Two UV-sensitive targets in dorsoanterior specification of frog embryos

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Summary

Previous work has shown that ultraviolet (UV) irradiation of fertilized frog eggs yields embryos that lack dorsal and anterior structures. The eggs fail to undergo the cortical/cytoplasmic rotation that specifies dorso-ventral polarity, and they lack an array of parallel microtubules associated with the rotation. These eggs can be rescued by tilting with respect to gravity, and normal dorsoanterior development occurs. We find here that UV irradiation of *Xenopus* prophase I oocytes or *Rana* metaphase I oocytes also causes the dorsoanterior deficient syndrome, but the UV target is different from that in fertilized eggs. Tilting eggs, irradiated as oocytes, with respect to gravity, does not rescue dorsoanterior development, although lithium treatment does. The UV dose required to produce dorsoanterior deficiency for *Rana* metaphase I oocytes is much less than that for fertilized eggs, and the oocytes can form the array of parallel microtubules and undergo the cortical/cytoplasmic rotation after fertilization. Despite these features of normal development, no dorsoanterior structures form. While the UV target in fertilized eggs is thought to be the parallel microtubules (Elinson & Rowning, 1988; *Dev. Biol.* 128, 185-197), the UV target in the oocytes may be a dorsal determinant.

Key words: frog, *Xenopus laevis, Rana pipiens*, dorsal development, ultraviolet irradiation, oocyte maturation, grey crescent.

Introduction

The dorsoventral axis of the frog embryo is specified during the first cell cycle by a rotation of the egg cortex relative to the cytoplasm (Ancel & Vintemberger, 1948; Vincent et al. 1986). Through early cleavage, a few blastomeres contain dorsal information, and these cells induce others to become notochord and dorsal mesoderm (Nieuwkoop, 1969; Gimlich & Gerhart, 1984; Yamana & Kageura, 1987). A transforming growth factor activity has been implicated in the induction of dorsal mesoderm development (Kimelman & Kirschner, 1987; Rosa et al. 1988; Smith et al. 1988).

It is possible to interfere experimentally with dorsal specification in a variety of ways. Embryos with excessive dorsoanterior development can be produced by treating 32-cell embryos with lithium (Kao et al. 1986). All of the mesoderm is converted to dorsal mesoderm and differentiates into notochord (Kao & Elinson, 1988). Lithium appears to act on animal cells, so that when they are induced by vegetal cells, they form dorsal mesoderm (Slack et al. 1988; Kao & Elinson, 1989; Cooke et al. 1989). The extreme embryo produced by lithium has radial symmetry with radial dorsoanterior structures including eye, cement gland, notochord and heart.

Conversely, radial ventral embryos lacking all dorsoanterior structures are produced by irradiation of the vegetal half of the fertilized egg with ultraviolet (UV) light (Malacinski et al. 1975; Scharf & Gerhart, 1980). The critical period for this effect is the first half of the first cell cycle, prior to the cortical/cytoplasmic rotation that specifies dorsoventral polarity (Manes & Elinson, 1980; Vincent & Gerhart, 1987). UV irradiation prevents the rotation as well as the formation of an array of parallel microtubules associated with the rotation. We have suggested that the parallel microtubules are part of a mechanism for driving the cortical/cytoplasmic rotation, and their absence would account for the UV inhibition of rotation (Elinson & Rowning, 1988).

UV-irradiated eggs can be rescued by tilting the eggs with respect to gravity (Scharf & Gerhart, 1980). Gravity causes a cytoplasmic rearrangement which mimicks some aspect of the cortical/cytoplasmic rotation (Ubbels et al. 1983; Vincent & Gerhart, 1987) and the embryos develop the normal complement of dorsoanterior structures. Rescue of UV-irradiated eggs by gravity demonstrates that no essential component for dorsoanterior development is destroyed by UV, although the normal means for activating those components via the cortical/cytoplasmic rotation has been blocked by UV.

Recently, Holwill et al. (1987) found that UV irradiation of prophase I oocytes led later to dorsoanterior deficient embryos. UV irradiation up to two days prior to fertilization also had this effect, so it seems likely that the targets for UV in the prophase I oocyte and in the fertilized egg are different, even though they
both result in the dorsoanterior deficient syndrome. We present evidence here showing that is the case.

