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Original Article

Dorsoventral polarization and formation of dorsal axial structures in *Xenopus laevis:* analyses using UV irradiation of the full-grown oocyte and after fertilization

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ABSTRACT ***Xenopus laevis*** **embryos which had been UV-irradiated as full-grown oocytes (UV-O) or after fertilization (UV-F) showed typical UV syndrome, namely dorsal axial deficiency. Morphological comparison revealed that UV-O embryos showed a clear dorsoventral polarity from early cleavage to gastrula stage, but UV-F embryos showed radially symmetrical development throughout embryogenesis. Although UV-O embryos developed morphologically normal-looking dorsal lips of the blastopore, they failed to develop dorsal axial structures at later stages. Implantation of dorsal lips demonstrated that the dorsal lip of UV-O embryos had less activity as Spemann's organizer than the dorsal lip of normal embryos. It is thus suggested that a morphological differentiation of the dorsal lip of the blastopore does not necessarily imply a functional differentiation of Spemann's organizer. Dorsal or ventral cytoplasm from normal embryos at the 8-16 cell stage was injected into a blastomere of UV-F and UV-O embryos at the same stage as the donor. The injection of the dorsal cytoplasm could rescue partially the UV syndrome of UV-F but not of UV-O embryos.**

KEY WORDS: *Xenopus laevis, UV irradiation, dorsoventral polarity, Spemann's organizer, dorsal cytoplasm*

Introduction

The basic body plan in *Xenopus laevis* embryos is initially specified by a cortical rotation within the first cell cycle following sperm penetration (Vincent *et al.*, 1986; Elinson and Rowning, 1988), and then finally established by acquisition of potency for dorsal blastomeres to induce future dorsal axial structures (Gimlich and Gerhart, 1984; Kageura and Yamana, 1984; Gimlich, 1986). The dorsal vegetal blastomeres of early embryos have been shown to contain "dorsal determinant(s)" as cytoplasmic factor(s) (Yuge *et al.*, 1990). It is essential to know the properties and behavior of the "dorsal determinant(s)" to elucidate the body patterning of embryos (Wakahara, 1986; 1989). Thus, the properties of the dorsal cytoplasm were investigated using UV- (ultraviolet) irradiated embryos as a tool.

It is well known that UV irradiation to the vegetal region of full-grown oocytes (UV-O) and of fertilized eggs (UV-F) in amphibians results in dorsal axial deficiency (Grant and Wacaster, 1972; Malacinski *et al.*, 1975, 1977; Manes and Elinson, 1980; Scharf and Gerhart, 1980; Cooke and Smith, 1987). In UV-F embryos, the UV irradiation is believed not to destroy the essential components of the "dorsal determinant(s)" because the irradiated embryos can be rescued completely, although only by tilting the irradiated eggs before first cleavage (Scharf and Gerhart, 1980; Elinson and Pasceri, 1989). UV-O embryos, however, cannot be rescued by tilting (Elinson and Pasceri, 1989). These observations suggest that the UV target in UV-F embryos must be different from the target in UV-O embryos.

The UV-F embryos show radially symmetrical development throughout embryogenesis (Cooke and Smith, 1987; Kao and Elinson, 1988); they show a radially symmetric pigmentation pattern in the animal hemisphere at cleavage stages, a round blastopore at gastrulation, and they result in dorsally deficient embryos. In contrast, UV-O embryos show almost normal cortical rotation and the normal pigmentation pattern of the animal hemisphere, that is, less pigmented dorsal blastomeres and more darkly pigmented ventral blastomeres, probably as a result of the cortical rotation (Elinson and Pasceri, 1989). Whereas the UV-O embryos develop the dorsal lip of the blastopore as expected at gastrulation, at later stages, they eventually fail to form dorsal axial structures.

This study was carried out in three parts. First, morphological differences between UV-F and UV-O embryos were described during early development. Second, cytoplasmic transfer from

Abbreviations used in this paper: UV, ultraviolet; DAI, dorsoanterior index; UV-O, irradiation of full-grown oocyte; UV-F, UV irradiation of fertilized eggs.

