Arrest of intravitelline mitoses in *Drosophila* embryos by u.v. irradiation of the egg surface

By SHIN TOGASHI AND MASUKICHI OKADA

Institute of Biological Sciences, University of Tsukuba, Sakura-mura, Nihari-gun, Ibaraki 305, Japan

SUMMARY

The intravitelline mitosis in *Drosophila* was arrested at the anaphase within the span of a single cell cycle after irradiation with 300 nm u.v. Embryos at and before the 8-nucleus stage were influenced by the u.v. only when irradiated anteriorly, while at and after the 16-nucleus stage, embryos are sensitive to either anterior or posterior irradiation. In embryos anteriorly irradiated at or before the 8-nucleus stage all nuclei in the embryo were prevented from performing mitosis. When irradiated at or after the 16-nucleus stage, inhibition of the intravitelline mitosis is limited to the nuclei in approximately anterior-half region of embryos in anterior irradiation, and to those in approximately posterior-half region in posterior irradiation, resulting in incomplete blastoderm formation. Sites sensitive to 300 nm u.v. are postulated to be present in the peripheral cytoplasmic region of the embryo and not in the nucleus, because the half-attenuation thickness of 300 nm u.v. light for the *Drosophila* egg cytoplasm is 3 μm and nuclei are at least 50 μm away from the periphery at the stage of irradiation. In addition lateral irradiation of a portion of an egg where there is no nucleus underneath was also effective in arresting division of nuclei in the same egg. It is suggested that the effects of 300 nm u.v. may not be conveyed to the nuclei from the periphery by simple diffusion of a substance, and a hypothesis is proposed for the involvement of cytoskeletal elements associated with the u.v. sensitive sites on the surface to the control mechanism of the intravitelline mitosis of the *Drosophila* embryo.

INTRODUCTION

In *Drosophila* embryogenesis, 13 successive mitoses take place synchronously before cellular blastoderm formation (Zalokar & Erk, 1976). The first nine mitoses occur at 9-min intervals within the central yolk-rich portion of the embryo producing 512 nuclei. About 400 of these nuclei penetrate the periplasm, where the nuclei carry out four more mitoses. These mitoses may be controlled by a genetical mechanism. Some mutations that affect the intravitelline mitoses have been reported in *Drosophila melanogaster* (Zalokar, Audit & Erk, 1975; Mahowald, Caulton & Gehring, 1979; Niki & Okada, 1981; review by Wright, 1970).

During experiments to examine the effects of u.v. irradiation on *Drosophila* embryogenesis, we found that the irradiation of the egg surface sometimes inhibits the intravitelline mitosis. During the intravitelline nuclear multiplication stage, nuclei are shielded from a direct hit of u.v. light by a thick cytoplasmic

layer. Nevertheless, the mitosis is aborted shortly after u.v. irradiation. This suggests the possibility that u.v. light sometimes hits a mechanism that is responsible for normal division of nuclei in the early development of *Drosophila*.

Inhibitory effects caused by u.v. irradiation, such as sterility (Geigy, 1931; Graziosi & Micali, 1974; Graziosi & Marzari, 1976; Togashi & Okada, 1983), lethality and morphological abnormalities (Goldman & Setlow, 1956; Hathaway & Selman, 1961; Ghelelovitch, 1966; Bownes & Kalthoff, 1974; Bownes & Sander, 1976) have been reported in *Drosophila* embryos. Hathaway & Selman (1961) reported an immediate death of embryos irradiated by u.v. between wavelengths of 290 nm and 370 nm. However, no previous report dealing with effects of u.v. on *Drosophila* embryogenesis has described an influence of u.v. on intravitelline mitoses.

We report our findings that 300 nm u.v. light brings about the abortive intravitelline mitosis, and that the effects of u.v. are different depending on the stage of the embryos at irradiation. A possible mechanism that may regulate the intravitelline mitosis is discussed.

MATERIALS AND METHODS

Egg collection

Embryos used for this study were obtained from a *Drosophila melanogaster* strain with the mutations *mwh e^{11}*. About 500 male and female adults aged 2 to 3 days after the eclosion were kept in bottles and allowed to lay eggs at 25 °C on small plastic dishes filled with a 2 % agar solution containing 50 % Welch's grape juice, 1 % ethylalcohol, and 1 % acetic acid. The first 2-hour-precollection was discarded. The eggs were collected for an appropriate time and incubated at 25 °C until they reached the required stage.

Irradiation

Two types of irradiation were performed (Fig. 1). In parallel irradiation, embryos were irradiated from the anterior or posterior, called anterior irradiation and posterior irradiation, respectively. In lateral irradiation, embryos were irradiated from the left or right perpendicularly to the embryo axis.

