Effects of partial irradiation of monochromatic uv-light on the embryonic development of the centrifuged *Chironomus samoensis* eggs

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Introduction

By centifuging or uv-irradiating the chironomid eggs, various developmental types were obtained (Yajima, 1960, 1964; Kalthoff and Sander, 1967). The developmental types expected by centrifugation were modified by the subsequent uv-irradiation (Kalthoff and Jacle, 1982; Yajima, 1985). In the previous study, combining centrifugation of *Chironomus* egg and the successive uv-irradiation to the anterior end using germicidal lamp, Yajima obtained two modified patterns, normal larvae (NL) to double abdomen (DA) and double cephalon (DC) to inverted embryo (IE) (Yajima, 1985). In the former pattern, head and thorax are modified into posterior structures, namely abdomen. And, in the latter, anterior head of the two heads is replaced by abdomen. Thus, these modified patterns may be collectively considered as 'posteriorization' of the anterior half of embryos. Centrifugation causes some egg constituents to shift from the original places of the egg and the others not. In the present study, the anterior ends of centrifuged eggs were irradiated with monochromatic uv-light to examine such posteriorization depending on whether movable or immovable egg components, or both are damaged by uv-light.

Material and Methods

Eggs of *Chironomus samoensis* eggs used in the present study were obtained from females reared in the laboratory.

Centrifugation

The egg masses, containing the eggs at 2 pole cell stage, were centrifuged at 4,200 r.p.m. (2,600g) for 5 min as described previously (Yajima, 1983). In the present experiment, only anteriorly centrifuged eggs were used. In the eggs, the constituents are stratified into the anterior centrifugal yolky, intermediate clear cytoplasmic and posterior centripetal fatty zones. The anteriorly centrifuged eggs yield more double malformations than the posteriorly centrifuged eggs. Since the developmental types and their frequencies after the combined centrifugation and uv-irradiation change depending on the condition of centrifuging, the data of the irradiated eggs, the centrifuged controls of which yield DC at about 50%, were pooled to minimize the differences among the single experiments.

UV-irradiation

Irradiation of the anterior end of centrifuged eggs, in the direction along the long axis, with monochromatic uv-light from 245 nm to 305 nm at 10 nm interval with 3,000 J/m^2 was performed using a small glass vessel as previously reported (Yajima, 1983). The irradiation was done with a high-pressure mercury lamp and a grating monochromator (NX-25C, Jasco).

UV-irradiation was done either at 30 min or at 150 min after the centrifugation. The former treatment will be called 'early irradiation' and the latter 'late irradiation' thereafter. At the early irradiation, the stratified egg constituents are at almost the same positions with those at the stage just after the centrifugation. As a result, the uv-irradiation from the anterior side damages the anterior yolky end, while the clear cytoplasmic zone with the cleavage nuclei is shielded from the uv-beam by the yolky zone. On the other hand, by the late irradiation stage, the clear cytoplasm carrying the cleavage nuclei have redistributed along the egg surface towards the both ends of the egg and the tips have arrived at 2/3 of the entire process of redistribution. Thus, the tips of the clear cytoplasm should be exposed to the uv-light as well as the yolky end.

Photoreversion treatment

After the uv-irradiation, a half of the irradiated eggs was transferred to a light-proof box and another half was placed under a fluorescent lamp for photoreversion-treatment.

Incubation of the centrifuged eggs

After the treatments of the centrifuged eggs with uv, the eggs were incubated over 24 hr at 20℃ until the developmental types were able to examine.

Results and Discussion

Early uv-irradiation

As shown in Table 1, the uv-irradiation of centrifuged *Chironomus* eggs at 245 nm and 255 nm at 30 min after the centrifugation increased frequencies of both DAs and IEs 30% more than these found in the centrifuged controls, while DC and NL decreased in reverse proportion to the increment of the DAs and IEs. At 265 nm, the frequencies of DAs and IEs were 19.7% and 25.4%, respectively. In the uv-irradiation at 275 nm to 305 nm, the frequencies of these two types of abnormal embryos decreased gradually with increasing of the wavelengths. Furthermore, these uv-damages after the early irradiation were not photorecovered (data were not shown). These results coincide with the early uv-irradiation of the centrifuged eggs with the germicidal lamp (predominantly 254nm) (Yajima, 1985).