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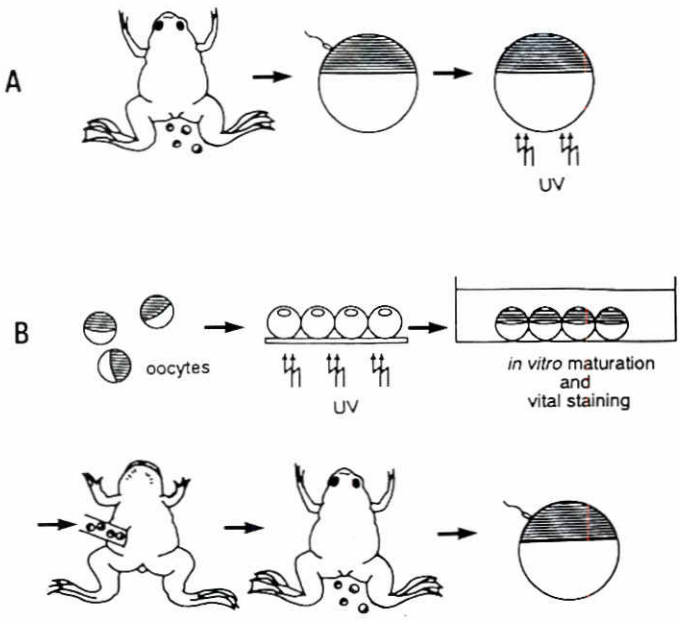

Fig. 1. Schematic illustration of experimental designs for obtaining UV-O and UV-F embryos. *In the case of UV-F embryos* **(A)**, *vegetal halves of fertilized eggs of Xenopus laevis were simply irradiated with UV before first cleavage and then allowed to develop. In the case of UV-O embryos* **(B)**, *full-grown oocytes were defolliculated, irradiated with UV, and then allowed to mature with progesterone. UV-irradiated and unirradiated oocytes were stained with different vital dyes and then transferred into a host female previously primed with HCG. After spawning, colored eggs were inseminated with a sperm homogenate.*

normal embryos to both UV-F and UV-O embryos was done, and the embryos' ability to form dorsal axial structures was scored according to the dorsoanterior index (Kao and Elinson, 1988). Third, the activity of the dorsal lip of UV-O embryos as Spemann's organizer was investigated using a classical technique, a blastocoelic implantation of the dorsal lip.

Results

Developmental differences between UV-O and UV-F embryos

Fig. 1 shows the experimental designs used for obtaining UV-O and UV-F embryos. In the case of UV-F embryos, fertilized eggs were irradiated with UV in the vegetal region before first cleavage and allowed to develop. In the case of UV-O embryos, full-grown oocytes were manually defolliculated, irradiated with UV, allowed to mature with progesterone, stained with vital dyes, and then transferred to a host female. UV-O embryos and their controls, which had been spawned by one female, were easily distinguished according to their staining properties.

Fig. 2 shows the external views of UV-O and control (unirradiated) embryos, which were stained with different vital dyes.

Fig. 3 shows the external morphology of normal control, UV-F, and UV-O embryos. UV-O embryos at cleavage stages showed a well-polarized pigmentation pattern with a clear dorsoventral polarity (Fig. 3C), which was identical to that in normal, unirradiated embryos in showing less pigmented dorsal and more darkly pigmented ventral blastomeres (Palecek *et al.*, 1978) (Fig. 3A). In contrast, the dorsoventral polarity of UV-F embryos at early cleavage stages was not clear (Fig. 3B).

Blastopore formation in both UV-F and UV-O embryos occurred almost simultaneously with their normal siblings (approximately 24 h after fertilization at 15°C). Typical UV-F embryos formed a faint, round blastopore without a clear dorsoventral polarity (Fig. 3E). Thus, it was difficult to determine the dorsal lip of the blastopore and to predict the dorsal side of the UV-F embryos. In contrast, UV-O embryos showed well-polarized and clear dorsal lips (Fig. 3F) similar to those of normal embryos (Fig. 3D). Thus, UV-O and UV-F embryos were completely different during early embryogenesis with respect to dorsoventral polarity. After the neurula stage, however, they became very similar in appearance; no clear neural folds were observed in either UV-O or UV-F embryos (Fig. 3H and I) when normal embryos showed a clear neural fold (Fig. 3G).

