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Pattern Abnormalities Induced in *Drosophila* Imaginal Discs by an Ultraviolet Laser Microbeam

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Treatment of portions of excised imaginal leg discs with an uv laser microbeam was shown to kill cells in the treated region. Treated discs cultured in the abdomens of adult females underwent pattern regulation, either regenerating, duplicating, or triplicating, depending on the precise portion of the disc which was treated. The laser-induced pattern of regulation is similar to that induced by cell removal and by cell-lethal mutations, supporting the hypothesis that pattern regulation in response to all forms of wounding is controlled by the same pattern-forming system.

INTRODUCTION

The imaginal discs of *Drosophila* are capable of extensive pattern regulation in response to wounding. This regulation is epimorphic (Morgan, 1901), with the missing cells being replaced by cells from a localized zone of proliferation (a blastema) which forms near the wound surface (Bryant, 1975; Karpen and Schubiger, 1982). Pattern regulation has been induced by surgical bisection of discs, either *in situ* (Bryant, 1971) or after excision from the larva (Schubiger, 1971; Bryant, 1975), and by cell death induced *in situ* using radiation (Postlethwait and Schneiderman, 1973; Postlethwait, 1975) or cell-lethal mutations (Russell, 1974; Postlethwait, 1978; Girtton, 1981). From the morphological similarities of the pattern abnormalities induced by these two types of wounding (regeneration, duplication, or triplication) it has been suggested that cell death and cell removal have the same effect on pattern formation in imaginal discs (Postlethwait and Schneiderman, 1973; Russell, 1974; Bryant *et al.*, 1981). However, there are important morphological differences between abnormalities induced by cell death and by cell removal which suggest that this might not be so.

Pattern duplications are produced surgically by removing a large portion of an imaginal disc, such as the upper medial quarter of the prothoracic leg disc, and culturing the remaining fragment in the abdomen of an adult female (Schubiger, 1971). During metamorphosis the cultured fragment will form two mirror-image symmetrical sets of those structures normally formed by the cells in the fragment, and will be missing all of those structures normally formed by the excised (upper medial quarter) cells. Pattern duplications of legs produced by radiation (Postlethwait and Schnei-

derman, 1973) or temperature-sensitive cell-lethal mutations (Russell, 1974; Russell *et al.*, 1977; Postlethwait, 1978; Jürgens and Gateff, 1979) are also usually deficient for structures formed by cells in the upper medial quarter of the leg disc and duplicated for structures formed by lateral cells. However, the extent of the deficiencies accompanying these duplications is extremely variable and is often much less than the entire upper medial quarter. In a significant number of cases the duplication is complete, with no detectable medial deficiency (Russell *et al.*, 1977). No experimental evidence has been presented which resolves the question of whether this difference in the deficiencies accompanying duplications is due to a different response of imaginal disc cells to the death as opposed to the removal of their neighbors.

We have attempted to address this question by determining the effects on pattern formation of defined patches of cell death induced in imaginal discs using an uv laser microbeam. Microirradiation has a long history as a successful technique for removing or killing cells or portions of cells *in situ* (Kaltoff, 1971; Berns, 1974; Lohs-Schardin *et al.*, 1979; Berns *et al.*, 1981). The precise control over the size, shape, and location of the induced cell death available with the laser microbeam offer many advantages for the study of cell death in imaginal discs. In this report we present the results of an initial study designed to determine whether laser-induced cell death in imaginal discs can induce pattern abnormalities such as duplications and triplications.

MATERIALS AND METHODS

Wild-type larvae and adults of the Oregon R strain were used as hosts. Similar strains homozygous for the

FIG. 1. An imaginal leg disc which has been treated with the uv laser microbeam and then stained with trypan blue. The semicircular treated region (arrow) stains heavily.

cuticular marking mutations *y* (yellow), *f* (forked bristles), or *sn* (singled bristles) were used as donors (for abbreviations see Lindsley and Grell (1968)). All stocks and experimental animals were raised at 25°C on a standard cornmeal, yeast, agar medium.