The following procedures were performed in an 18 °C room with red-light illumination. Embryos were dechorionated with a pair of watchmaker's forceps on double-stick Scotch tape attached to a microscope slide. The dechorionated embryos were fixed, by double-stick Scotch tape, to a second slide to be irradiated. The embryos were placed parallel to each other with either their anterior or posterior poles facing a u.v. source in parallel irradiation. When irradiated at various wavelengths (see Results), the time of irradiation was calculated so that embryos subjected to different wavelengths of radiation received the same total fluence. Correction of the fluence for quantum energy for each wavelength was made by calculation when necessary.
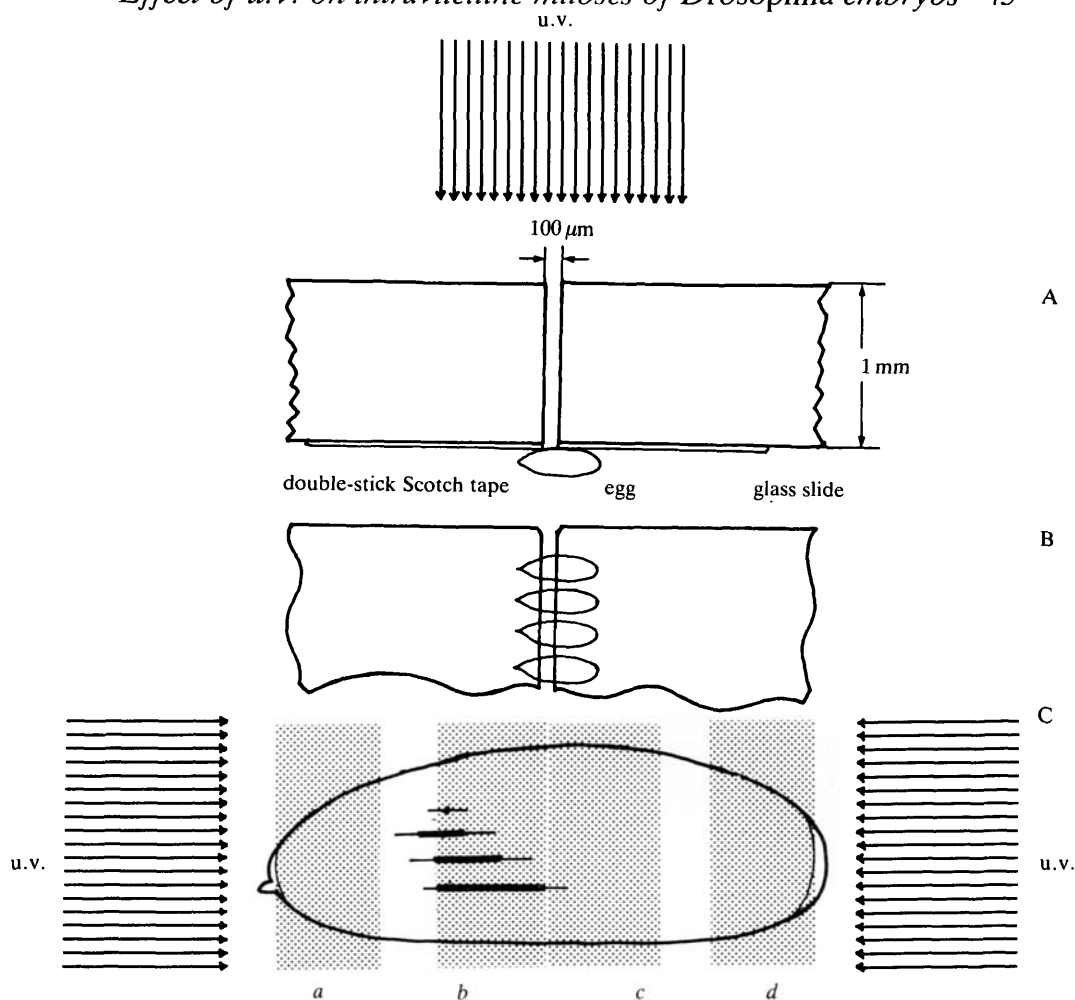
Fig. 1. Diagrams showing how eggs are irradiated. A and B, Lateral irradiation of eggs. Two glass slides 1 mm thick are placed side by side to make a 100-μm wide slit between them. Eggs were placed across the slit with double-stick Scotch tape on the slides, so that the eggs are irradiated only at the portion under the slit. A, A horizontal view; B, a vertical view. C, Portions laterally irradiated are indicated by screen (*a*, *b*, *c*, and *d*). Bars show ranges with standard deviation (thin bars) of anteroposterior distribution of nuclei in embryos at intravitelline nuclear multiplication stages; from the top, 1-, 2-, 4-, and 8-nucleus stage. Parallel arrows in A and C show fluxes of u.v. light.

The u.v. source used was a Hitachi 203 fluorescence spectrophotometer, which had a band pass of 10 nm, and emitted a parallel light flux to irradiate a rectangular area with the dimensions of 1 mm × 12 mm. As we did not use a microbeam, embryos were irradiated not only at the pole but also on sides (Fig. 1C), although fluence on the sides should be much lower than that at the poles; according to a simple calculation, the fluence on the ventral side was 6 % of that at the pole, on

the lateral sides, 1·2 %, and on the dorsal side, 0 %. Fluence rates at the pole facing the u.v. source in W/m^2 at wavelengths used were 0·44 at 230 nm, 2·65 at 254 nm, 1·84 at 280 nm, 3·60 at 295 nm, 5·38 at 300 nm, 6·30 at 305 nm, 4·52 at 320 nm, 8·21 at 360 nm, and 2·68 at 400 nm. The fluence rates were measured for each wavelength with IL 700 Research Radiometer (International Light INC.) with PT171C photodiode as the detector in a plane at right angles to the u.v. flux and exactly in the plane where embryos to be irradiated are placed. Values were corrected for each wavelength on the spectral sensitivity curve of the detector.

Lateral irradiation was performed as shown in Fig. 1A and B. Embryos were shielded by two glass slides between which was a 100 μm-wide slit, under which embryos were irradiated. Consequently, an embryo was irradiated in a 100 μm-wide region across the embryo at a portion *a*, *b*, *c*, or *d* as shown in Fig. 1C.

Postirradiation treatment

Immediately after irradiation, embryos were covered with a drop of paraffin oil and incubated in a dark, moist-chamber at 25 °C until 150 min after egg laying (AEL). By this time control (not irradiated) embryos had developed to the syncytial blastoderm stage. Nuclei in the periplasm are observable with a compound microscope installed with Nomarski differential interference optics. The irradiated embryos were scored at 150 min AEL for the presence or absence of nuclei in the periplasm. According to our preliminary observations, nuclear penetration of the periplasm never occurred in the irradiated embryos if it had not occurred by 150 min AEL.

Wholemount preparation

Embryos were fixed in heptane–alcohol–formalin–acetic acid, and stained with basic fuchsin according to the method of Zalokar & Erk (1977). The embryos were whole mounted and observed with a Nikon Apophoto microscope.

Transplantation of cytoplasm

Apparatuses and procedures used for transplantation of cytoplasm were basically the same as those described in the previous paper (Okada, Kleinman & Schneiderman, 1974a). Cytoplasm was taken from à single egg and transplanted into another single egg. The glass capillary was stuck at an angle into a donor egg from one side of the anterior region toward the anterior pole until the tip of the capillary hit the inner surface of the vitelline membrane at the anterior pole, and cytoplasm was taken from the very surface of the periplasm while observing the procedure under a microscope. The volume of cytoplasm transplanted varied between 0·1 and 0·2 nl per egg. After injection the recipient embryos were covered with a drop of paraffin oil and incubated in a moist chamber at 25 °C until scored.

RESULTS

Development of Drosophila *embryos discontinues when irradiated in the anterior pole region by u.v.*

The anterior pole region of *Drosophila* embryos at 35 min ± 20 min AEL when the embryos are at intravitelline nuclear multiplication stages were irradiated with 230, 254, 280, 295, 300, 305, 320, 360 or 400 nm radiation. Differences in the effect of the irradiation were found with u.v. of different wavelengths (Fig. 2). Approximately half of the embryos irradiated at 300 nm of the wavelength did not develop to the stage of nuclear penetration of the periplasm (Fig. 3B). Similar effects were observed in 295 nm and 305 nm radiation, although the effects were less than in 300 nm radiation. While the majority of embryos irradiated at the other wavelengths developed normally (Fig. 3A). In controls in which embryos were dechorionated but not irradiated, the frequency of the embryos that died before the blastoderm stage was approximately 20 % in this
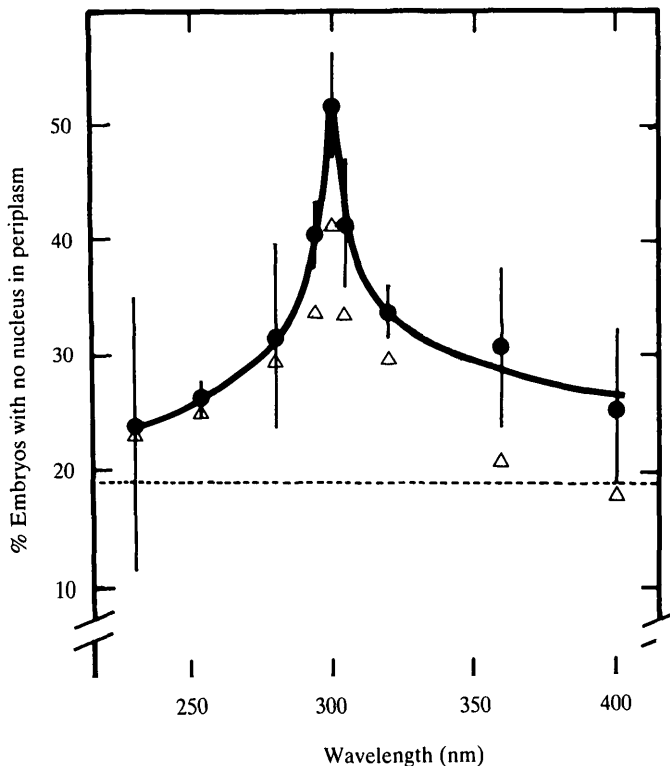