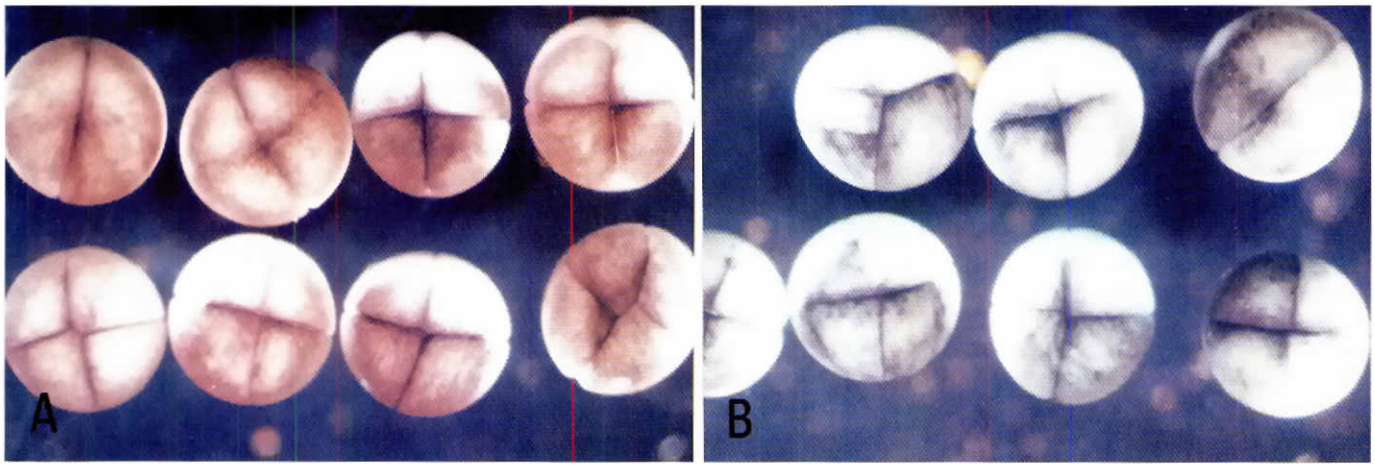

Fig. 2. External views of normal (unirradiated) and UV-O (embryos UV-irradiated at the oocyte stage) embryos at the 4-8 cell stage. (A) *UV-O embryos which had been irradiated with UV at the oocyte stage, stained with neutral red, matured in vitro, transferred into a host female, and then fertilized.* **(B)** *Controls which had been stained with Nile blue, matured in vitro, transferred into the same host female, and then fertilized.*