Late third-instar donor larvae were collected from the sides of the culture container and the prothoracic leg discs were excised in insect Ringers (Ephrussi and Beadle, 1936). For purposes of orientation the two leg discs from one larva were not severed from the brain, and were handled as one unit. The discs were arranged on a standard glass slide in a drop of Ringers and were covered by a quartz coverslip, which was slightly raised to prevent excessive flattening of the discs. An uv laser microbeam (266 nm, fourth harmonic from a YAG laser, Berns *et al.*, (1981)) was directed through a Zeiss photomicroscope with a 32× quartz objective, and was focused on the disc. Manipulation of the microscope stage moved the disc under the beam, allowing a predefined, contiguous patch to be treated. The disc was observed during treatment via closed circuit television. Control discs were isolated and the region corresponding to the

laser-treated area was surgically removed using sharpened tungsten needles.

After treatment the discs were injected into late third-instar larvae for metamorphosis either immediately or after being cultured in the abdomens of adult females for 7–10 days at 25°C. The adult cuticular structures formed by each disc were recorded (see Schubiger (1968) and Strub (1977) for a complete list of prothoracic leg structures, and fate maps of the prothoracic leg disc).

RESULTS

The ability of the uv laser microbeam to kill imaginal leg disc cells was tested using two criteria for cell death. First, treated discs were stained with the exclusion dye trypan blue immediately after treatment. The regions exposed to the laser stained heavily and uniformly within 3–5 min (an example is shown in Fig. 1). The stain did not spread beyond the treated region, suggesting that the laser's effect is highly localized. Second, to determine whether the cells in the treated region were capable of further development, a series of treated discs were injected into late third-instar larvae immediately after treatment, where they underwent metamorphosis with the host. Discs in which the upper medial quarter or entire medial half had been treated generally did not form the adult structures fate-mapped to these regions (Fig. 2B). As a control, these same regions were surgically removed from a second series of leg discs which were also injected into late third-instar larvae. The pattern of adult structures formed by the surgically bisected discs was very similar to that of the laser-treated discs (Fig. 2A). Structures fate-mapped near the edge of the treated region were occasionally observed in both experiments, most likely due to errors in treatment.

The ability of laser-induced cell death to stimulate pattern regulation was tested in two experiments. First the medial half, upper medial quarter, or upper medial one-eighth portions of a series of leg discs were laser treated, and the discs were cultured *in vivo* for 7–10 days before metamorphosis. As a control, these same regions were surgically removed from a second series of discs which were also cultured prior to metamorphosis. In both the laser-treated and the control series pattern duplications of lateral structures occurred in the medial half and upper medial quarter treated/removed series but not in the upper medial one-eighth series (Figs. 3A, B). The duplications contained two mirror-image sets of lateral structures and were deficient for a large portion of the medial structures normally formed by the cells in the treated portions (Fig. 4). The frequency of duplications resulting from laser

treating the upper medial quarter was lower than that from the surgical controls.

In the second experiment the effects of an internal patch of cell death (one which does not intersect the edge of the disc) were tested. These patches were thin and semicircular, extending across the upper half of the disc in the presumptive tibial/tarsus region. Figure 1 shows an example of such a patch. This treatment configuration was not chosen at random. Analysis of the morphology of pattern triplications induced by a temperature-sensitive cell-lethal mutation (Girton, 1981) suggests that leg triplications may be induced by cell death patches of this size, shape, and location. This laser treatment was chosen as a test of whether the laser-induced cell death could induce pattern regulation similar to that induced by the cell-lethal mutation. The 35 treated discs which were recovered usually contained

FIG. 2. Diagrams showing the results of implanting surgically bisected (A) and laser-treated (B) imaginal discs into late third-instar larvae, without a period of growth in culture. The histograms indicate the percentage of the cuticular markers normally produced by the cells in that portion of the prothoracic leg disc (Schubiger, 1968; Strub, 1977) which were formed by the treated discs. The cross-hatching indicates the extent of the laser-treated area.