Late uv-irradiation

In the uv-irradiation at the 150 min after the centrifugation, 'posteriorized types' were not obtained at the same rate as those found in the early irradiation. The frequencies of IEs were produced at the rates from 6.2% to 10.6% of the irradiated eggs at the wavelengths from 245 nm to 265 nm and DAs at the rates from 10% to 13.6% at these wavelengths (Table 1). The frequencies of IEs at the 285 nm and 295 nm irradiations were 5.9% and 7.4%, respectively, and these of DAs at the two wavelengths were 12.0% and 10.3%, respectively. The uv-irradiations either at 275 nm or at 305 nm did not induce any of these types. Since the uv-sensitivity of the anterior yolky end did not change between the early and late uv-irradiation (Yajima, 1985), these low frequencies of IEs and DAs after the late irradiation should be ascribed to the uv-damage to the tips of redistributing cytoplasm as well as the anterior yolky end.

Although the uv-effect after the late uv-irradiation were changeable by the successive photoreversion treatment, the results is somewhat different from usual photoreversion. By the late uv-irradiation, except at 275 and 305 nm, frequencies of normal larvae were significantly reduced to about half of the centrifuged controls in the embryos irradiated at 245 and 295 nm (in a χ^2, $P<0.05$) and at 255, 265, and 305 nm ($P<0.01$). Furthermore, although the late uv-irradiation to the centrifuged embryos did not induce a significant decrease of double cephalons, the subsequent photoreversion treatment caused a significant reduce of DC yields in the embryos irradiated at 245 and 285 nm from 42.7 to 25.8% ($P<0.05$) and from 48.9 to 28.5% ($P<0.05$), respectively. A slight decrease in the DC frequency also occurred in the embryos irradiated at 295 nm (from 42.7 to 31.2%: $0.10<P<0.20$). However, the successive exposure of the irradiated embryos to photorecovering light did not significantly recover the uv-damages. Frequencies of the posteriorized types (inverted embryos and double abdomens) were significantly increased from the centrifuged controls by the late uv-irradiation except at 275 and 305 nm ($P<0.001$). However, the subsequent photoreversion caused a significant increase of the posteriorized types only in the embryos irradiated at 285 nm ($P<0.05$). Since the developmental results after the early uv-irradiation were not changed by the successive photoreversion treatment, the changes of the effects of the late uv-irradiation by the photoreversion treatment should again ascribe to redistribution of the constituents of the centrifuged eggs to the range of uv-irradiation.

Since the early uv-irradiation of the yolky end of the centrifuged eggs causes the induction of IEs and DAs at the expense of NLs and DCs, the uv-light must damage some immovable factors necessary for the development of the anterior part of embryo (Table 1). However, whereas the posteriorized developmental types were induced in high frequencies after the early irradiation, the frequencies of those types after the late irradiation were below half of the early treatment. In this connection, the redistributing cytoplasm or the original clear cytoplasmic zone should contain another factor which promotes the development of the posterior parts of

Table 1 UV-irradiation of the centrifuged eggs and the photoreversion.

		NL(%)	IE(%)	DC(%)	DA(%)	OT(%)	UD(%)
cent.	(control)	45.5	0	48.5	0.8	3.0	2.3
245 nm	E.Dark	15.8	32.8	11.7	30.1	5.7	3.9
	L.Dark	23.5	9.4	42.7	13.6	5.3	5.5
	L.Light	28.8	15.9	25.8	20.9	5.8	2.9
255 nm	E.Dark	4.5	37.0	7.2	34.9	8.4	8.0
	L.Dark	22.0	6.2	47.3	11.5	5.5	7.6
	L.Light	20.9	7.6	37.9	15.9	9.1	7.9
265 nm	E.Dark	21.1	25.4	22.0	19.7	7.4	4.5
	L.Dark	23.9	10.6	42.1	10.0	7.3	6.1
	L.Light	25.5	13.6	36.7	13.3	4.2	6.7
275 nm	E.Dark	34.6	11.4	39.8	6.8	4.2	3.2
	L.Dark	34.2	2.7	47.4	2.7	6.1	6.8
	L.Light	33.3	8.3	42.7	6.1	4.2	5.3
285 nm	E.Dark	36.1	7.4	34.3	12.3	3.3	6.6
	L.Dark	21.5	5.9	48.9	12.0	7.0	4.7
	L.Light	26.1	13.6	28.5	21.1	8.5	2.3
295 nm	E.Dark	35.5	7.4	40.9	5.9	2.9	7.5
	L.Dark	28.0	7.4	42.7	10.3	6.1	5.5
	L.Light	32.4	13.6	31.2	16.7	0.8	5.3
305 nm	E.Dark	39.4	3.0	44.8	1.5	5.0	6.0
	L.Dark	43.3	0	49.8	0	3.5	3.3
	L.Light	43.9	1.5	50.0	0.6	2.4	1.5