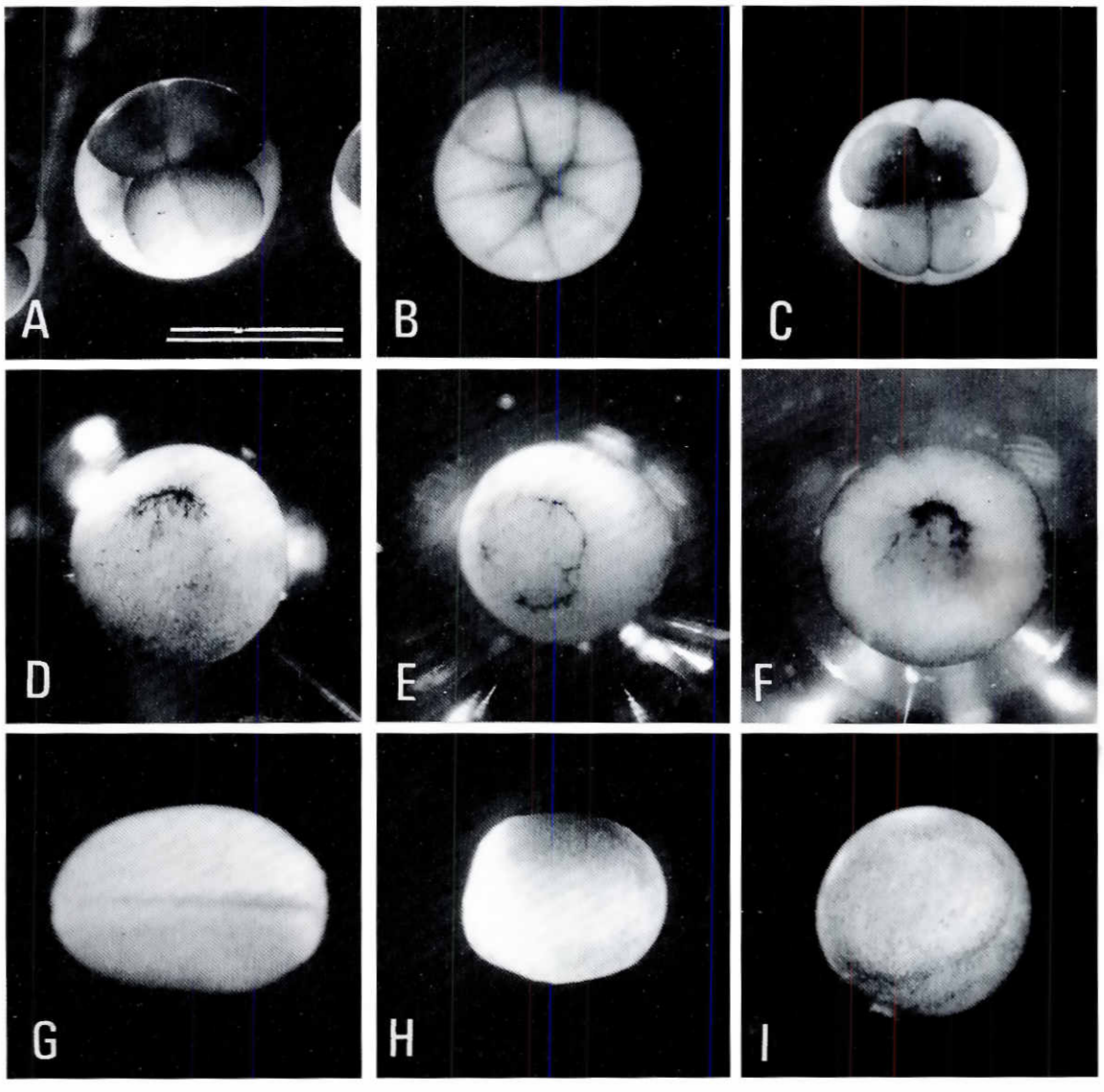

Fig. 3. External views of normal, UV-F (UV-irradiated embryos after fertilization) and UV-O (embryos UV-irradiated at oocyte stage) embryos. **(A,B,C)** *Animal-pole views of 8-cell stage embryos. The UV-O embryo* **(C)** *shows a well-polarized pigmentation pattern similar to a typical normal embryo* **(A)**, *which shows less pigmented dorsal blastomeres and more darkly pigmented ventral blastomeres. In contrast, UV-F embryos never show any indication of dorsoventral polarity in the pigmentation pattern of the animal blastomeres* **(B). (D,E,F)** *Vegetal-pole views of stage 11 (early gastrula). Normal* **(D)** *and UV-O* **(F)** *embryos show clear dorsal lips. The UV-F embryo has a faint, round blastopore without any dorsoventral polarity* **(E). (G,H,I)** *Dorsal views of early neurula embryos. Normal neurula embryo* **(G)** *with a clear neural fold, and UV-F* **(H)** *and UV-O* **(I)** *embryos at corresponding stages. Scale bar, 1 mm.*

Fig. 4 shows typical internal morphologies at gastrula and neurula stages of normal, UV-F, and UV-O embryos. In UV-O embryos, a dorsal lip was detected clearly at stage 11 (Fig. 4C, arrowhead). At stage 12, the involution of mesoderm with a dorsoventral polarity could still be detected, but a clear archenteron was not observed (Fig. 4F). The invaginating mesoderm was clearly observed in UV-O embryos (Fig. 4I). In contrast, the internal morphology of UV-F embryos was considerably different; at stage 10, a dorsal lip could hardly be detected, and symmetrical invagination of the mesoderm was observed at stage 11 (Fig. 4B, arrowheads). At stage 12 epiboly of the ectoderm and invagination of the mesoderm were still radially symmetrical. Thus, a typical differentiation of chordamesoderm, which was already differentiated in normal embryos (Fig. 4D,G), was not found (Fig. 4E,H). A blastocoel largely remained, but no archenteron was observed in UV-F embryos.

Dorsal cytoplasmic transfer to UV-F and UV-O embryos

At the neurula stage, normal embryos showed clearly differentiated notochord and neural tissue (Fig. 4J). In UV-F embryos, however, no axial structures were differentiated as a result of the radially symmetrical involution of the chordamesoderm (Fig. 4K). In