Materials and methods

Animals and embryos

Procedures for maintaining *Xenopus laevis*, induction of ovulation, insemination and dejellying were described by Kao & Elinson (1988). Comparable procedures for *Rana pipiens* were described by Elinson (1983). Normalized time was used to compare events in the first cell cycle of fertilized eggs. Insemination is time 0, first cleavage is 1-0, and intervening times are given as decimal fractions.

**UV irradiation and the dorsoanterior index**

Vegetal halves of oocytes and eggs were irradiated by UV through a quartz slide using a short-wave Mineralight Lamp (UVP, San Gabriel, CA). Exposure times were determined empirically to give embryos that cleaved normally and gastrulated but which lacked dorsoanterior development. Dosage of UV was measured with a Blak-Ray Short Wave UV Meter (UVP).

The various abnormalities were scored using the Dorsoanterior Index or DAI (Kao & Elinson, 1988). Embryos with no dorsoanterior development are DAI 0 while DAI 1 through DAI 4 represent progressively more dorsoanterior development. Normal embryos are DAI 5 while embryos with enhanced dorsoanterior development receive scores of DAI 6 to DAI 10. The DAI 10 embryo is radially symmetric with a radial retina and cement gland and large amounts of notochord. The dorsoanterior deficient embryos (DAI 1–4) are produced by UV, while the dorsoanterior enhanced embryos (DAI 6–10) are produced by lithium.

**Oocyte transfer**

For *Xenopus*, the oocyte transfer procedures of Holwill et al. (1987) were used. Pieces of ovary were removed surgically from a pregnant mare serum-primed female into OR-2 (Wallace et al. 1973) adjusted to pH 7.8 and containing 400 μg/ml−1 bovine serum albumen and 50 μg/ml−1 penicilin. Oocytes were dissected from ovarian follicles using watchmaker’s forceps and stored in modified OR-2 at 15°C until use. When sufficient numbers of oocytes were collected, some were UV-irradiated, and then all were induced to mature with a 10 min treatment in 10 μg/ml−1 progesterone in OR-2. Host females were injected with human chorionic gonadotropin at 3 p.m. at 23–1°C, and fertilization was performed the next day.

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**Results**

**Meiotic stage and UV-induced dorsoanterior deficiencies**

Holwill et al. (1987) reported that UV irradiation of full-grown *Xenopus* oocytes at prophase I caused them to develop as dorsoanterior deficient embryos following oocyte maturation and fertilization. We have repeated this experiment and have confirmed their results (Table 1). Prophase I oocytes were usually UV-irradiated 0.5–6 h before progesterone treatment and 16–22 h (at 18°C) before insemination. Most of the UV-irradiated prophase I oocytes lacked dorsoanterior structures (average DAI = 1.3). The most extreme embryos lacked an axis and appeared identical externally to DAI 0 embryos. Large numbers of red blood cells could be seen pooled in the living embryo, and a
inseminated after one day at 15-18°C. The resulting cytological
sections. Following UV irradiation, the sufficient to activate the oocyte. This compromise meant
vegetal half down on a quartz slide, UV-irradiated phase I oocytes to
metaphase II oocytes and oocytes had given off the first polar body and were at
8-10 h later than the metaphase I oocytes. These
embryos exhibited the usual dorsoanterior
deficiencies (Table 1).

UV irradiation had to be balanced between that necess-
ary to cause dorsoanterior deficiencies, with that insuf-
ficiencies with the procedure. The jelly absorbed UV
irradiation of
metaphase II oocytes caused
Xenopus oocytes. In order to UV irradiate
Rana
metaphase II oocytes, the dorsoanterior de-
deficiencies when applied to
prophase I oocytes damaged by
UV at earlier stages can be rescued by gravity. Tilting was usually begun at 0-3-0-4 of the first
cell cycle. For
Xenopus fertilized eggs in the first
half of the first cell cycle.