Fig. 2. Frequencies in per cent of embryos that cease development before the stage of nuclear penetration of the periplasm plotted against wavelengths of u.v. (●). The embryos, 35 ± 20 min AEL, were anteriorly irradiated at various wavelengths with a constant fluence of 100 J/m^2. Results from four independent experiments are combined, and each point represents at least 60 embryos, varying from 60 to 190. Values corrected for dosage at 10^{20} photons/m^2 are also shown (△). Vertical lines show s.e.m. Lethality in controls is shown by dotted line.
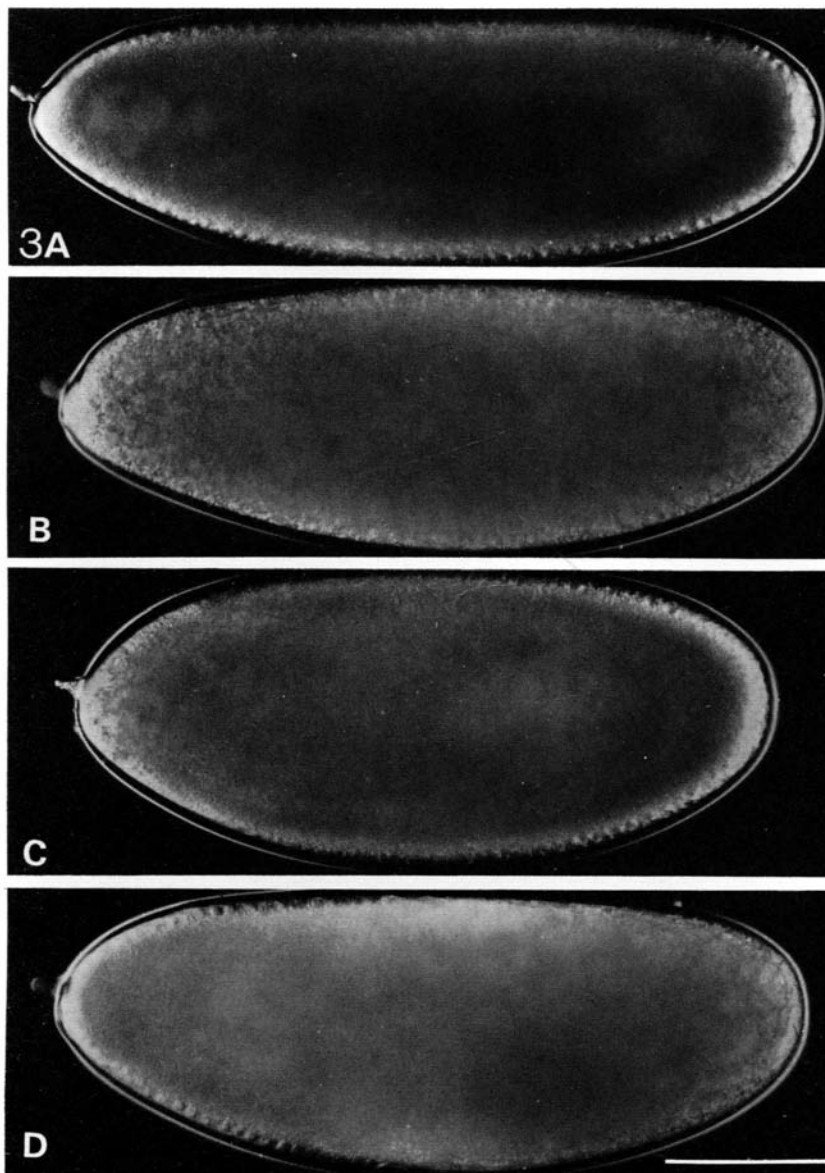
Fig. 3. Micrographs of living embryos. The anterior poles to the left. (A) A normal embryo at the syncytial blastoderm stage. (B) An embryo that has been anteriorly irradiated at the 8-nucleus stage and incubated for a period of time sufficient for a normal embryo to develop into the cellular blastoderm stage. Note that the syncytial blastoderm is not formed. (C) and (D), Incomplete blastoderms developed from embryos irradiated at the 16-nucleus stage anteriorly (C) or posteriorly (D). Magnification is the same for all pictures, and bar represents 100 μm.

series of experiments (Fig. 2, dotted line). This is rather a high level of lethality for controls; probably being caused by the dechorionated embryos being left uncovered in the laboratory as long as the time of exposure to u.v. in experimentals.

Some embryos receiving 295, 300 or 305 nm radiation developed into incomplete blastoderms, in which nuclei did not penetrate the periplasm of the anterior half of the embryo; in the posterior half of the embryo nuclear penetration of the periplasm and cellularization took place normally (Fig. 3C). No incomplete blastoderms were obtained from irradiation with the other wavelengths. When embryos of the same age (35 min ± 20 min AEL) were irradiated posteriorly, no developmental arrest occurred. This is consistent with our previous results (Togashi & Okada, 1983).

To compare the effect of u.v. at 295 nm and 300 nm the fluence-response relations were established (Fig. 4). In all five independent experiments, the development of embryos aged 35 ± 10 min AEL were inhibited more strongly with 300 nm radiation than with 295 nm radiation. The inhibition rate with 300 nm radiation at 200 J/m^2 reached a maximum at about 85 %, while with 295 nm radiation 400 J/m^2 was required to induce the same degree of inhibition.
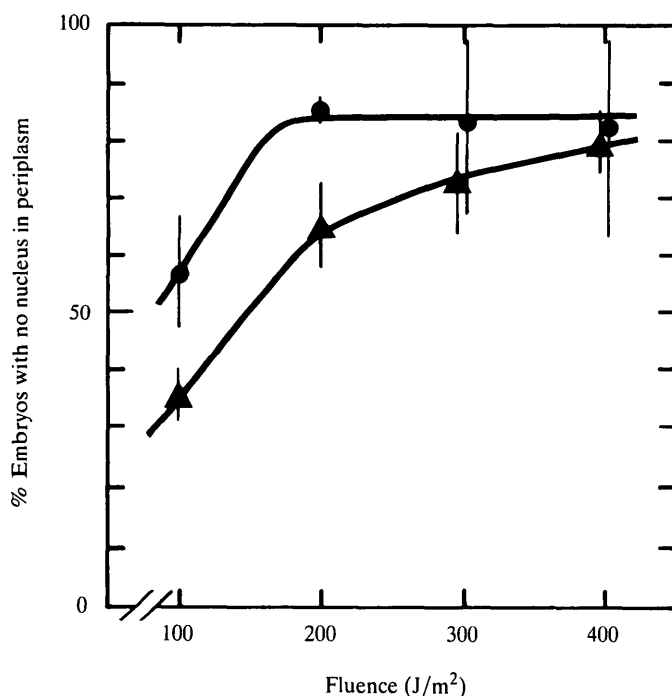