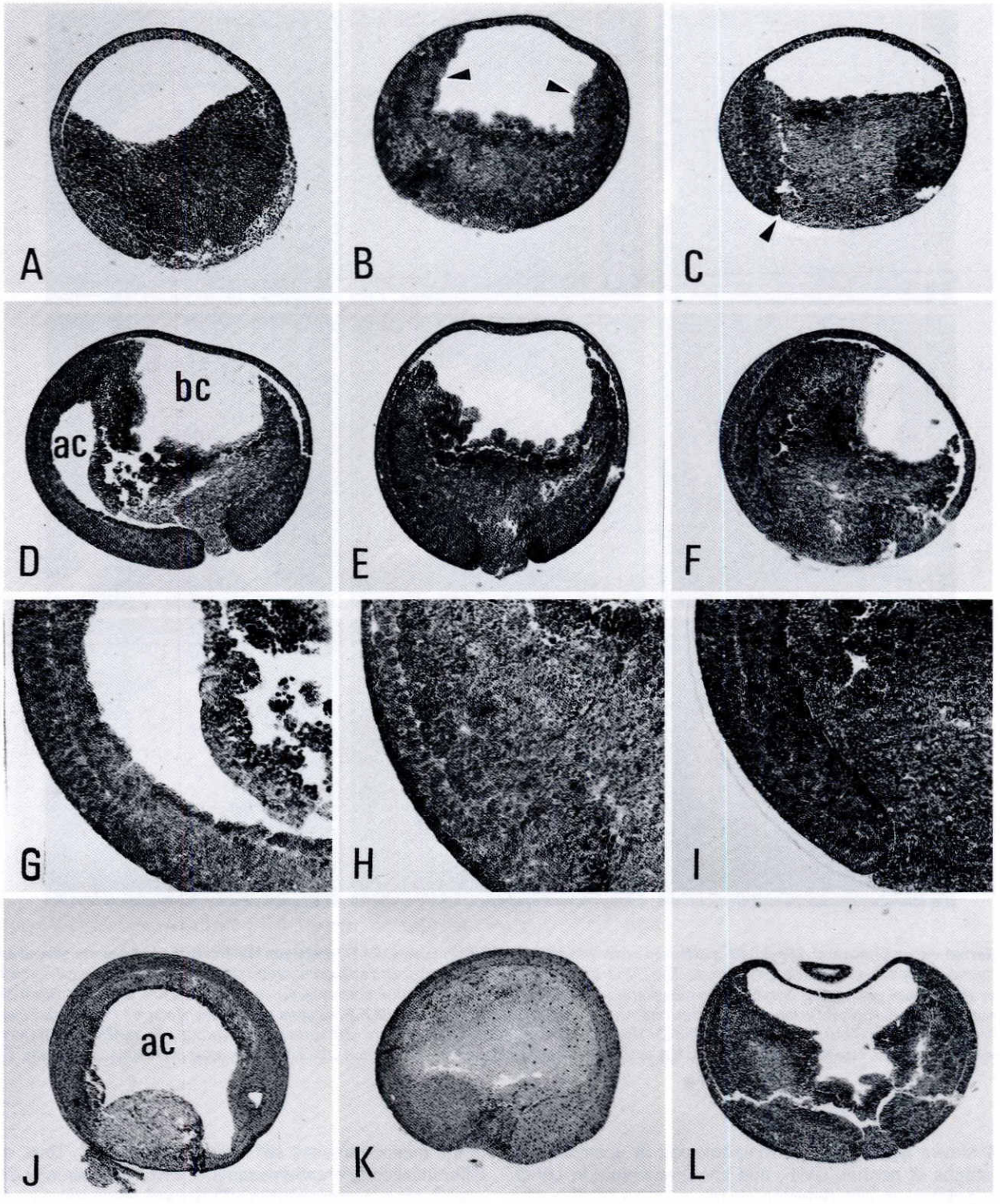

Fig. 4. Internal morphology of normal, UV-F and UV-O embryos. (A,B,C) *Normal, UV-F, and UV-O embryos at stage 11;* **(D,E,F)**, *normal, UV-F, and UV-O embryos at stage 12;* **(G,H,I)**, *enlargement of D, E, and F, respectively;* **(J,K,L)**, *normal, UV-F, and UV-O embryos at stage 19. Normal* **(A)** *and UV-O* **(C)** *embryos show epiboly of ectoderm and an invagination of mesoderm on the dorsal side of stage 11 embryos. In contrast, the invagination in UV-F embryos (arrowheads in* **B**) *is radially symmetrical. The normal embryo at stage 12* **(D)** *shows a conspicuous involution of dorsal mesoderm and a well-developed archenteron (ac), but the UV-F* **(E)** *embryo shows radially symmetrical gastrulation. In the UV-O embryo, no archenteron space is observed, but a blastocoel (bc) still remains* **(F)**. *At stage 19, when the normal embryo* **(J)** *shows well-differentiated internal structures, no dorsal axial structures are observed in UV-F* **(K)** *and UV-O* **(L)** *embryos. The UV-F embryo shows no blastocoel, whereas a large blastocoel (bc) is still visible in UV-O embryos. Stained with Delafield's hematoxilin and eosin. Scale bars, 1 mm in A-F and J-L; 100 μm in G-I.*

TABLE 1

DORSOANTERIOR INDEX OF UV-F EMBRYOS INJECTED WITH DORSAL OR VENTRAL CYTOPLASM FROM NORMAL EMBRYOS

Treatment[a]	No. of embryos		Dorsoanterior index[b]						
	total	survival	0	1	2	3	4	5	mean±SD[c]
UV control (No cytoplasm injected)	174	157	104	17	24	8	1	3	0.7±1.1[d]
D cytoplasm injected	129	105	27	26	25	25	2	0	1.5±1.2[e]
V cytoplasm injected	86	73	45	14	13	1	0	0	0.6±0.8[f]
Normal control	161	151	0	1	0	0	18	132	4.9±0.4

[a]Fertilized eggs were irradiated with UV before first cleavage. When the irradiated embryos had developed to the 8-16 cell stage, they were injected with dorsal (D) or ventral (V) cytoplasm from normal embryos at the same stage as the recipients. [b]According to Kao and Elinson (1988). [c]Mean DAI was calculated by summing individual indexes and dividing by the total number of individuals. [d]Not statistically significant compared with [f]; [e]statistically significant compared with [f] ($p<0.001$).