Gravitational rescue of dorsoanterior deficiencies
The dorsoanterior deficiencies caused by UV ir-
radiation of
Xenopus fertilized eggs can be prevented by
tilting the irradiated eggs with respect to gravity
during the first cell cycle (Schaff & Gerhart, 1980; Table 1). We have asked whether oocytes damaged by
UV at earlier stages can be rescued by gravity.

Oocytes were UV-irradiated as described in the
previous section, fertilized and tilted 90° with respect to
gravity. Tilting was usually begun at 0-3-0-4 of the first
cell cycle and stopped at first cleavage (1-0).

Xenopus eggs, UV-irradiated at prophase I, were not rescued
later by gravity (Table 1). This result suggests that the UV
target is different in the prophase I oocyte com-
pared to the fertilized egg.

Unlike those irradiated at prophase I,
Xenopus eggs, UV-irradiated at metaphase II, were rescued by gravity (Table 1). This result can be interpreted in several
ways, since the unilted eggs had some dorsoanterior
development (average DAI = 2-6). If the UV target

Table 1. Gravity rescue of UV-irradiated Xenopus oocytes and eggs

<table>
<thead>
<tr>
<th>Stage at irradiation</th>
<th>Control</th>
<th>UV</th>
<th>UV+Tilt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prophase I</td>
<td>5 (67)</td>
<td>0-28 (24)</td>
<td>0-17 (12)</td>
</tr>
<tr>
<td>Prophase I</td>
<td>4-6 (106)</td>
<td>1-8 (58)</td>
<td>2-3 (67)</td>
</tr>
<tr>
<td>Prophase I</td>
<td>5 (124)</td>
<td>0-71 (31)</td>
<td>0-58 (31)</td>
</tr>
<tr>
<td>Prophase I</td>
<td>4-9 (126)</td>
<td>2-3 (19)</td>
<td>2-9 (60)</td>
</tr>
<tr>
<td>Prophase I</td>
<td>4-95 (64)</td>
<td>0 (7)</td>
<td>0 (9)</td>
</tr>
<tr>
<td>Average DAI</td>
<td>4-9</td>
<td>1-3</td>
<td>1-9</td>
</tr>
<tr>
<td>Metaphase II</td>
<td>5 (41)</td>
<td>2-5 (28)</td>
<td>4-8 (14)</td>
</tr>
<tr>
<td>Metaphase II</td>
<td>4-7 (38)</td>
<td>2-3 (19)</td>
<td>4-3 (12)</td>
</tr>
<tr>
<td>Metaphase II</td>
<td>5 (44)</td>
<td>2-9 (42)</td>
<td>4-9 (37)</td>
</tr>
<tr>
<td>Metaphase II</td>
<td>5 (38)</td>
<td>2-5 (82)</td>
<td>5 (18)</td>
</tr>
<tr>
<td>Average DAI</td>
<td>4-9</td>
<td>2-6</td>
<td>4-8</td>
</tr>
<tr>
<td>Fertilized (0-27)</td>
<td>5 (69)</td>
<td>0-56 (89)</td>
<td>4-6 (27)</td>
</tr>
<tr>
<td>Fertilized (0-26)</td>
<td>5 (58)</td>
<td>0-92 (53)</td>
<td>4-5 (49)</td>
</tr>
<tr>
<td>Fertilized (0-29)</td>
<td>5 (84)</td>
<td>0-37 (73)</td>
<td>5-0 (53)</td>
</tr>
<tr>
<td>Average DAI</td>
<td>5</td>
<td>0-58</td>
<td>4-7</td>
</tr>
</tbody>
</table>

small clump of striated muscle was revealed by histo-
logical examination.

