The development of *Drosophila* embryos after partial u.v. irradiation

By M. BOWNES and K. SANDER

*From the Zoologisches Institut der Universitat, Freiburg*

**SUMMARY**

U.v. irradiation of the anterior pole of nuclear multiplication stage *Drosophila* eggs produces embryos with defective anterior structures. At a low frequency embryos resembling some phenotypes of the bicaudal syndrome of *Drosophila* were observed. These embryos had no head or thorax and the eight abdominal segments were spread to the anterior of the embryo. Sometimes spiracles, characteristic of the most posterior embryonic segment were observed at the anterior of the embryo. The development of these embryos was followed, and abnormalities occurred as early as blastoderm formation. The extent of the blastoderm defects correlated well with the final abnormality in the embryo.

**INTRODUCTION**

Previous experiments u.v.-irradiating specific regions of the early *Drosophila* embryo produced defective embryos; the type of defect was correlated to the initial site of irradiation (Bownes & Kalthoff, 1974). This final abnormality, however, does not provide any information about the initial type of damage caused by the u.v. irradiation. Although a structure may be absent at the time of hatching the factors involved in the determination of this structure need not necessarily have been destroyed directly by the u.v. Other factors needed for normal morphogenesis, or materials needed for interactions or the induction of these organs may have been damaged, yet the resulting embryo would be similar. One way of attempting to find the primary effect of u.v. irradiation on development is by watching the reaction of embryos immediately after localized u.v. irradiation and seeing how the development of the treated region differs from surrounding areas and from normal embryogenesis. We will describe here the development of embryos irradiated at the anterior pole using the technique of time-lapse photography.

Irradiation at the anterior pole was chosen because of its interesting effects on the development of the embryo. Normally embryos result with abnormal head formation, or an anterior yolk patch with gut extruded at the anterior and

---

1. Author's address: Department of Biology, University of Essex, Wivenhoe Park, Colchester, CO4 3SQ, Essex, England.

2. Author's address: Zoologisches Institut der Universitat, 78 Freiburg i Br. Katharinenstrasse 20, West Germany.
a varying number of abdominal and thoracic segments at the posterior. Occasionally embryos consisting of one abdomen occupying the whole egg were found. These embryos occurred with a low frequency (15/186 after irradiation at the nuclear multiplication stage irradiation and 4/47 after irradiation the syncitial blastoderm stage). None were found after blastoderm irradiations. Irradiation of the anterior pole region in *Smittia* embryos produces 'double abdomen' embryos; with two abdomens in mirror-image symmetry (Kalthoff, 1968). In *Drosophila* a mutant *bicaudal* also produces embryos with this appearance (Bull, 1966). It is interesting that the embryos with one abdomen closely resemble one of the other classes of embryos described as part of the *bicaudal* syndrome, considered to represent a different stage in the reversal of polarity in the anterior of the egg. The experiments described here look in particular detail at these embryos with one abdomen to see if there is any indication (e.g. reversed segments, or anterior spiracles) that anterior u.v. irradiation can produce some polarity reversal in *Drosophila* even though double abdomens cannot be induced.

**MATERIALS AND METHODS**

*Origin and preparation of eggs*

All eggs were of Oregon-K stock. Eggs were collected at 25 °C on agar plates coated with a paste of fresh yeast and sugar. Eggs were collected over 30 min intervals and used immediately or incubated at 25 °C until they reached the required age. Subsequently the eggs were dechorionated in 3 % sodium hypochlorite for 5 min, then washed with water and placed in a mild detergent to prevent the eggs from clumping together and from floating on the surface of solutions during experimental procedures. Eggs of the following developmental stages were selected using a dissecting microscope for experimental use:

Stage 'NM' was during nuclear multiplication. The nuclei are shielded from incident radiation by periplasm and yolk material. In these embryos there are gaps between the egg cell and the vitelline membrane at both poles.

Stage 'B1' was after formation of a cellular blastoderm, but before the appearance of the ventral or cephalic furrows and before invagination of the pole cells. These correspond to stages 2 and 6 respectively in Bownes (1975), where more detailed descriptions and photographs of these eggs can be seen.

*Generation and measurement of u.v. irradiation and irradiation procedure*

See Bownes & Kalthoff (1974) for details of apparatus and procedure.

Eggs at all stages were irradiated with 285 nm wavelength in order to aim at targets located in the periplasm. NM eggs were irradiated for 2 min at 25 A; no filters. B1 eggs were irradiated for 3 min at 25 A; no filters. These doses (11424 and 13464 ergs/mm² respectively) gave 100 % defective embryos.
Following the development of individual eggs

After irradiation, eggs were placed in individual depression slides, coded, and observed submerged in 0-9 % sodium chloride. Irradiated eggs were observed at 20-30 min intervals after irradiation until morphogenetic movements had commenced. At each observation the egg was described and often photographed. The eggs were then floated on the sodium chloride and incubated at 25 °C for 24 h. The final defects were observed by placing the eggs on a slide, and covering it with a coverslip, which was supported by pieces of coverslip to prevent the eggs from bursting.