Fig. 4. Fluence-effect relation of radiation at 300 nm (●) and 295 nm (▲). Effects of u.v. are presented in frequencies of embryos that cease development before the syncytial blastoderm stage. Results from five independent experiments are combined, and average number of embryos for each point is 100. Vertical lines show S.E.M.
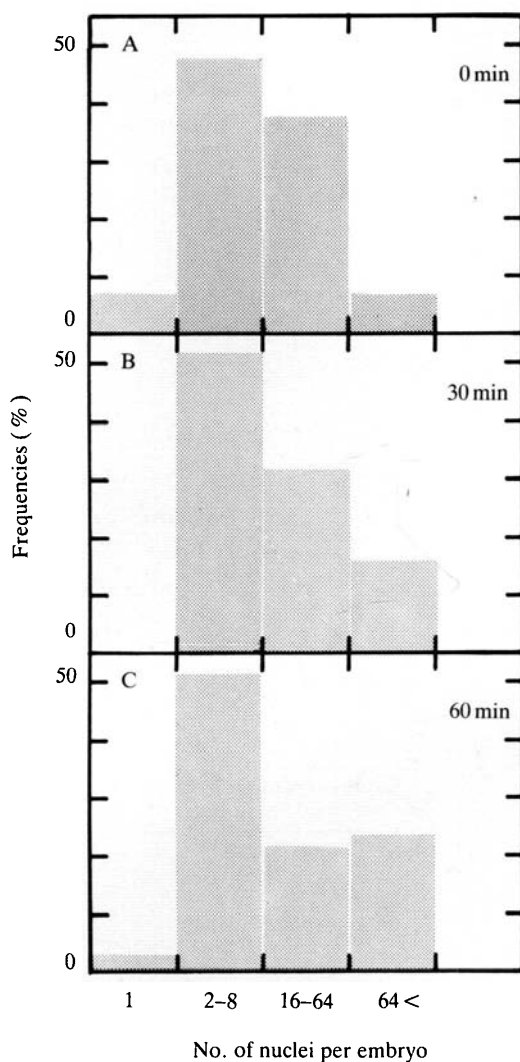
Fig. 5. Histograms showing difference in distribution in the number of nuclei per embryo depending on time from irradiation to fixation. Embryos were irradiated at 35 min ± 20 min AEL, and fixed immediately after irradiation (A), 30 min after irradiation (B), 60 min after irradiation (C). The number of embryos observed was 41, 21, and 41, in A, B, and C, respectively. For explanation in detail see text.

In this series of experiments, the time of irradiation was adjusted so that embryos subjected to different wavelengths of radiation received the same total fluence of $100 \, \text{J/m}^2$, but not the same total quantum energy. From the fluence-response curves for 295 nm and 300 nm we calculated responses at 10^{20} photons/m^2; and obtained 34 % and 42 %, for 295 nm and 300 nm, respectively. Since in these curves the responses changed linearly against quantum energy within a range smaller than 2×10^{20} photons/m^2, responses at 10^{20} photons/m^2 were estimated for other wavelengths, assuming the responses changing linearly in this

range of quantum energy. Estimated values are plotted in Fig. 2. Effects seem to be lower in the range of wavelengths longer than 300 nm. However, the peak of the response was at 300 nm in both curvés.

Cessation of development in the irradiated embryos is caused by discontinuance of intravitelline mitoses

To elaborate the causes that prevent the irradiated embryos from developing, the embryos were fixed and stained for the cytological observation. Embryos aged 35 ± 20 min AEL were irradiated in the anterior pole region with 300 nm radiation at a fluence of $250 \, \text{J/m}^2$ and incubated at 25 °C.

The irradiated embryos were divided into three groups. The first group of embryos was fixed immediately after the irradiation, the second group 30 min after irradiation, and the third group 60 min after the irradiation. The nuclei were counted in each wholemount embryo. The first group included embryos at 2-,
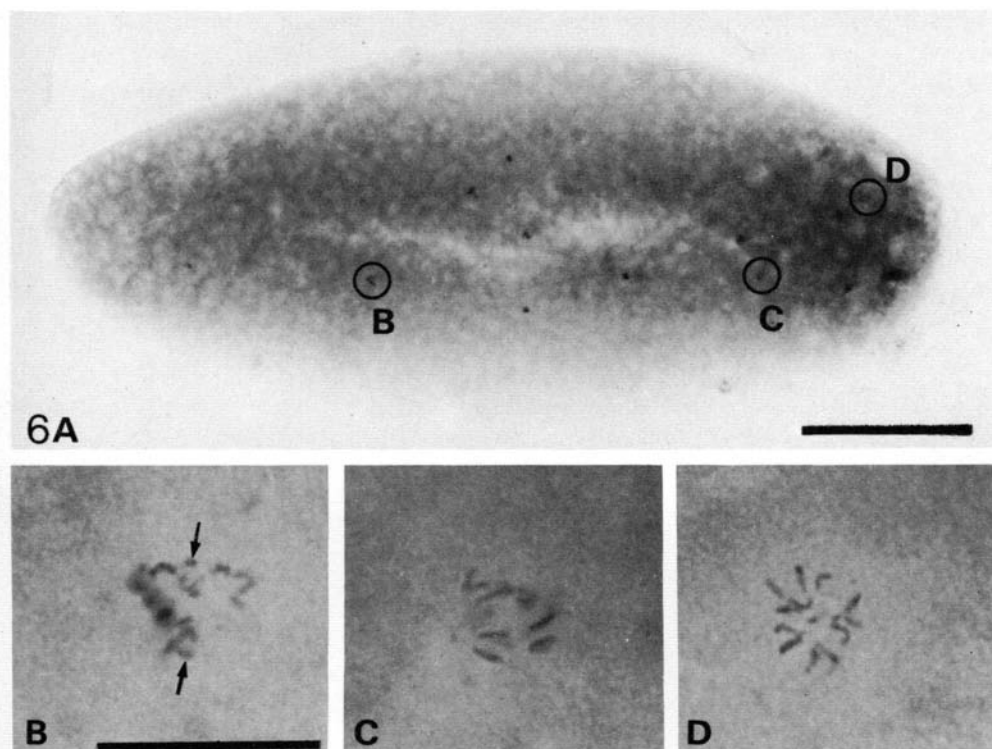

Fig. 6. (A) A wholemount preparation of an embryo, irradiated anteriorly with 300 nm u.v. at $250 \, \text{J/m}^2$ at the 16-nucleus stage, fixed and stained 15 min after irradiation. The anterior pole to the left. Bar represents $100 \, \mu\text{m}$. (B), (C) and (D), Higher magnification of mitotic figures encircled in (A). Note that the mitotic figure in the anterior half of the embryo is abnormal (arrows point to disintegrated chromosomes), but the mitotic figures in the posterior half have normal features. Bar in (B) represents $10 \, \mu\text{m}$. Magnification is the same in (B), (C) and (D).