We have extended the analysis from
Xenopus pro-
phase I oocytes to
Xenopus metaphase II oocytes and to
Rana oocytes. In order to UV irradiate
Xenopus oocytes at metaphase II, jellied oocytes were stripped from the female’s ovisac into 200% Steinberg’s to
preserve fertilizability. They were oriented with the vegetal half down on a quartz slide, UV-irradiated vegetally, and then inseminated. There were several difficulties with the procedure. The jelly absorbed UV and underwent partial dissolution, particularly over the vegetal half. Sometimes, the jelly change altered the oocyte’s orientation. More importantly, however, was the fact that UV irradiation activated the oocytes, rendering them subsequently unfertilizable. The time of UV irradiation had to be balanced between that necessary to cause dorsoanterior deficiencies, with that insuf-
ficient to activate the oocyte. This compromise meant that it was possible to obtain groups of embryos with an average DAI of 2-3 (Table 1), but difficult to obtain large numbers of DAI 0 embryos. Nonetheless, as Chung & Malacinski (1980) previously mentioned, UV irradiation of
Xenopus metaphase II oocytes caused
dorsoanterior deficiencies (Table 1).

For
Rana, oocytes were collected from the female’s body cavity shortly after ovulation. These oocytes were at prometaphase I or metaphase I as confirmed by
cytological sections. Following UV irradiation, the oocytes were transferred to host females and usually inseminated after one day at 15-18°C. The resulting embryos exhibited the usual dorsoanterior deficiencies, and a DAI of less than one was easily obtained (Table 2).

Rana oocytes were also collected from donors about
8-10 h later than the metaphase I oocytes. These oocytes had given off the first polar body and were at
metaphase II, as shown cytologically. A variable fraction, from 10% to 70% in different experiments,

activated in response to a needle prick. This suggests that the
Rana metaphase II oocytes used here were not fully mature, and therefore slightly younger developmentally than the jellied
Xenopus metaphase II oocytes described above. As with
Rana metaphase I oocytes, UV irradiation of
Rana metaphase II oocytes led to dorsoanterior deficient embryos (Table 2).

In summary, UV irradiation caused later dorsoanterior deficiencies when applied to
Xenopus pro-
phase I, Xenopus metaphase II, 
Rana metaphase I, and
Rana metaphase II oocytes. The dorsoanterior def-
cicient syndrome is similar to that produced by UV irradiating
Xenopus or Rana fertilized eggs in the first
half of the first cell cycle.

Gravity rescue of dorsoanterior deficiencies
The dorsoanterior deficiencies caused by UV ir-
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Table 2. Gravity rescue of UV-irradiated Rana oocytes and eggs

<table>
<thead>
<tr>
<th>Stage at irradiation</th>
<th>Control</th>
<th>UV</th>
<th>UV+Tilt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prophase I</td>
<td>5 (42)</td>
<td>0-54 (64)</td>
<td>0-24 (34)</td>
</tr>
<tr>
<td>Prophase I</td>
<td>5 (30)</td>
<td>0-43 (37)</td>
<td>0-37 (30)</td>
</tr>
<tr>
<td>Prophase I</td>
<td>4-8 (56)</td>
<td>0 (11)</td>
<td>0 (11)</td>
</tr>
<tr>
<td>Prophase I</td>
<td>5 (31)</td>
<td>0-33 (12)</td>
<td>0-50 (10)</td>
</tr>
<tr>
<td>Prophase I</td>
<td>5 (58)</td>
<td>0 (38)</td>
<td>0 (28)</td>
</tr>
<tr>
<td>Prophase I</td>
<td>4-9 (30)</td>
<td>0-22 (22)</td>
<td>0-16 (32)</td>
</tr>
<tr>
<td>Prophase I</td>
<td>5 (13)</td>
<td>0-90</td>
<td>1-3 (42)</td>
</tr>
<tr>
<td>Prophase I</td>
<td>4-9 (80)</td>
<td>0-88 (17)</td>
<td>0-14 (21)</td>
</tr>
<tr>
<td>Average DAI</td>
<td>5-0</td>
<td>0-41</td>
<td>0-34</td>
</tr>
<tr>
<td>Metaphase II</td>
<td>4-8 (21)</td>
<td>0-88 (9)</td>
<td>1-3 (14)</td>
</tr>
<tr>
<td>Metaphase II</td>
<td>5 (35)</td>
<td>0-26 (23)</td>
<td>0-59 (22)</td>
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<td>0 (8)</td>
<td>0-95 (19)</td>
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<td>Metaphase II</td>
<td>5 (26)</td>
<td>3-0 (14)</td>
<td>2-5 (15)</td>
</tr>
<tr>
<td>Average DAI</td>
<td>5-0</td>
<td>1-0</td>
<td>1-4</td>
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<tr>
<td>Fertilized (0-31)</td>
<td>5 (22)</td>
<td>1-2 (18)</td>
<td>3-8 (18)</td>
</tr>
<tr>
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<tr>
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<td>5 (12)</td>
<td>0-24 (25)</td>
<td>4-6 (29)</td>
</tr>
<tr>
<td>Fertilized (0-35)</td>
<td>5 (6)</td>
<td>0 (7)</td>
<td>4-7 (7)</td>
</tr>
<tr>
<td>Average DAI</td>
<td>5-0</td>
<td>1-0</td>
<td>4-6</td>
</tr>
</tbody>
</table>
were a dorsal determinant, a DAI of 2-6 would imply that the determinant was only partially destroyed. Tilting might generate a sufficiently concentrated localization of the determinant, so that the embryos would develop normally. This was not the case with prophase I oocytes, since batches with intermediate DAI's of 1-8 and 2-3 showed little rescue with tilting (Table 1). The UV target in the metaphase II oocyte, therefore, appears different from that in the prophase I oocyte.

