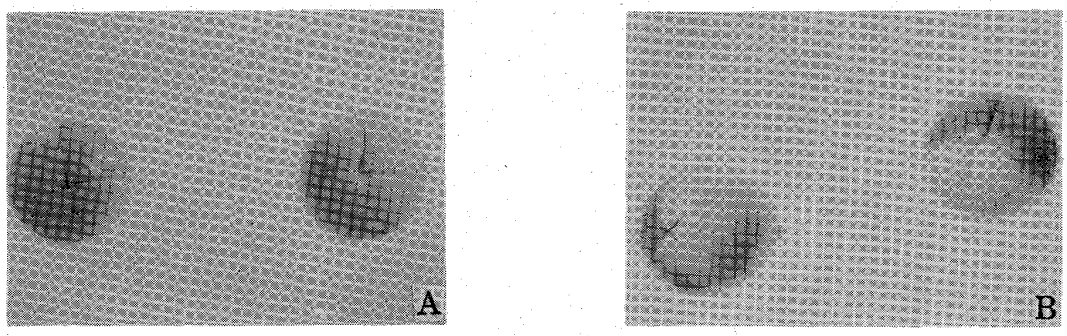

Fig. 2. 4-cell stage eggs
A, control B, vegetal halves were irradiated before insemination.

(Fig. 2) and, in spite of the abnormal pattern of cleavage, most of these eggs could continue to cleavage and develop further.

Transmission of the activating stimulation

The velocity of transmission of the stimulus by insemination or by pricking was compared by measuring the phase of the 2nd maturation division in fixed eggs at 3, 6, 9, 15 and 20 min after either one of these two activation methods.

1) The fertilized eggs

The progression of the 2nd maturation division in eggs which were normally inseminated after UV-irradiation of the animal half (UAF) or of the vegetal half (UVF) is shown in Table 1, together with four kinds of the parthenogenetic eggs. In the control eggs, while about half of the eggs were at metaphase of the 2nd maturation division 3 min after insemination, 7 out of 17 eggs had already reached the early and late anaphases. At 9 min, more than half of the eggs had reached the late anaphase and most of the eggs were found at the telophase and the stage of polar body formation 15 and 20 min after insemination. UAF eggs progressed similarly to the control eggs, but a slight retardation was found until 15 min. They also eventually completed the 2nd maturation division at 20 min after insemination. On the other hand, in UVF eggs, the progression was severely retarded and many eggs could not complete their 2nd maturation division even at 20 min after insemination.

2) Eggs activated by pricking

As seen in Table 1, the velocity of the 2nd maturation division of pricked eggs was always faster than that of the inseminated eggs whether the eggs were pricked at the animal or the vegetal hemisphere and whether they were irradiated or not.

a) Non irradiated eggs: Up to 6 min after pricking, most of the CAP eggs reached the stage of early or late anaphase and the formation of the 2nd polar body was completed in all eggs by 15 min. In the CVP eggs, the 2nd maturation division also progressed faster as found in the CAP eggs. There was no difference in the velocity between the two kinds of eggs, viz. those pricked at the animal hemisphere and those at the vegetal one. Accordingly, it may be said that the activating stimulation can be transmitted through the cortex of the vegetal hemisphere as well as through the cortex of the animal hemisphere.

b) Irradiated eggs: In both eggs of which the animal or vegetal halves were pricked after irradiation, the velocity of the 2nd maturation division was not affected by irradiation. From a detailed comparison among these eggs, it was found that the progression was more reduced in the UVP eggs than in the UAP eggs (Table 1). Even in these irradiated eggs, however, the progression occurred faster than that of the control eggs (CF) and it showed that the efficiency of the activating stimulation was greater by pricking than by insemination.

Table 1. Progression of the 2nd maturation division

Kinds of eggs	Time* (min)	No of eggs	Metaphase	Early anaphase	Late anaphase	Telophase	2nd polar body	Unidentified
CF	3	17	8	3	4	—	—	2
	6	14	3	3	7	—	—	1
	9	18	3	3	10	—	—	2
	15	16	1	—	5	8	1	1
	20	20	—	—	—	2	16	2
UAF	3	15	8	4	1	—	—	2
	6	18	9	4	4	—	—	1
	9	17	3	5	9	—	—	1
	15	12	3	3	5	1	—	—
	20	13	2	—	—	—	11	—
UVF	3	14	13	—	—	—	—	2
	6	13	11	2	—	—	—	1
	9	14	9	4	1	—	—	1
	15	15	4	4	5	—	—	—
	20	15	5	1	2	4	1	1
CAP	3	15	4	5	4	—	—	2
	6	22	1	7	13	—	—	1
	9	19	—	—	2	8	8	1
	15	16	—	—	—	—	15	1
	20	20	1	—	—	—	18	1
CVP	3	14	2	2	9	—	—	1
	6	17	2	3	9	1	1	1
	9	15	1	—	2	7	5	—
	15	15	—	—	—	—	13	2
	20	15	—	—	—	—	14	1
UAP	3	15	11	2	—	—	—	2
	6	12	2	2	7	—	—	1
	9	14	—	—	2	4	8	—
	15	13	—	—	—	2	9	2
	20	14	1	1	—	2	10	—
UVP	3	16	9	2	4	—	—	1
	6	14	8	3	1	—	—	2
	9	14	—	—	5	6	2	1
	15	15	—	—	2	1	12	—
	20	16	—	—	—	2	14	—