4-, 8-, 16-, 32- and 64-nucleus stages; the frequency of the embryos of each stage was about the same. Consequently, half of the embryos were at the 8-nucleus or younger stages, and the other half were at older stages (Fig. 5A). In the second group, embryos with 2, 4, or 8 nuclei appeared at the same frequency as that in the first group, while the frequency of the embryos with between 16 and 64 nuclei had decreased and the embryos with more than 64 nuclei had increased (Fig. 5B). It was noticed that in embryos with more than 16 nuclei the number of nuclei per embryo deviated from expected number of nuclei (i.e. 2 to a certain degree of power). In the third group, the frequency of the embryos containing 2, 4, or 8 nuclei remained the same as the first group, but the number of embryos with more than 64 nuclei had further increased at the cost of those with 16 to 64 nuclei (Fig. 5C).

In *Drosophila*, the number of nuclei per embryo doubles every 9·5 min in normal development. Therefore, if the irradiated embryos maintain a normal developmental schedule each embryo in the second group (in which the youngest embryo is 45 min AEL when fixed) is expected to contain more than 16 nuclei; with the same logic the embryos in the third group (75 min AEL or older when fixed), should all contain more than 64 nuclei. Thus the number of nuclei in wholemounts of irradiated embryos differs considerably from the numbers of nuclei expected in unirradiated controls.

In the second and third groups, in the embryos with more than 16 nuclei more nuclei were found in the posterior than in the anterior half (Figs 6A, 7). Nuclei in the anterior half were always at anaphase; in some nuclei chromosomes seemed to have just started segregation (Fig. 8A), and in others are in the process of segregation (Figs 6B, 8B, C). In many cases chromosomes seemed to be abnormal in that some chromosomes are partly pycnotic, some are disintegrated (shown by arrows in Figs 6, 8), and segregation of chromosomes is incomplete (Fig. 8B) and chromosome arrangement deviates from that of the normal anaphase (Figs 6B, 8C). Nuclei in the posterior half of embryos anteriorly irradiated varied in their mitotic phases from embryo to embryo, and their chromosomes seemed normal (Figs 6C, D). In embryos that were irradiated in the anterior pole region at or before the 8-nucleus stage and fixed 15 min later, all nuclei were at anaphase with abnormal chromosomes (Fig. 9).

The above-mentioned observations show that 300 nm radiation to the anterior region of embryos at or before the 8-nucleus stage prevents all nuclei in the embryos from proceeding beyond anaphase. This inhibition of mitosis causes the embryos to cease development; and this arrest of mitosis probably occurs within a span of a single cell cycle after irradiation. In embryos irradiated anteriorly at the 16-nucleus or older stages, nuclei in the approximately anterior-half of the embryos discontinue their division, while those in the approximately posterior-half continue to divide and migrate into the periplasm, where cellularization occurs. This explains the formation of incomplete blastoderms in these embryos. The relation between the stages of irradiation and the effect of irradiation is
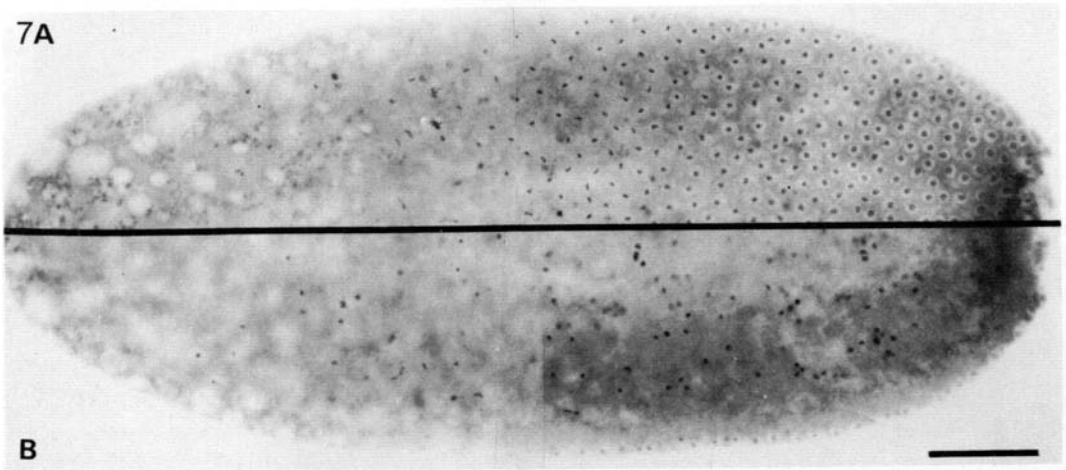
Fig. 7. A wholemount preparation of an embryo anteriorly irradiated at the 256-nucleus stage with 300 nm u.v. at $250 J/m^2$, fixed and stained 15 min after irradiation. Pictures of the same preparation focusing on the surface (A), and on interior (B). The anterior pole to the left. Bar represents 50 μm.
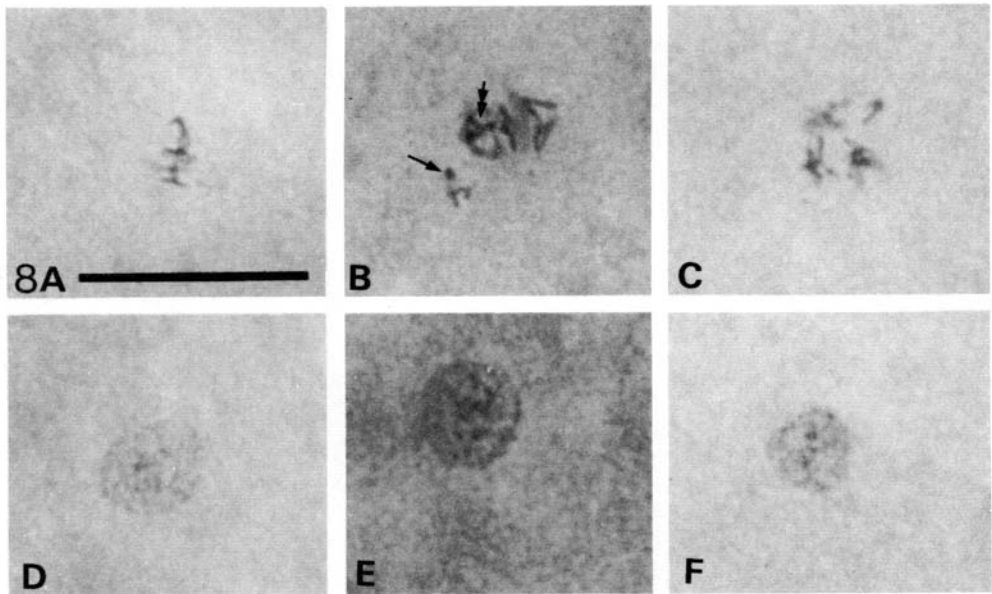