Number of treated eggs for each wavelength: 250~300.
NL: Normal, IE: Inverted embryo DC: Double Cephalon, DA: Double Abdomen, OT: Other Types, UD: Undifferentiated, cent. (control) : centrifuged control, E.Dark: Early Dark (irradiated at 30 min after centrifugation and incubated in dark), L.Dark: Late Dark(irradiated at 150 min after centrifugation and incubated in dark), L.Light: Late Light (irradiated at 150 min after centrifugation and incubated in light).

embryo. Thus, even if the anterior determinative factor was damaged with uv-light, the posteriorized development did not assure as far as the posterior factor remains inactive as in the late irradiation. The development of 'posteriorized' types was resumed by reactivating the posterior factor by the successive photoreversion treatment.

Furthermore, effective wavelengths causing various types of development differs between the early and late irradiations. The posteriorized types after the early irradiation were induced in a narrow range of wavelengths around 255 nm, while those after the late irradiation were produced in more wider range, showing two peaks at 255 and 285 nm. Since photoreversion occurred only in the uv-damage after the late irradiation, the photoreversible uv-targets should be sensitive to the wavelengths around 285 nm. This again suggests that another factor carried by the recovering cytoplasm participates in inducing different spectrum of sensitive wavelengths in the late irradiation.

Considering together the present results of the early and late irradiations, the following conclusion may be obtained: there are two factors concerning the induction of inverted embryos and double abdomens. The first one is immovable by the centrifugation and the inactivation by uv-irradiation causes induction of IEs and DAs. The uv-damage of this factor is not photorepairable. The second one is movable by the centrifugal force used in the present experiments (2, 600g). The inactivation of this factor by uv inhibits the occurrence of IEs and DAs. But the re-activation by the successive photoreversion treatment after the uv-irradiation increases the frequencies of IEs and DAs.

It has been generally accepted that uv-light damages either morphogenetic determinants such as RNA or cell component such as cytoskeleton anchoring or transporting the determinative factors (see reviews: Wall, 1990; Slack, 1991). Kalthoff suggested the involvement of nucleic acid moiety in the uv-targets for the induction of *Smittia* double abdomen by the detailed action spectrum and the photoreversibility (1973). Furthermore, Kandler-Singer and Kalthoff (1976) obtained double abdomen by application of RNase (RNase A, Sigma) to the anterior pole of uncentrifuged *Smittia* embryo.

In a preliminary experiment applying RNase A (Sigma, 0.5μg/ml) to the anterior pole of *Chironomus* embryo by the method described by Kandler-Singer and Kalthoff (1976), we obtained 'posteriorized developmental types' at a rate of 11.5 and 8.7% of the treated eggs by the application at 30 and 150 min after the centrifugation, respectively. Similar experiment using 0.02μg of colchicine (Sigma) per ml were also effective to induce 'posteriorized types' at a rate of 12.5 and 16.0% of the treated eggs by applying at 30 and 150 min after the centrifuging, respectively. These results show that occurrence of posteriorized development may be ascribed to the damage to RNA or microtubles at the anterior pole of the centrifuged embryo.

In connection with this, Kuhn *et al.* (1987) established a strain of *Chironomus samoensis* which yields double abdomens in a great number. They suggested that the occurrence of DA in this strain should be ascribed to gene alteration in the fourth chromosome affecting cytoskeletal components involved in anchoring anterior determinant and segregating them into the anterior blastoderm cells.

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