* Time (min) after insemination or pricking

Discussion

Kemp and Istock (1967) thoroughly studied the cortical changes in the eggs of *Rana pipiens* and reported that the activating stimulation caused a wave-like break down of cortical granules during 1-1.5 min after pricking and during 10-15 min after insemination. Grey *et al.* (1974) have found in *Xenopus* eggs that the disruption of cortical granules began at 3 min after insemination and was completed during the 9-10 min after. They also found a multilayered arrangement of two kinds of granules in the cortex of the vegetal hemisphere and some of these granules remained intact in the fertilized or pricked eggs. The same fact had also reported by Kotani *et al.* (1973).

From the results of the present experiment, the velocity of the 2nd maturation division after insemination or pricking seemed to coincide with the time of the propagation of the cortical granules disruption as reported by these authors. As seen in Table 1, while most of the eggs entered the anaphase stage of the 2nd

maturation division at 9 min after insemination in CF and UAF, most of the CAP, CVP, UAP and UVP eggs had already entered the telophase at the same time after pricking. This fact showed that the progression of the 2nd maturation division advanced faster in the pricked eggs than in the inseminated eggs as reported by Katagiri (1959) and Kemp and Istock (1967). In comparing the velocity of the 2nd maturation division between UAF and UVF, it may be said that UV-irradiation damaged more severely the cortex of the vegetal hemisphere than that of the animal hemisphere and, by that, the propagation of the activating stimulation was more impeded in the cortex of the vegetal half. The difference in UV-ray sensitivity seemed to be due to the difference in constitution of the cortex of the vegetal hemisphere as pointed out by Grey *et al.* (1974). The same presumption may also be derived from the following fact: The cleavage retardation and slow progression of UAF and UVF were caused by irradiation damage to the cortex and such damage was more severe in eggs in which vegetal halves were irradiated.

It was found that the pattern of cleavage was affected by UV-irradiation before insemination in the UVF eggs. It may be estimated that the UV-rays obstructed the disruption of cortical granules on the vegetal cortex, thereby the formation of the perivitelline space was suppressed and eggs could not rotate. Recently, Wolf (1974) reported in detail on the cortical granules substance, but a difference in granules between the animal and vegetal cortices was not mentioned. To understand the mechanism of propagation of the activating stimulus, the protoplasmic constituents on the overall cortex of the egg and the difference between the animal and vegetal cortices remains to be studied.

References

ALLEN, R.D. AND J.L. GRIFFIN (1958) The time sequence of early events in the fertilization of sea urchin eggs. I. The latent period and cortical reaction. *Exptl. Cell Res.* **15**: 163-173.

AUSTIN, C.R. (1956) Cortical granules in hamster eggs. *Exptl. Cell Res.* **10**: 533-540.

GREY, R.D., D.P. WOLF AND J.F. HEDRICK (1974) Formation and structure of the fertilization envelopes in *Xenopus laevis*. *Develop. Biol.* **34**: 46-61.

KATAGIRI, CH. (1959) Cortical changes at fertilization in the eggs of the grass frog, *Rana temporaria*. *J. Fac. Sci. Hokkaido Univ. Ser. VI.* **14**: 166-174.

KEMP, N.E. AND N.L. ISTOCK (1967) Cortical changes in growing oocytes and in fertilized or pricked eggs of *Rana pipiens*. *J. Cell Biol.* **34**: 112-122.

KOTANI, M., K. IKENISHI AND K. TANABE (1973) Cortical granules remaining after fertilization in *Xenopus laevis*. *Develop. Biol.* **30**: 228-232.

MOTOMURA, I. (1952) Cortical granules in the egg of the frog. *Annot. Zool. Japon.* **25**: 238-241.

SUGIYAMA, M. (1956) Physiological analysis of the cortical response of the sea urchin egg. *Exptl. Cell Res.* **10**: 364-376.

WOLF, D.P. (1974) On the contents of the cortical granules from *Xenopus laevis* eggs. *Develop. Biol.* **38**: 14-29.

YAMAMOTO, T. (1956) Physiology of fertilization in the Medaka (*Oryzics latipes.*) *Exptl. Cell Res.* **10**: 387-393.