The dorsoanterior deficiency following UV irradiation of *Rana* fertilized eggs can also be prevented by tilting 90° with respect to gravity (Table 2). Tilting was usually begun at 0-35-0-45 of the first cell cycle and stopped at first cleavage (1-0). *Rana* eggs, UV-irradiated at metaphase I, were not rescued later by tilting (Table 2), similar to results with *Xenopus* prophase I oocytes. *Rana* eggs, UV-irradiated at metaphase II, were also not rescued by tilting, unlike the results with *Xenopus* metaphase II oocytes. This difference may be a species one, but further experiments would be necessary using *Xenopus* eggs with DAI 0-1 and ensuring that the eggs of the two species were the same developmental age. As discussed earlier, the *Rana* metaphase II oocytes may have been UV-irradiated at a slightly earlier stage compared to the *Xenopus* metaphase II oocyte.

**UV dose and gravity rescue**

The UV doses used for most of the experiments in Tables 1 and 2 were determined empirically as the dose that yielded an average DAI of less than one when applied to *Xenopus* or *Rana* fertilized eggs. Holwill *et al.* (1987) mentioned that lower doses produce dorsoanterior deficiency in *Xenopus* prophase I oocytes compared to *Xenopus* fertilized eggs, so the doses that we used may be high. Accordingly, we examined *Rana* metaphase I oocytes with respect to the UV dose required for dorsoanterior deficiency. A UV dose of 3-4×10⁻⁴ J mm⁻² was sufficient to yield an average DAI of less than one when applied to

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**Fig. 1.** Effect of UV irradiation at different times. Oocytes arrested at prophase (pro I) are stimulated to mature by progesterone (prog). They proceed to metaphase I (meta I), give off the first polar body, and arrest at metaphase II (meta II). The metaphase II arrest is broken by sperm entry, and the sperm pronucleus surrounded by a large aster migrates inward (0-4). By 0-8, the egg reaches metaphase of the first cell cycle. UV irradiation of the vegetal half causes later axis (dorsoanterior) deficiency (+) at the times indicated, but has no detrimental effect (−) when applied at 0-8. Tilting eggs in the first cell cycle with respect to gravity can rescue dorsoanterior development (+) of previously UV-irradiated oocytes and eggs in some cases but not in others (−). Lithium treatment of 32-cell embryos always rescues dorsoanterior development (+).
Rana metaphase I oocytes (5 experiments), while a dose of $1.7 \times 10^{-4} \text{J mm}^{-2}$ had little effect (4 experiments). In contrast, a UV dose of $10-16 \times 10^{-4} \text{J mm}^{-2}$ was required to produce an average DAI of less than one for Rana fertilized eggs (5 experiments). These results indicate that 3-4 times more UV is necessary to produce dorsoanterior deficiency for Rana fertilized eggs compared to Rana metaphase I oocytes.

