Abnormal Morphogenesis of Sea Urchin Embryo Induced by UV Partial Irradiation Given at Cleavage Stage

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(Received December 25, 1982)

UV partial irradiation/Sea urchin embryo/Morphogenesis/Exogastrula/Permanent blastula

Morphological abnormalities due to UV partial irradiation to 8 or 16 cell embryos were studied in the sea urchin (Hemicentrotus pulcherrimus) embryo. UV irradiation on the animal hemisphere of 8, 16 cell embryos inhibited normal development at the gastrula stage and caused the formation of exogastrula. UV irradiation of the vegetal hemisphere arrested the normal development at the blastula stage and inhibited the gastrulation and the skeleton formation, giving rise to permanent blastula.

INTRODUCTION

Ultraviolet light (UV) has been used for better understanding of the morphogenesis in the early development of various animals. The pattern of the body segment of a harlequin fly, Chironomus dorsalis altered drastically by UV irradiation (Yajima, 1964; Kalthoff, 1971, 1973). Two types of double malformation, ‘double cephalon’ and ‘double abdomen’ are formed by UV partial irradiation to either the posterior or the anterior half of Chironomus eggs, respectively. In the early amphibian development, UV irradiation to the vegetal half of fertilized frog eggs prior to the first cleavage delays gastrulation and inhibits the formation of grey crescent and the neural induction (Grant and Wacaster, 1972; Malacinski, 1975; 1977; Ijiri, 1978; Manes and Elison, 1980; Youn and Malacinski, 1981). In Lymnaea UV irradiation to the vegetal hemisphere causes exogastrulation (Labordus, 1970). In a sea urchin, morphological abnormality due to the UV irradiation to the sperms or embryos and its modification by photoreactivation were studied (Shioda and Shiroya, 1974; Akimoto and Yajima, 1981; Ejima and Shiroya, 1982). The UV irradiation to the animal hemisphere of sea urchin blastula causes exogastrulation (Czhak, 1962; Ame-miya, personal communication). The induction of morphological abnormalities

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by UV in sea urchin embryos might be useful as an analytical tool for the study of morphogenesis.

In the present paper, we will report on the morphological abnormalities caused by UV partial irradiation to the animal or the vegetal hemisphere of 8 or 16 cell embryos of a sea urchin.

MATERIALS AND METHODS

Sea urchin.

The sea urchin used was *Hemicentrotus pulcherrimus*, from which the gametes were obtained by an intracoelomic injection of 0.55M KCl to it. The eggs were fertilized and the embryos were cultured at 20°C in the filtered sea water. The following procedures were carried out in a dark room kept at 20°C under the pure yellow fluorescent lamps (Matsushita Electric, FL 20 Y-F) to avoid uncontrolled photoreactivation (PR). The each experiment using about 400 embryos was repeated three to four times.

UV irradiation.

Irradiation was done with a low pressure germicidal lamp (Toshiba Electric, GL-10, 254 nm). The fluence rate was 4 J/m²/sec which was estimated by an UV monitor (Topcon UVR-254). An embryo was submerged in the sea water on a hollow slide glass and covered with a quartz cover glass. By sliding the quartz cover glass on the hollow slide, the animal pole or the vegetal pole of the embryo was turned upward and irradiated through the quartz cover glass. The direction of the incident radiation was perpendicular to the hollow slide glass. The irradiation was carried out at 8 cell stage or 16 cell stage of the embryo, since at these stages the animal pole or vegetal pole can be distinguished easily by both the size and the arrangement of blastomeres. After irradiation the embryos were transferred into a petri dish (15 mm in diameter) and incubated at 20°C under

| Table 1 | Induction of exogastrula by UV irradiation of animal hemisphere |
|---------|---------------------------------------------------------------|
| irradiated stage | percentage of exogastrula | 180 J/m² | 225 J/m² | 270 J/m² | 315 J/m² |
| 8 cell stage     | 32 %                  | 48       | 82       | –        |
| 16 cell stage    | 66                    | 62       | 65       | 57       |
Fig. 1a. Effect of varying UV fluence (abscissa) on embryos expressed as arrest of development at gastrula stage. With increasing UV fluence, the percentage of embryos which arrest development at gastrula stage (ordinate) increased. Animal hemisphere of 8 cell embryos (○) or 16 cell embryos (●) was irradiated. At low UV fluence (≤180 J/m²) gastrula, prism and pluteus, which proceed over gastrula stage, were also observed. Observation was done 48 hr after fertilization when control embryo reach pluteus stage (Fig. 2a). About 30 embryos were used for each group.