FIG. 3. Diagrams showing the results of implanting surgically bisected (A) and laser-treated (B) imaginal discs into late-instar larvae after a period (7-10 days) of *in vivo* culture. As in Fig. 2, the histograms indicate the percentage of cuticular structures formed.

a complete set of leg structures, and 11 contained additional structures located in a cuticular process similar to those of the cell-lethal induced triplications (an example is shown in Fig. 5). In each case the cuticular

FIG. 4. A photograph of the tarsal structures formed by a disc whose medial half region was laser treated. After treatment the disc was cultured for 10 days in the abdomen of an adult female prior to metamorphosis. Note the absence of sex comb teeth, and the presence of two claw organs (C) in mirror-image symmetry.

process contained two mirror-image symmetrical partial sets of tarsal structures. The number of bristles, hairs, sensilla, etc., contained in these processes varied greatly, and only those cases which could be clearly distinguished from the folded, unevered leg cuticle in the implants were counted.

DISCUSSION

The staining of regions of imaginal discs with the exclusion dye trypan blue is commonly used as evidence for localized cell death in mutant and in wild-type imaginal discs (Fristrom, 1968, 1969; James and Bryant, 1981). We interpret our results in a similar manner: treatment with the uv laser kills imaginal discs cells, and the death is limited to the treated region. This conclusion is strongly supported by the observation that laser-treated cells are incapable of forming adult structures during metamorphosis. These results are analogous to those of James and Bryant (1981) who observed localized staining of *vestigial* wing discs in precisely the region (wing pouch) which fails to develop into adult structures.

Our results support the hypothesis that the differences in the deficiencies accompanying cell removal-

and cell death-induced duplications are not due to inherent differences in response to the two types of wounding, as patches of laser-induced cell death of different size, shape, and location induced pattern abnormalities characteristic of both cell removal and cell death experiments. If a contiguous patch of cells including the upper medial quarter of the disc is laser-killed, the surviving lateral cells will regulate in culture, and during metamorphosis will form two, mirror-symmetrical sets of lateral structures (duplication) with no structures normally formed by cells in the killed region (deficiency). This same pattern was observed in our control (cell removal) discs and in previous cell removal experiments (Schubiger, 1971; Strub, 1977), suggesting that cell removal and cell death have similar effects on pattern formation in imaginal discs. While the specific parameters of cell death which result in leg triplication have not been defined in the *insitu* surgery (Bryant, 1971) or cell-lethal mutation (Postlethwait, 1978; Jürgens and Gateff, 1979; Girton, 1981) experiments done to date, it has been proposed that triplications result from cell death patches internal in the disc (Girton,

FIG. 5. A photograph of the tarsal structures formed by a disc which was given a semicircular internal laser treatment (similar to that shown in Fig. 1). After treatment the disc was cultured for 10 days in the abdomen of an adult female, and then transferred to a late third-instar larva for metamorphosis. Note the extra claw structures (C) in the culticular process (CP).

1981). The induction of extra structures similar to cell-lethal-induced triplications by laser-induced cell death patches internal in the disc supports this hypothesis and suggests that laser-induced cell death and cell-lethal-induced cell death have similar effects on pattern formation. However, a definitive conclusion on this point must wait for further studies of the cell-lethal-induced death patches which result in triplications.

Our finding that large wedge-shaped laser-induced cell death patches can induce duplications does not imply that no other cell death patches will induce duplication. Postlethwait (1978) suggests that any patch which removes a large portion of the upper medial edge of the disc may remove enough positional valves to induce duplication according to the rules of the polar coordinate model (Bryant *et al.*, 1981), even though the patch may remove few internal cells. Also, the recent findings of Karpen and Schubiger (1982) that isolated blastemas may regenerate an entire leg suggests that a cell death patch which is not complete (i.e., in which a small "island" of viable cells is surrounded by dead cells) may produce an isolated blastema which, through interaction with the remainder of the disc (see Postlethwait and Schneiderman, 1973), may produce a large duplication with a small deficiency. These and other hypotheses are currently being tested using our ability to induce cell death patches of different size and shape in any desired location using the uv laser microbeam.

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