Use of high UV on Rana metaphase I oocytes, however, was not the reason that gravity failed to rescue them. The last three entries for Rana metaphase I oocytes in Table 2 are based on doses of 5-1, 3-4 and $3.4 \times 10^{-4} \text{J mm}^{-2}$, respectively; yet gravity rescue was unsuccessful.

We also considered that UV irradiation of fertilized eggs made it easier for gravity to rearrange the cytoplasm and to rescue development. In two experiments, Rana fertilized eggs, previously irradiated at metaphase I with $3.4 \times 10^{-4} \text{J mm}^{-2}$, were irradiated again with $13-8 \times 10^{-4} \text{J mm}^{-2}$ prior to tilting. The second UV irradiation did not help, and all of the tilted embryos lacked dorsoanterior development.

Lithium rescue of dorsoanterior deficiencies

Dorsoanterior deficient Xenopus embryos can also be rescued by treatment with lithium at the 32-cell stage (Kao et al. 1986). We wanted to see whether lithium could rescue dorsoanterior development in Xenopus eggs, UV-irradiated earlier at prophase I. Xenopus prophase I oocytes were UV-irradiated, progesterone-treated and later fertilized. When they reached the 32-cell stage, they were treated with 0-3 M LiCl in 20% Steinberg’s for 6-10 min. In three experiments, lithium promoted enhanced dorsoanterior development (Table 3). Most embryos approached the radial extreme with radial pigmented retina, a radial cement gland, and a reduced or absent tail and trunk (DAI 8–10). There was no rescue in one experiment, perhaps due to a lower sensitivity to lithium found in some batches of eggs.

Lithium also caused dorsoanterior development in Rana. In preliminary experiments, Rana fertilized eggs were UV-irradiated and treated with Li+ at early cleavage stages to determine a dose. Patterns of enhanced dorsoanterior development, similar to those described for Xenopus (Kao & Elinson, 1988), were obtained by treating Rana 32-cell embryos with 0.4–0.5 M Li+ for 6 min. The Li+ concentration is higher than the 0.3 M Li+ usually used for Xenopus embryos.

Rana metaphase I oocytes were UV-irradiated, transferred to a host female and later inseminated. When they reached the 32-cell stage, they were treated with 0.3-0.5 M Li+ for 6 min. Most of the embryos had enhanced dorsoanterior development (Table 3), and twins, Janus twins and radial proboscis embryos were produced. Dorsoanterior development was strongly evoked by lithium even though tilting of Rana eggs, UV-irradiated at metaphase I, did not rescue dorsoanterior structures.

The lithium rescue results are summarized in Fig. 1.

Grey crescent formation in UV-irradiated oocytes

UV irradiation of fertilized eggs prevents the cortical/cytoplasmic rotation that produces the grey crescent. Rana eggs, UV-irradiated at metaphase I, were examined for grey crescents at the time of first cleavage in order to see whether UV treatment of oocytes had the same effect as treatment of fertilized eggs. Grey crescents were clear on transferred control eggs and measured 0-52 ± 0-13 mm at their maximum height between animal and vegetal halves (Table 4). This height corresponds to a 35° rotation of the cortex relative to the cytoplasm, similar to the 30° found on Xenopus eggs by the dye imprinting method (Vincent et al. 1986).

Grey crescents were also clear on eggs, UV-irradiated at metaphase I, although there was a 25% reduction in the height of the grey crescent (Table 4). The UV dose was sufficient to inhibit dorsoanterior development completely, since sibling eggs developed with an average DAI of less than one. The embryos lacked dorsoanterior structures even though they had undergone most of the normal rotation of the cortex relative to the cytoplasm.