UV-O embryos, the animal cap was not lined with mesoderm because the invagination of mesoderm was not complete (Fig. 4L). A large blastocoel still remained, but no archenteron was observed. Although UV-F and UV-O embryos resembled each other in external morphology from late gastrula to neurula stages, their internal structures were quite different at the gastrula stage.

Dorsal cytoplasmic transfer to UV-F and UV-O embryos

The dorsal or ventral cytoplasm from normal embryos at the 8-16 cell stage was transferred to vegetal blastomeres of UV-F or vegetal dorsal blastomeres of UV-O embryos at the same stage as the donor embryos. Their "dorsal-axial-structure-forming activity" was assayed by scoring the dorsoanterior index (DAI: Kao and Elinson, 1988).

Table 1 shows 7 independent experiments of dorsal or ventral cytoplasmic transfer from normal to UV-F embryos. The mean DAI of embryos injected with ventral cytoplasm was 0.6 and was not statistically different from the DAI of embryos only irradiated with UV at the pre-cleavage stage (mean DAI= 0.7). The mean DAI of embryos injected with dorsal cytoplasm was 1.5 and was statistically different from the mean DAI of both UV-irradiated controls and ventral cytoplasm-injected embryos ($p<0.001$). Dorsal cytoplasm injection caused a decrease in the proportion of embryos which lacked dorsal axial structures completely (DAI 0), and an increase in the proportion of embryos which had partial dorsal axial structures (DAI 3). Some embryos injected with dorsal cytoplasm could develop their dorsal axial structures to the level of DAI 4.

UV-O embryos at the 8-16 cell stage were also injected with dorsal and ventral cytoplasm from normal 8-16 cell embryos into one of the vegetal blastomeres of the less pigmented side. The results of 7 independent experiments are summarized in Table 2. Almost all controls (embryos developed from unirradiated oocytes transferred to the host female and then fertilized) developed normally (mean DAI= 4.9). The UV-irradiated embryos failed to develop normally; many of them developed into dorsal axis-deficient embryos. However, some UV-irradiated embryos developed dorsal axial structures with various degrees of development

TABLE 2

DORSOANTERIOR INDEX OF UV-O EMBRYOS INJECTED WITH DORSAL OR VENTRAL CYTOPLASM FROM NORMAL EMBRYOS

Treatment[a]	No. of embryos		Dorsoanterior index[b]						
	total	survival	0	1	2	3	4	5	mean±SD[c]
UV control (No cytoplasm injected)	70	43	19	10	6	2	4	2	1.3±1.5[d]
D cytoplasm injected	66	46	16	13	9	5	3	0	1.3±1.2[d]
V cytoplasm injected	39	24	15	6	1	1	1	0	0.6±1.0[d]
Normal control	84	57	0	0	0	1	8	50	4.9±0.4

[a]Defolliculated oocytes were irradiated with UV, matured *in vitro*, transferred into a body cavity of a hormone primed host female, and then spawned. After fertilization, D (dorsal) or V (ventral) cytoplasm from normal 8-16 cell embryos were injected into a vegetal cell of the UV-O embryos at 8-16 cell stage. [b]According to Kao and Elinson (1988). [c]Mean DAI was calculated by summing individual index and dividing by the total number of individuals. [d]Not significant compared with each other.

TABLE 3

SECONDARY AXIS INDUCTION AFTER DORSAL LIP IMPLANTATION FROM UV-O TO NORMAL EMBRYOS

Origin of dorsal lips	No. of embryos		Degree of induction[a]		
	implanted	survived	I	II	III
UV-O	39	35	10 (28.6%)	19 (54.3%)	6 (17.1%)
Normal	39	34	20 (58.8%)	7 (20.6%)	7 (20.6%)

[a]Degree of induction: I, embryos with two clear heads or anterior dorsal axes; II, embryos with secondary tails or a process-like structure; III, no induced secondary axis.

because the dose of UV used in this experiment was relatively low, and because it was difficult to place the defolliculated oocytes on a quartz slide glass with the correct orientation (with their vegetal halves facing down). Thus, the mean DAI of UV-O embryos was relatively high (DAI= 1.3).

The mean DAI of UV-O embryos injected with dorsal cytoplasm was 1.3, which was identical to that of UV-irradiated controls and was not different from that of ventral cytoplasm-injected embryos (DAI= 0.6). The injection of cytoplasm from normal embryos into UV-O embryos did not change the dorsal axial development of the injected embryos.