Fig. 8. Mitotic figures in wholemount preparations of embryos, irradiated anteriorly at the 16-nucleus stage with 300 nm u.v. at $250 J/m^2$, fixed and stained 15 min after irradiation. The mitotic figures that are present in the anterior half of three different embryos are shown in (A), (B), and (C); nuclei in the posterior half of the respective embryos are shown in (D), (E) and (F). An arrow indicates a disintegrated chromosome, and a double arrow points to pycnotic chromosomes. Magnification is the same in all pictures. Bar represents 10 μm.
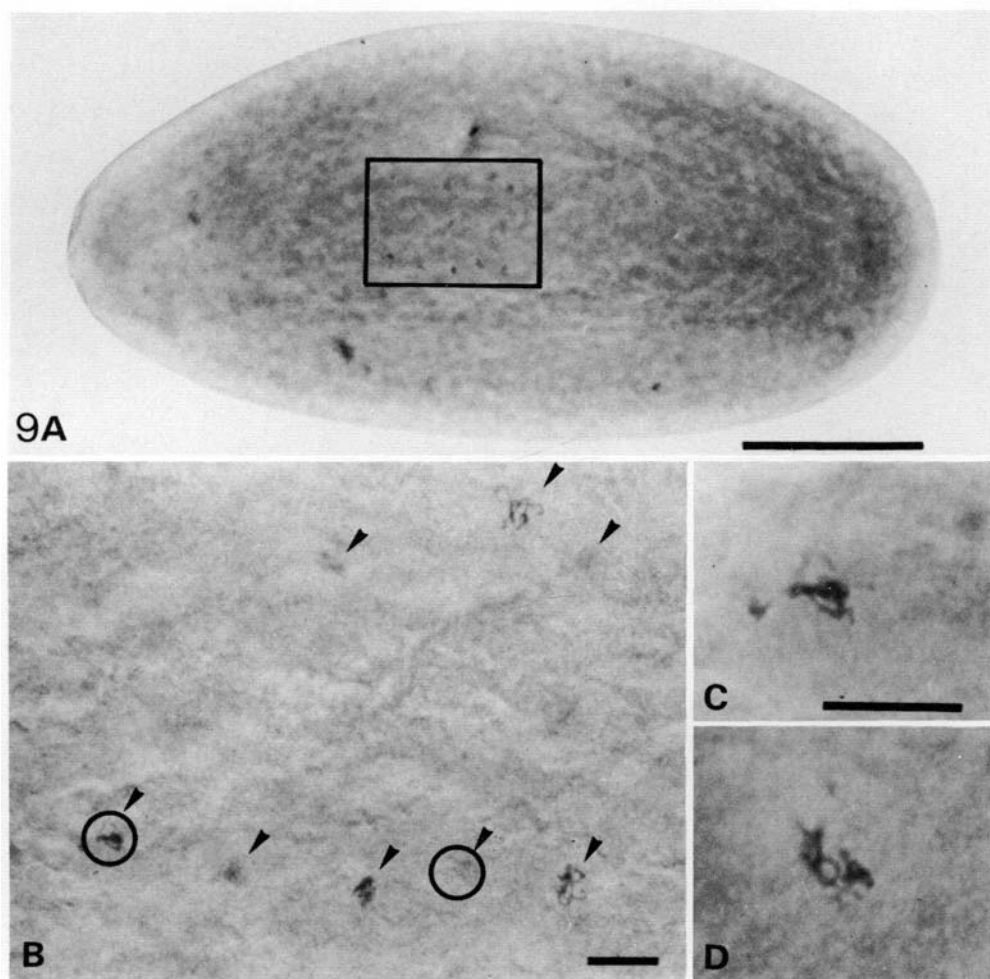
Fig. 9. (A), A wholemount preparation of an embryo, irradiated anteriorly with 300 nm u.v. at 250 J/m² at the 8-nucleus stage, fixed and stained 15 min after irradiation. The anterior pole to the left. Bar represents 100 μm. (B), Higher magnification of the area enclosed in rectangle in (A); all eight nuclei in the embryo are marked by arrowheads, although some are out of focus. (C) and (D), Highly magnified mitotic figures circled in (B) showing abnormal chromosomes; (C), one in the left bottom corner, and (D) one in the right bottom corner. Bars in (B) and (C) both represent 10 μm.

shown in Fig. 10A. The frequency of embryos without nuclear penetration of the periplasm is shown as representing the frequency of embryos with abortive mitoses. This frequency decreased as the developmental stages at irradiation proceeded and reached almost 0 % after 60 min ± 5 min AEL, when most of the embryos are at 32-nucleus stage. Lethality in controls was very low in this series of experiments; probably because this series was performed in the rainy season and our laboratory is not controlled for humidity. Complementary to the

decrease in the frequency of embryos ceasing development before nuclear arrival in the periplasm the frequency of incomplete blastoderms increased and all embryos irradiated at or later than 60 min ± 5 min AEL became incomplete blastoderms. Thus, in anterior irradiation with 300 nm, embryos either stop
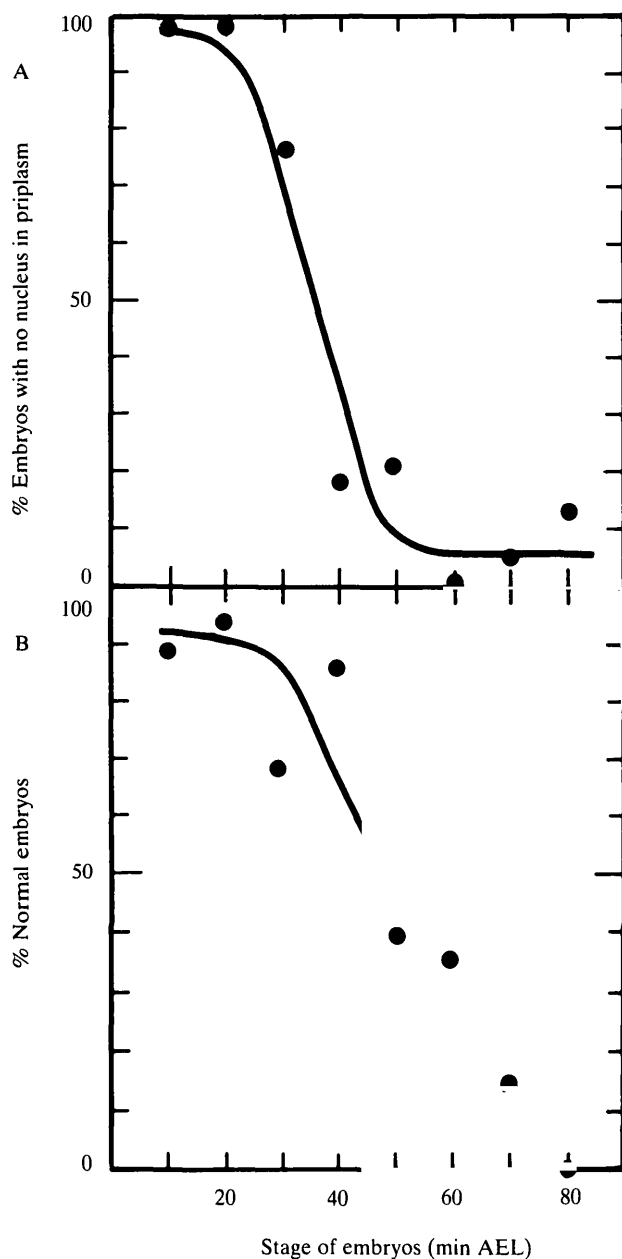