Some eggs were photographed using time lapse photography. These eggs were placed on a slide, under a coverslip supported by a rod to prevent bursting. They were photographed until morphogenesis had started, then removed from the slide and floated on 0-9 % sodium chloride in a depression slide and treated as above.

RESULTS

The normal segmentation of the embryo at the time of hatching

Fig. 5(a) shows the segmentation pattern of the Drosophila embryo at the time of hatching. The most anterior segment is part of the invaginated head and contains the hooks of the mouth-parts. Posterior to this are three thoracic and eight abdominal segments. The anterior border of each segment contains several rows of small chaetae. The belts of chaetae marking the abdominal segments are much thicker and darker than those marking the thoracic segments. The last abdominal segment contains a pair of spiracles which the tracheal trunks open into.

Immediate effects of anterior pole u.v. irradiation on development, and correlations between the initial defect and final embryonic abnormality

Nuclear multiplication stage. A number of different responses to anterior u.v. irradiation were observed, described in Fig. 1, apparently reflecting a variation in the intensity of effect. (1) Development may fail completely at once. (2) Nuclei migrate into the posterior periplasm of the embryo, but not into the anterior regions. A few blastoderm cells may form at the posterior, but morphogenesis is never initiated and the egg soon decays (Fig. 1a). (3) Nuclei migrate to all regions of the periplasm, though their arrival is often delayed at the anterior. Blastoderm formation in these embryos begins at the posterior. Behaviour at the anterior of these embryos is variable. Blastoderm cells in some instances eventually formed at the anterior and the embryo appeared normal (Fig. 1b). Sometimes cleavage furrows began to form at the anterior but were never completed, gaps resulted in the blastoderm at the anterior, and yolk was extruded (Fig. 1d). Occasionally blastoderm cells moved in to close up this gap when morphogenetic
movements commenced, sometimes excluding a small amount of yolk material from the embryo (Fig. 1c). In most cases as morphogenesis commenced, however, an anterior patch of yolk was seen. It was not possible to see from time lapse films or observing living embryos what happened to the nuclei which migrated to the anterior, but did not become incorporated into blastoderm cells.

Fig. 1. A diagrammatic representation of the effects of u.v. irradiation on NM stage embryos. (a) Nuclei fail to migrate into the irradiated region. Blastoderm cells form at the posterior only. (b) Blastoderm formation is delayed at the anterior of the egg. (c) Nuclei migrate into the irradiated region, but blastoderm cells do not form there. Later the blastoderm cells move to make a complete blastoderm layer. (d) Nuclei migrate into the irradiated region, but blastoderm cells do not form there. Yolk is extruded at the anterior. For list of abbreviations see p. 408.

There was good correlation between the initial defects observed before morphogenesis commenced and the final embryonic defect observed at the time of hatching. The data described here is based on following the initial development (excluding embryos which died immediately after irradiation) and subsequently observing the final defects of 121 embryos. For this study the dose of u.v. irradiation was adjusted so that no normal larvae were ever observed.

Embryos forming a complete blastoderm initially usually showed abnormal head formation in the resulting embryo. An example of this type of development is shown in Fig. 2. When a complete blastoderm was formed by movement of cells to fill in a gap (Fig. 1b), either embryos with abnormal head formation resulted (Fig. 3) or embryos with one abdomen occupying the whole embryo (Figs. 4, 5b). Occasionally during subsequent development the cells of a complete blastoderm break apart again at the anterior and produce an embryo with an anterior yolk patch situated in front of all the abdominal and some thoracic segments (Fig. 5). Eggs where a gap in the B1 remains at the anterior, produce
Fig. 2. (a) Nuclei migrate to all regions of the periplasm. (b) A complete blastoderm is forming; but it is irregular at the anterior and B1 formation is delayed at the anterior. (c) Morphogenesis has commenced. The blastoderm is now complete, but remains irregular at the anterior. (d) The final embryo has abnormal head formation, and abnormal mouth-parts.
Fig. 3. (a) After irradiation nuclei are migrating into all regions of the periplasm. (b) The blastoderm has not formed at the anterior and yolk is extruded. (c) Blastoderm cells move in to make a complete layer, cutting off a small yolk patch in the process. (d) The final embryo shows abnormal head formation. No mouth-parts are present.
Development of Drosophila embryos after u.v. irradiation

embryos with an anterior yolk patch and a well formed posterior containing varying numbers of abdominal segments (Fig. 6).

Blastoderm stage. Within 15 min of u.v.-irradiating blastoderm eggs defects could often be seen. The development of 33 eggs were followed. Some eggs began morphogenesis with the blastoderm intact, but in others a gap appeared at the anterior of the blastoderm. It was not possible to elucidate using either time-lapse films or direct observation of living embryos what happened to the cells in this region. The eggs with a complete blastoderm usually produced embryos with abnormal head formation. When a break occurred in the blastoderm the resulting embryos had an anterior yolk patch and a well-formed posterior containing varying numbers of abdominal segments (Fig. 7).