Dorsal lip implantation

UV-O embryos formed a dorsal lip normal in appearance, but many of them developed into embryos without dorsal axial structures. To investigate the Spemann's organizer activity of the dorsal lip of the UV-O embryo, an implantation of the dorsal lip was done (Table 3). When the dorsal lips from unirradiated embryos (controls) were implanted into blastocoels of normal embryos, 20 embryos out of 34 (58.8% of the recipients) developed into double embryos or embryos with secondary axes. In contrast, when the

dorsal lips from UV-O embryos were implanted into blastocoels of normal embryos, secondary axes were induced at a lower frequency (10 embryos out of 35, 28.6%). These results suggest that the dorsal lip of UV-O embryos has low Spemann's organizer activity in spite of the normal-looking morphology.

Discussion

UV-irradiation of the vegetal surface of either full-grown oocytes (UV-O) or fertilized eggs (UV-F) leads to a similar UV syndrome, namely dorsal axial deficiency (Malacinski *et al.*, 1975; Holwill *et al.*, 1987; Elinson and Pasceri, 1989). Although UV-F embryos showed a radially symmetrical pattern throughout embryogenesis, UV-O embryos showed a clear dorsoventral polarity until early gastrula stage (Figs. 2, 3). This observation supports the idea that UV targets are different between UV-F and UV-O embryos. Elinson and Pasceri (1989) have suggested that the UV target in fertilized eggs is a paralleled microtubules array, but the target in oocytes is a "dorsal determinant". Because the machinery for driving the cortical rotation (Vincent *et al.*, 1986; Elinson and Rowning, 1988) has been reported to be destroyed by UV in UV-F embryos (Elinson and Pasceri, 1989), they cannot polarize dorsoventrally. It is thus assumed that UV-F embryos fail to form their dorsal axial structures in spite of the intact presence of the "dorsal determinant(s)". This concept is consistent with three different findings showing (1) that the UV-F embryos can be rescued completely only by tilting the irradiated eggs before first cleavage (Scharf and Gerhart, 1980; Elinson and Pasceri, 1989), (2) that the dorsal axis-inducing activity, which has initially been reported to be localized to the dorsal equatorial region of 8-16 cell embryos (Yuge *et al.*, 1990), remains near the vegetal pole beyond the first cell cycle and does not appear in the equatorial region in UV-F eggs (Fujisue *et al.*, 1993), and (3) that vegetal cortical cytoplasm from UV-O embryos does not retain the dorsalizing activity, whereas the cytoplasm from UV-F embryos does (Holowacz and Elinson, 1993).

UV-O embryos were able to construct a dorsoventral polarity at a morphological level during early cleavage and to form normal-looking dorsal lips at the gastrula stage, but failed to develop dorsal axial structures. Dorsal lips from UV-O embryos showed low activity of Spemann's organizer when they were implanted into the blastocoels of normal embryos (Table 3), though they looked normal at a morphological level (Fig. 3F,I). It is thus evident that morphological differentiation of dorsal lips of the blastopore does not imply functional differentiation of Spemann's organizer. A similar phenomenon was reported in the embryos injected with RNA of BMP-4; they showed clear dorsal lips, but later developed into ventralized embryos similar to UV-O embryos (Dale *et al.*, 1992; Jones *et al.*, 1992). These results suggest that there are some important events between morphological differentiation of the dorsal lip and subsequent successful neural induction.

A characteristic of UV-0 is the absence of a normally developed archenteron (Fig. 4F and I). A similar abnormality has been reported in *Xenopus* embryos in which a gene coding G-protein α subunit was overexpressed (Otte *at al.*, 1992), and also in urodele embryos. In the latter an interaction between fibronectin and its receptors was prevented by microinjection either of antibodies to fibronectin or of a competitive peptide inhibitor of fibronectin function (Boucaut *et al.*, 1984). Thus, UV irradiation to the vegetal region of oocytes is assumed to destroy or inactivate some vegetally localized molecules participating in the signal transduction cascades which are involved in neural induction, or it is assumed to affect molecules participating in the interaction between fibronectin and the receptors which promote mesodermal cell migration.

RNA molecules of the *Wnt* family are known to be able to induce a secondary axis and rescue UV-F embryos (Smith and Harland, 1991; Sokol *et al.*, 1991; Chakrabarti *et al.*, 1992). It has also been reported that the RNA molecule *noggin* can rescue UV-F embryos (Smith and Harland, 1992). Furthermore, Hainski and Moody (1992) reported that the RNA fraction derived from animal-dorsal blastomeres can induce a secondary axis in normal embryos and rescue the dorsal development in UV-irradiated embryos. Thus, the activity of dorsal cytoplasm resembles that of these RNAs.