Fig. 10. Stage-dependent responses of embryos to irradiation with 300 nm u.v. at 250 J/m². Embryos were irradiated anteriorly (A), or posteriorly (B). Inaccuracy of ±5 min resulting from 10-min egg collection is included in stages of embryos. Each point represents at least 50 embryos.

development shortly after irradiation or develop into incomplete blastoderms. These blastoderms can continue development and produce larvae with abnormal segmentation.

Results were different when embryos were posteriorly irradiated. When embryos at early stages were irradiated with $250 \, \text{J/m}^2$ at 300 nm, they all elicited normal development. As the stage of embryos at irradiation increased, the frequency of embryos that developed normally decreased, and complementary to this the frequency of incomplete blastoderms increased (Fig. 10B). In these incomplete blastoderms, cellularization occurred only in the anterior half of the embryos (Fig. 3D). These results indicate that a u.v.-sensitive area is not present in the posterior region of embryos in early developmental stages, but it is present in the posterior region in the later stages. Observations of wholemount preparations indicate that the critical stage is the 16-nucleus stage.

Lateral irradiation of partly shielded embryos also arrests mitoses

When embryos at stages of intravitelline nuclear multiplication were irradiated laterally at a fluence of $300 \, \text{J/m}^2$, none of the embryos developed to the syncytial blastoderm. Shielding experiments were designed to examine if the periplasm is sensitive to u.v. only when it has nuclei underneath. Embryos at or before the 8-nucleus stage were irradiated at the region a, b, c, or d as shown in Fig. 1. Distribution of nuclei was surveyed in embryos from the same stock used for these experiments, and exhibited in Fig. 1C. There seems to be no possibility that nuclei are in the yolk mass or in the periplasm in the regions a and d, and the probability that nuclei are present in the region c seems to be very low. The results of the shielding experiments are shown in Table 1. The results from the irradiation of the region a were a little different from those of the other regions.

Table 1. *Lateral irradiation of partly shielded embryos*

| | Lateral | | | | | |
Irradiation*	a†	b	c	d	Anterior	Posterior
No. of embryos treated	104	39	38	55	14	27
No. of embryos ceasing development§	38 (36·5%)	38 (97·4%)	23 (60·5%)	6 (11·1%)	14 (100%)	5 (18·5%)
Significances§§	$P < 0·01$	$P < 0·01$	$P < 0·01$	$P > 0·05$	$P < 0·01$	—

* Fluence was $300 \, \text{J/m}^2$.
† For a, b, c, and d, see Fig. 1C.
§ Number of embryos in which no nucleus arrived in the periplasm by 150 min AEL was scored.
§§ Statistical significance was calculated against posterior irradiation by χ^2 test using a computer program (Ishii, 1983).

Table 2. *Cytoplasmic transplantation between normal and irradiated embryos*

Recipient*	Injected Material*	No. of Embryos Treated	No. of Embryos Cellularized
Irradiated Embryos†	None	54	16§ (29·6%)
	Intact Cytoplasm	76	21§ (27·6%)
Normal Embryos	Irradiated Cytoplasm†	39	36§§ (92·3%)

* Cytoplasm was transplanted from the anterior region of a single donor embryo to the anterior region of a single recipient embryo.

† Embryos were irradiated anteriorly with 300 nm radiation at 250 J/m².

§ Incomplete blastoderms, in which cellularization takes place only in the posterior region of the embryo (*cf.* Fig. 3C).

§§ Normal blastoderms.

Embryos irradiated at *b*, *c*, or *d* either developed normally or ceased development before nuclear arrival in the periplasm. Among 104 embryos irradiated at the region *a*, 38 stopped development promptly and 66 developed into blastoderms. However, among the 66 embryos 37 followed the normal time schedule of the development, while 29 embryos developed very slowly and they did not have their nuclei arriving in the periplasm until 150 min AEL, when normal embryos have initiated cellularization. Further development of these embryos seemed normal. Since no control embryos exhibited such a delay in early development, this is certainly the effect of u.v. These results indicate that the region d is not sensitive to u.v. at these stages, while the regions a, b, and c are sensitive to u.v. Therefore, the sensitivity of the periplasm to u.v. may have no correlation with presence of nuclei underneath. The sensitive area is probably restricted to the anterior two thirds at the 8-nucleus stage, and it has extended to all periplasm at the 16-nucleus stage.

Cytoplasmic transplantation cannot rescue embryos from developmental arrest

Cytoplasmic transplantation was carried out immediately after irradiation in order to examine the possibility that mitotic inhibition is caused by a toxic factor generated by irradiation, or by any diffusible factor essential for the mitosis being damaged by irradiation. Cytoplasm from the anterior periplasm of a normal embryo was transplanted into the anterior region of an embryo immediately after the irradiation. Donor and recipient embryos aged 35 ± 5 min AEL were taken from the same agar plate. The recipient eggs were anteriorly irradiated with 300 nm radiation at a fluence of 250 J/m². A reciprocal transplantation was also performed. The results are summarized in Table 2. In about 70 % of embryos that were irradiated and injected with intact cytoplasm, nuclei did not penetrate the periplasm; in the remainder, nuclear penetration of the periplasm took place only in the posterior region resulting in incomplete blastoderm formation. These

results were consistent with those of the control experiment, in which embryos were irradiated but not injected. Cytoplasm from the irradiated anterior region of a donor embryo was transplanted into a recipient egg that was not irradiated. More than 90 % of the recipient embryos developed into normal blastoderms. The volume of cytoplasm transplanted was approximately 1 % of the egg volume, and technical difficulties prohibited us from transplanting larger amount of cytoplasm.