Detailed analysis of embryos with only an abdomen

In no case was a bicaudal embryo found after anterior u.v. irradiation which had spiracles and attached tracheal trunks at both poles of the embryo. One embryo (Fig. 9) looked very similar in appearance to a bicaudal, the abdominal segments appeared to have a symmetry about the middle, although the bristle rows towards the anterior did not have an obvious polarity reversal and mal-pighian tubules and spiracles were not observed at the anterior. Several embryos were found with a small amount of material anterior to the last abdominal bristle row resembling spiracles, typical of the most posterior abdominal segment (Figs. 4, 5b), no trachael tissue was connected to this structure however. Some embryos had a large patch of disorientated bristles near the anterior which were not arranged in the usual rows, but occupied a large patch, indicating some abnormal organization near the anterior.

DISCUSSION

These results showed very clearly that there was a good correlation between the immediate effects of u.v. irradiation on development and the final appearance of that embryo. U.v. irradiation did not prevent the migration of nuclei into the anterior periplasm, but in many cases migration was delayed. The u.v. must damage some factors at the anterior of the egg which are essential for the subsequent interactions needed to form the anterior of the embryo. In the cases where the blastoderm cells physically moved to compensate for a gap in the cell layer at the anterior, it was clear that this could not compensate for the effects of the u.v. irradiation, since embryos with abnormal heads still formed.

Those embryos containing one large abdomen, occupying the whole embryo, with no head or thorax present, could have formed in a number of ways. The information needed to form the head may have been destroyed directly by the u.v. irradiation, so that no anterior forms, or the u.v. may have interfered in a more general way with development so that the nuclei migrating late into the anterior fail to pick up the developmental cues needed to initiate the development
Development of Drosophila embryos after u.v. irradiation

of the anterior of the embryo. Subsequent to this it is possible that the cells at the posterior, destined to be abdomen, spread over the whole egg during development, whilst the thoracic cells failed to divide or decayed due to toxic substances from the irradiated area. The existence of embryos where the anterior of the embryo contains spiracles characteristic of the most posterior abdominal segment.

Fig. 5. Drawing of (a) normal segmentation just prior to hatching, (b) of embryo in Fig. 4 at time of hatching to show details of abdominal segments and the head and thorax replaced by spiracles characteristic of the posterior.

Fig. 4. (a) Nuclei have migrated into all regions of the periplasm and blastoderm cells are forming at the posterior of the egg. (b) The blastoderm is somewhat irregular at the anterior. (c) The blastoderm is complete at the anterior some time after morphogenesis has begun. (d) The final embryo has no head or thorax, but all eight abdominal segments are intact, the most anterior segment appearing near the anterior of the embryo. Two spiracle-like structures are present at the anterior of the egg along with a mass of undifferentiated cells which appear in place of the head and thorax. (e) Detail of spiracle-like structure, typical of the posterior of the embryo.
Fig. 6. (a) Nuclei have migrated to all regions of the periplasm. (b) There is a gap in the blastoderm at the anterior. (c) The gap in the blastoderm has closed. (d) The final embryo has all eight abdominal segments and parts of two thoracic segments. There is a large yolk patch at the anterior.
Fig. 7. (a) Nuclei migrated to all regions of the periplasm and blastoderm cells are forming at the posterior. (b) There is a gap in the anterior blastoderm where yolk is extruded. (c) The gap remains as morphogenesis proceeds. (d) The final embryo has all eight abdominal segments and one thoracic segment. Anterior is a mass of extruded yolk material.
suggest that an alternative mechanism may be involved in their occurrence. These embryos closely resemble those observed by Bull (1966) as a less extreme example of polarity reversal than the true ‘bicaudal’ embryos. It is possible that the u.v. irradiation destroys ‘anterior factors’ (Sander, 1971, 1975; Herth & Sander, 1973; Lohs-Schardin & Sander, 1976), and that the destruction of this permits the remainder of the egg to be influenced only by ‘posterior factors’. This may result in a reorganization of the pattern specifying machinery such that embryos with small polarity reversals develop.
Fig. 9. This embryo is very similar in appearance to a bicaudal embryo. The abdominal segments appear to have a symmetry about the middle although spiracles were not observed at the anterior of the embryo.

We would like to thank Dr Klaus Kalthoff for his constant assistance, advice and discussions during these experiments. M. Bownes would like to thank both Professor K. Sander and Dr K. Kalthoff for making the visit to Freiberg possible. This research was supported by the Deutsche Forschungsgemeinschaft (SFB 46).

REFERENCES


(Received 18 May 1976)
ABBREVIATIONS

abn, abnormal; abs, absent; Abs 1–8, abdominal segments one to eight; ant, anterior; Asp, anterior spiracle; Bl, cellular blastoderm; Ch, chaetae; CLF, cleavage furrow; HS, head segment; M, micropyle; MP, mouth parts; N, nucleus; post, posterior; sp, spiracle; Syn Bl, syncitial blastoderm; T, tracheae; TS1, prothoracic segment; TS2, mesothoracic segment; TS3, metathoracic segment; UV, ultraviolet irradiation; VM, vitelline membrane; Y, yolk.