Higher UV doses led to greater inhibition of grey

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Control</th>
<th>UV+ 0.3 M Li+</th>
<th>UV+ 0.4 M Li+</th>
<th>UV+ 0.5 M Li+</th>
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<tbody>
<tr>
<td>(A) Xenopus prophase I oocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>5 (37)</td>
<td>0-10 (29)</td>
<td>8-3 (36)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>4-4 (10)</td>
<td>0 (9)</td>
<td>8-2 (6)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>5 (41)</td>
<td>2-6 (26)</td>
<td>7-5 (15)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>4-9 (21)</td>
<td>0-81 (27)</td>
<td>1-2 (35)</td>
<td></td>
</tr>
<tr>
<td>(B) Rana metaphase I oocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>5 (33)</td>
<td>0 (21)</td>
<td>7-2 (18)</td>
<td>9-9 (10)</td>
</tr>
<tr>
<td>6</td>
<td>5 (66)</td>
<td>0-61 (33)</td>
<td>0-85 (33)</td>
<td>4-9 (20)</td>
</tr>
<tr>
<td>7</td>
<td>4-9 (39)</td>
<td>0-13 (38)</td>
<td>1-7 (27)</td>
<td>6-3 (27)</td>
</tr>
</tbody>
</table>

* All Li treatments were 6 min except for experiments 1 and 3 where 10 min was used.
† Only one embryo survived; the rest exogastrulated.
Table 4. Grey crescents on UV-irradiated Rana metaphase I oocytes

<table>
<thead>
<tr>
<th>Female</th>
<th>Control</th>
<th>UV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grey crescent (mm)</td>
<td>Dose ($\times 10^{-4}$J mm$^{-2}$)</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>4-6</td>
</tr>
<tr>
<td>2</td>
<td>4-7</td>
<td>3-4</td>
</tr>
<tr>
<td>3</td>
<td>4-9</td>
<td>5-1</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>3-4</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>5-1</td>
</tr>
<tr>
<td>Average</td>
<td>0.52±0.13</td>
<td>0-39±0.11</td>
</tr>
</tbody>
</table>

crescent formation. The lamp output was not measured in these experiments, but the doses were similar to those used for fertilized eggs. In the most extreme experiments, two thirds of the eggs (17/26) lacked grey crescents. UV irradiation of metaphase I oocytes can inhibit later grey crescent formation, but at a dose greater than that required to eliminate dorsoanterior development.

Parallel microtubules in UV-irradiated oocytes

A transient array of parallel microtubules appears at the vegetal surface of eggs undergoing the cortical/cytoplasmic rotation, and UV irradiation of fertilized eggs eliminates the array (Elinson & Rowning, 1988).

We have asked whether fertilized eggs, developing from UV-irradiated Rana metaphase I oocytes, have parallel microtubules. Oocytes were irradiated with low doses of UV (2-6-6-9$\times 10^{-4}$J mm$^{-2}$), sufficient to eliminate dorsoanterior development. After fertilization, the eggs were fixed during the first cell cycle for immunocytochemistry of microtubules. UV-irradiated eggs had parallel microtubules (Fig. 2A), comparable to those found in unirradiated eggs. Eggs fixed at 0-73, about midway in the rotation period, had nice patterns, while in three of four experiments, irradiated eggs fixed at 0-62 had microtubules that were often less organized than unirradiated eggs. Despite the possibility of a later formation, UV irradiation of metaphase I oocytes permits the appearance of parallel microtubules, even though dorsoanterior development is inhibited.

With higher doses of UV, more disruption of the parallel array was found. Many eggs had cobweb-like meshworks of microtubules (Fig. 2B), or areas of meshwork with patches of parallel microtubules. The parallel patches were often near the equator. We suspect that the higher UV dose is causing a general disruption of the cortical area, and this prevents the organization of microtubules into a parallel array.

Discussion

UV irradiation of the vegetal halves of frog oocytes and eggs eliminates dorsoanterior development whether applied at prophase I, metaphase I, or just after fertilization. The targets for UV inactivation appear to differ depending on the stage of the egg. UV irradiation of fertilized eggs stops the cortical/cytoplasmic rotation leading to grey crescent formation (Manes & Elinson, 1988).