Fig. 1b. Effect of varying UV fluence (abscissa) given to vegetal hemisphere of embryos expressed as arrest of development at blastula stage. With increasing UV fluence, the percentage of embryos which arrest development at blastula stage (ordinate) increased. Below 200 J/m² of UV fluence, prism and pluteus, which proceed over blastula stage, were also observed.
the dark condition. At 48 hr after fertilization when control embryo reached pluteus stage (Fig. 2a), the effects of UV irradiation on the morphology of the embryos were examined microscopically. UV light was absorbed mainly by animal or vegetal hemisphere of the embryo, because cleavage delay and chromosomal abnormality were observed in the blastomeres of UV irradiated hemisphere.

RESULTS

UV irradiation of animal hemisphere of 8, 16 cell embryos induced the arrest of normal development at gastrula stage. With increasing UV fluence, embryos which arrested development at gastrula stage increased (Fig. 1a). The development of almost all of the embryos arrested at gastrula stage and some of them formed exogastrula at the range of UV fluence over 180 J/m² (Fig. 2b, Table 1). We rarely observed exogastrula at the range of UV fluence under 180 J/m² or over 350 J/m². No significant differences in morphology were found between groups irradiated at 8 cell and 16 cell stages.

UV irradiation of vegetal hemisphere of 8, 16 cell embryos caused the arrest of normal development at blastula stage. With increasing UV fluence, the percentage of embryos which arrested development at blastula stage increased (Fig. 1b). About 90% of embryos arrested development at blastula stage at the range of UV fluence over 200 J/m² and became permanent blastulae (Fig. 2c). Gastrulation and spicule formation never occurred in the permanent blastula. In morphology of the embryos, clear difference was observed between embryos irradiated on animal hemisphere and those on vegetal hemisphere.

Fig. 2. Embryos 48 hr after fertilization; a) normal pluteus derived from UV unirradiated embryos. b) Exogastrula derived from the embryos UV irradiated on the animal hemisphere at 16 cell stage. a; archenteron c) permanent blastula derived from the embryos irradiated on the vegetal hemisphere at 16 cell stage. Bottom bar, 50 µm.
DISCUSSION

In this study using 8, 16 cell embryos, exogastrula and permanent blastula were induced by UV partial irradiation of animal hemisphere and vegetal hemisphere respectively. Exogastrula induced by UV irradiation was observed in the range of 180—315 J/m². Within this range the percentage of exogastrula formation increased up to about 80% with UV influence given to animal hemisphere of 8 cell embryos, while it was almost constant (about 60%) in the case of UV irradiation of 16 cell embryos. Several reasons could be considered why exogastrulation occurs by UV irradiation of animal hemisphere of embryos at 8, 16 cell stage: 1) the interaction between the pseudopodia from the top of archenteron and the inside of body wall near animal pole is disturbed. 2) a number of mesenchyme like cells fill blastocoel, so mechanically archenteron cannot invaginate. We are doing scanning electron microscopic observation to examine these possibilities.

Permanent blastula was formed by UV irradiation of vegetal hemisphere of 8, 16 cell embryos. The descendants of blastomeres of vegetal hemisphere of 8, 16 cell embryos form spicule, oesophagus, stomach, intestine, coelom and a part of ectoderm (Hörstadius, 1939). UV may affect development by disrupting cell division or damaging cytoplasmic and nucleic components involved in gastrulation, thus forming permanent blastula.

We have not determined the depth of UV penetration into the embryo. We assume however almost UV energy absorbed in embryo is located in the hemisphere irradiated, because cleavage delay and chromosomal abnormality, that is chromosomal bridges at anaphase, were observed in the blastomeres of UV irradiated hemisphere (unpublished).

Stage sensitivity to UV was almost the same between 8 cell embryo and 16 cell embryo. It must be considered that UV sensitivity of sea urchin embryo varies as cell cycle proceeds (Rustad, 1961).

Experiments of the effect of visible light given after UV irradiation has shown high effectiveness for photoreactivation of abnormal morphogenesis mentioned above (Akimoto, Shiroya and Sutherland, 1983). Thus the primary lesions responsible for morphological abnormalities reported in this study are most likely to be pyrimidine dimer formation in DNA of the blastomeres in animal or vegetal hemisphere.

ACKNOWLEDGMENT

We thank Dr. Hideo Yajima, Ibaraki University, for the discussions and valuable suggestions. We are also grateful to Dr. Takashi Ito, College of General Education University of Tokyo, for critical advices.
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