DISCUSSION

The present results suggest that u.v. irradiation (300 nm, 200 J/m^2) on the egg surface causes the intravitelline nuclei to discontinue their mitotic cycle at the anaphase. As previously reported, the half-attenuation thickness of u.v. for the *Drosophila* egg cytoplasm is approximately 3 μm (Togashi & Okada, 1983). It is calculated that a given dose of u.v. decreases to approximately 0·001 % of the original dosage at 50 μm from the egg surface. Nuclei are never found within 50 μm from the egg surface in embryos at the stages we used in the experiments. Besides, mitotic arrest was observed even in embryos irradiated laterally in the region *a*, where no nucleus is present (Fig. 1C). Therefore, it is probable that the mitotic arrest resulted from irradiation by 300 nm u.v. is not caused by direct hit of nuclei, but by effects of the u.v. on the peripheral cytoplasm. There have been several reports that may support this assumption. It has been suggested that u.v. of the range of wavelengths between 290 and 400 nm affects the activity of microbial and plant cells by hitting the cell membrane (Moss & Smith, 1981; Klamen & Tuveson, 1982; Watanabe, Ito & Ito, 1982; Imbrie & Murphy, 1983). Jagger (1981) indicated that the target of near u.v. may be chromophores on the cell surface. Mitotic delay observed in *Physarum* cells that have been irradiated with 163 nm vacuum u.v. at late G2 is ascribed to the damage by u.v. in the surface layer of the plasmodia (Matsumoto, Ito & Ito, 1981).

The following question arises, if 300 nm u.v. affects only the very surface region of the egg, how can the effect of this u.v. be conveyed to the nuclei that are present at least 50 μm below the egg surface? It may be possible that a toxic substance is generated by the u.v. in the surface region and it diffuses to reach the nuclei. The time required for molecules with the molecular weight of 1000 to migrate by diffusion to a certain point starting from a source at an end of a biological system was calculated using the equation given by Crick (1970) assuming the diffusion constant of the molecules in the cytoplasm as $0·8 \times 10^{-6}$ cm^2/s. According to the calculation the time required for the molecules to diffuse 50 μm and 100 μm from the periphery is 15 min and 62 min, respectively. These values would be the minimum expected, even when the substance is produced in a wide area of the periplasm from which it diffuses inward, because Crick's equation deals with one-dimensional biological system, while the *Drosophila* embryo is a three-dimensional system. Based on this calculation, in embryos at 4- and

8-nucleus stages irradiated laterally in the region *a* or *c* (Fig. 1C) the time when nuclei are affected by the toxic substance diffusing from the periphery is anticipated to be different in the anteriormost nucleus compared with the posteriormost nucleus. However, the results from our experiments do not support this theory. Delay in blastoderm formation in approximately 30 % of the embryos irradiated in the region *a* could indicate the existence of a diffusible toxic substance. However, if so, the fact that no such a delay occurred in irradiation in the region *c* cannot be explained. In addition, judging from observation of wholemount preparations the mitotic arrest must have occurred within a time period of one mitotic cycle, 9·5 min, after irradiation. This is an insufficient period for a substance to diffuse and accumulate at nuclei that are at least 50 μm away from the periphery. Thus, there is no evidence to support the theory that the effects of u.v. are due to the generation of a diffusible toxic substance in the periplasm.

The results from the cytoplasmic transplantation are not conclusive by themselves, since they are negative and the possibility that the volume of transplanted cytoplasm was too small could not be ruled out. However, the results at least indicate that u.v. irradiation does not produce in the cytoplasm a substance toxic enough to cause abortive mitosis when cytoplasm of an amount corresponding to 0·7 %–1·5 % of the egg volume is transplanted. This volume of cytoplasm from a wild-type egg has been shown to be sufficient to rescue an embryo from lethality laid by females homozygous for rudimentary mutation (Okada *et al.* 1974*b*), and 1 % egg volume of cytoplasm taken from polar plasm has also been shown to restore fertility to a u.v.-sterilized embryo (Okada *et al.* 1974*a*).

Recently cytoskeletons have been shown to participate in several functions of cells, such as rapid transport of molecules and organelles, stimulation and inhibition of cell growth (McClain & Edelman, 1980), and intercellular signalling through receptors in the cell membrane during embryogenesis (reviewed by Tucker, 1981). Zalokar & Erk (1976) reported that treatment of *Drosophila* embryos with colchicine at a concentration of 1 μg/ml resulted in poor separation of daughter nuclei, while at 10 μg/ml, mitoses were arrested at metaphase. Since we find difficulties in attributing the mitotic arrest in intravitelline nuclei to either direct hit by u.v. of nuclei or to toxic and diffusible substances generated by u.v., it is tempting to speculate that cytoskeletal complexes are involved in a mechanism that regulates synchronous intravitelline mitoses in the early development of *Drosophila*, and that u.v.-sensitive sites in the peripheral cytoplasm or in the cell membrane of the egg are included in this mechanism.

Our results may be explained by assuming: 1) There exist structures connecting intravitelline nuclei with the periphery of an egg; these structures have to be intact for the nuclei to complete a mitotic cycle. 2) The peripheral ends of these structures are sensitive to 300 nm u.v. or associated with u.v.-sensitive sites; when the peripheral ends are damaged, the nuclei that are on the other ends of the structures cannot continue the mitotic cycle beyond the anaphase. 3) The

posterior region (within 20 % of the egg length from the posterior pole) lacks
u.v.-sensitive sites at and before the 8-nucleus stage; the sites are present in this
region at or after the 16-nucleus stage. Intravitelline nuclei are associated with
plural u.v.-sensitive sites, but not with sites distant from them.

The arrest of intravitelline mitoses caused by 300 nm u.v. radiation induces
developmental defects in the irradiated embryos. Previous authors have
described these developmental defects in their experiments including irradiation
of embryos with u.v., but without noticing the effect of u.v. on the intravitelline
mitosis. Inactivation of *Smittia* embryos with u.v. has been reported by Kalthoff
(1976) and a similar phenomenon has been reported by Hathaway & Selman
(1961) in *Drosophila*. Inferring from their descriptions and from the wavelengths
of u.v. and the developmental stages of embryos they used for their experiments,
the inactivation they observed probably represents the instant intravitelline
mitotic arrest we have described. Bownes & Sander (1976) have given drawings
and photographs of *Drosophila* embryos that they irradiated anteriorly with
285 nm wavelength at 1142·4 J/m^2; these seem similar to the incomplete blas-
toderms we obtained in the present work.

We hypothesize the involvement of subcellular structures in controlling
nuclear division in early development of *Drosophila*. Unfortunately, however,
the cytoskeletal construction of *Drosophila* eggs has not yet been disclosed.
Existence of structures that are sensitive to 300 nm u.v. and are located in the
periphery of cleavage embryos, and of cytoskeletal elements connecting these
structures with intravitelline nuclei is to be investigated. In the present experi-
ments a specific wavelength of u.v., 300 nm, was effective in inhibiting the
intravitelline mitosis. We have yet to discover what specific molecules in the
embryo are the target of the u.v. and result in the inhibition of intravitelline
mitosis.

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