Fig. 2. Immunofluorescent detection of microtubules in the vegetal cortex. (A) This Rana egg was UV-irradiated at metaphase I with 4-6$\times 10^{-4}$ J mm$^{-2}$. After maturation and fertilization, it was fixed at 0-69 normalized time and stained to reveal the characteristic array of parallel microtubules. Sibling eggs had no dorsoanterior development (DAI 0, 27 eggs), and grey crescents were present but reduced to 62% of control values. (B) This Rana fertilized egg (0-64) received a higher UV dose at metaphase I than the egg in A. A cobweb-like meshwork of microtubules is present. Scale lines = 25 μm.
1980; Vincent & Gerhart, 1987) and prevents the formation of an array of parallel microtubules associated with the rotation (Elinson & Rowning, 1988). Since these eggs can be rescued by gravity, the UV lesion is a failure of cytoplasmic rearrangement rather than the destruction of an essential dorsal determinant (Scharf & Gerhart, 1980; Chung & Malacinski, 1980).

In contrast, UV irradiation of Xenopus prophase I or Rana metaphase I oocytes inhibits later dorsoanterior development in a different way. This conclusion is derived from three results. First, the dose required to eliminate dorsoanterior development is much less for Xenopus prophase I (Holwill et al. 1987) and Rana metaphase I oocytes than for fertilized eggs. Second, eggs, irradiated as oocytes, cannot be rescued by a gravity-induced cytoplasmic rearrangement. Third, irradiated Rana eggs exhibiting parallel microtubules and grey crescent formation, nonetheless failed to develop dorsoanterior structures. There was a 25% reduction in the amount of cortical/cytoplasmic rotation, raising the possibility that an essential small movement was inhibited. This seems unlikely since gravity rescue failed and since a reduction by as much as 70% of the rotation following UV irradiation of fertilized Xenopus eggs permitted dorsoanterior development (Vincent & Gerhart, 1987).

The UV target in the oocyte is more sensitive than that in the fertilized egg. This indicates that the oocyte target must be moved or changed during oocyte maturation. Otherwise, the higher dose of UV used on fertilized eggs would inactivate the oocyte target as well, and gravity rescue would not be possible.

About 70% of the UV is absorbed within 60 μm of the egg surface (Youn & Malacinski, 1980). The vegetal cytoplasm within this region of Xenopus prophase I oocytes is special as it contains high concentrations of Vg1 mRNA, tubulin mRNA, and poly A(+) RNA (Melton, 1987; Larabell & Capco, 1988). Vg1 mRNA is a localized mRNA, which may be involved in dorsoanterior development. It codes for a TGF-β-like protein (Weeks & Melton, 1987), and TGF-β can induce animal cells in vitro to develop dorsal mesoderm (Kimelman & Kirschner, 1987; Rosa et al. 1988). Vg1 mRNA is localized close to the vegetal cortex in prophase I oocytes but disperses through the vegetal cytoplasm by the time of fertilization (Weeks & Melton, 1987), a property expected of the oocyte UV target. One hypothesis for the effect of UV on oocytes is that it alters an mRNA like Vg1 mRNA, preventing its later utilization in dorsoanterior development.

If UV irradiation of prophase I or metaphase II oocytes inactivates a mRNA or other dorsal factor, embryos would lack dorsoanterior development even with a normal or gravity-driven cytoplasmic rearrangement, as was found. Lithium, however, rescued dorsoanterior development, indicating that lithium affects a step downstream from the action of the factor. Lithium causes animal cells to interpret a ventral mesodermal signal as a dorsal signal (Slack et al. 1988; Kao & Elinson, 1989; Cooke et al. 1989). The dorsal factor could be a signalling molecule which acts through the same intracellular messenger system that lithium affects. In this way, eggs lacking the dorsal factor would be rescued by lithium but not by gravity.

UV is a relatively nonspecific agent with many targets; yet, it produces very specific effects on development. UV prevents dorsoanterior development by two different routes depending on the time of application. When applied to fertilized eggs, UV prevents the cortical/cytoplasmic rotation, likely due to an effect on microtubules. When applied to oocytes, UV permits the later cortical/cytoplasmic rotation but inactivates a factor required for dorsal development. In addition, UV activates unfertilized eggs and eliminates primordial germ cells. In a sense, UV behaves like many growth factors, hormones, or other signals in development. Specific signals are used to activate precise responses, but the response is a reflection more of the responding system than of the signal’s identity.

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References


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