Recently, Holowacz and Elinson (1993) have shown that dorsal axis formation can be rescued in UV-O embryos by injection of vegetal cortical cytoplasm from uncleaved UV-F eggs. In contrast, we failed to rescue the UV syndrome in UV-O embryos by injecting the dorsal vegetal cytoplasm from normal 8-16 cell embryos (Table 2). The basic difference between the two experiments was the stage of the donor embryos: vegetal cortical cytoplasm from uncleaved eggs (Holowacz and Elinson, 1993) or vegetal dorsal cytoplasm from 8-16 cell embryos (this study). The simplest explanation for the different results is that the dorsal cytoplasm from normal embryos at the 8-16 cell stages has already lost some factor(s) or activity indispensable for rescuing UV-O embryos. Rationally, an experiment to rescue the UV-O embryos by transplanting either vegetal cytoplasm of uncleaved eggs or vegetal cytoplasm from full-grown oocytes is absolutely necessary. Since the oocytes which had been irradiated with UV, matured *in vitro*, and then injected with dorsal or vegetal cytoplasm could not be recovered from the host females, this type of experiment was left for the future.

Materials and Methods

Animals and embryos

Animals used in this study were African clawed frogs, *Xenopus laevis*, reared in our laboratory. Fertilized eggs were obtained through artificial insemination (Neff *et al.*, 1983; Wakahara, 1986). Developmental stages of the eggs and embryos were determined according to Nieuwkoop and Faber (1967).

Oocyte transfer procedures

Oocyte transfer procedures of Heasman *et al.* (1991) were used with some minor changes (Fig. 1). Pieces of ovaries excised from a female were divided into small pieces in 70% Leibowitz medium (70% L-15: Flow Lab., McLean, VA, USA) with 0.04% bovine serum albumin (BSA), 5 mM HEPES (pH 7.4), 100 IU/ml penicillin G potassium and 100 μg/ml streptomycin sulfate. The ovary fragments were transferred to agar-coated dishes (1.5% agar in modified Barth's saline buffered with HEPES (MBSH, Gurdon, 1976) in 70% L-15. Then full-grown oocytes were defolliculated with two pairs of sharpened watchmaker's forceps.

Defolliculated oocytes were stimulated to advance to second meiotic metaphase by adding progesterone (1 μg/ml final concentration) to the 70% L-15. They were allowed to mature for 9 to 12 h at 15°C. After oocyte maturation, UV-irradiated and control (unirradiated) eggs were stained with 0.0025% neutral red and with 0.0005% Nile blue sulfate, respectively, for 15 min in order to distinguish experimental and host eggs.

A host female frog was injected with 800 IU HCG (human chorionic gonadotropin, Teikokuzoki, Tokyo, Japan) about 12 h before receiving donor (stained) eggs at 15°C. After the frog began to spawn her own eggs, she was anesthetized. The stained eggs were transferred into her body cavity through a small ventral incision using a Pasteur pipette. She began to spawn the colored eggs invested with host jelly coats about 2.5-3 h later.

UV irradiation

UV irradiation was done at two different stages: at the full-grown oocyte stage (UV-O) and after fertilization (UV-F). For UV irradiation to fertilized eggs, the procedures described by Scharf and Gerhart (1980) were employed. For UV irradiation at the oocyte stage, the procedures of Holwill *et al.* (1987) or of Elinson and Pasceri (1989) were used. Duration of UV irradiation was determined empirically to obtain embryos that lacked dorsoanterior structures and to allow only a low death rate (20000-30000 ergs/mm^2 for UV-F, and 5000-8000 ergs/mm^2 for UV-O). At stage 34-38, embryos developed from UV-irradiated oocytes and eggs were scored using a dorsoanterior index (DAI, Kao and Elinson, 1988).

Cytoplasmic transfer

Cytoplasmic transfer from normal embryos to UV-irradiated embryos was conducted according to the method basically described by Yuge *et al.* (1990). Normal embryos at the 8-16 cell stage were used as cytoplasm donors; they were placed on a paraffin bed, and a portion of the cytoplasm was sucked from their dorsal or ventral vegetal blastomere using a wide tip (30-60 μm) micropipette connected to a microinjector (IM-6, Narishige, Tokyo, Japan). The cytoplasm was injected into one of the vegetal blastomeres of UV-F or UV-O embryos at the same stage as the donor.

Implantation of dorsal lip

In order to analyze Spemann's organizer activity of dorsal lips from UV-irradiated embryos, dorsal lips were implanted into blastocoels of normal embryos according to conventional procedures (Malacinski *et al.*, 1977). The implanted embryos were allowed to develop to stage 22 or more, and the degree of induction of secondary axes was graded according to Cooke's classification (Cooke, 1989).

Histological studies

For morphological studies, materials to be sectioned were fixed in Bouin's solution overnight at room temperature. Then they were dehydrated in a graded ethanol series, embedded in paraffin, and sectioned serially at 10 μm. They were then stained with Delafield's hematoxilin and eosin.

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