Short Communication

Compartments and Distal Outgrowth in the Drosophila Imaginal Wing Disc

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Summary. Peripheral tissue of the imaginal wing disc gives rise to the proximal mesothoracic structures of the adult. Pieces of peripheral tissue, which have no regenerative capacity when cultured as intact fragments, are capable of distal outgrowth (regeneration) after dissociation and reaggregation. This ability depends on the region of the disc periphery from which the fragment is taken. Extensive distal outgrowth occurs in reaggregates of a fragment containing equal proportions of tissue from anterior and posterior developmental compartments. The extent of outgrowth decreases as the proportion of posterior tissue is reduced, so that a fragment containing only anterior tissue shows no regeneration after dissociation. Limited distal outgrowth occurs in reaggregates of a wholly posterior fragment, but the regenerative capacity is increased greatly when a small amount of anterior tissue is included. It is concluded that distal outgrowth in the wing disc requires an interaction between cells of the anterior and posterior compartments.

Key words: Drosophila — Imaginal discs — Compartments — Distal outgrowth.

Introduction

When pieces of tissue from different regions of an imaginal disc (or of some other appendages like cockroach or amphibian limbs) are apposed, they often interact so as to cause the regeneration of the missing intercalating tissue (Haynie and Bryant 1976; French 1978). If the pieces come from approximately the same position on the proximodistal axis of the appendage, the intercalation is known as circumferential regeneration. Fragments from different positions on the proximo-distal axis also interact in this way (Itten and Bryant 1975; Bohn 1976; Haynie and Schubiger 1979), but, in all these systems, proximal tissue can also regenerate distally in the absence of any distal stimulus (Strub 1977; see also French et al. 1976). Such distal outgrowth does not result in any obvious way from intercalation, and it was suggested that it occurred only if a complete circumference of tissue from a more proximal region of the appendage was present (French et al. 1976). Results obtained from studies of regeneration in both the imaginal leg disc and the amphibian limb have subsequently shown that such a ‘complete circle’ of proximal tissue is not necessary (Slack and Savage 1978; Stocum 1978; Schubiger and Schubiger 1978).

We have now investigated the requirements for distal outgrowth to occur in the Drosophila imaginal wing disc, by analyzing the structures regenerated in reaggregates of dissociated proximal wing disc fragments. Earlier experiments had indicated that, in the leg disc, regeneration was primarily proximal to distal after dissociation (Strub 1977). Our results confirm that this is also true for the wing disc. Further, they show that distal outgrowth does not require a ‘complete circle’ of proximal wing disc tissue. Rather it is dependent on the presence in the reaggregates of cells from both anterior and posterior developmental compartments (Garcia-Bellido et al. 1976). Schubiger and Schubiger (1978) reached similar conclusions from a study of the regenerative ability of undissociated leg disc fragments.

Materials and Methods

D. melanogaster imaginal wing discs were taken from late 3rd instar larvae which carried, as cuticle markers, either e11 or y; mwh (Lindsley and Grell 1968). 40–120 disc fragments (see Fig. 1) were dissociated in calcium-free insect Ringer solution containing
0.125 mg/ml of a collagenase preparation (Sigma type 1; this preparation contains other proteolytic activities). Dissociation at 25°C required 1'/2–2 min stirring, using a microstirrer. The dissociated cells were reaggregated by centrifugation, the pellet drawn up into a thin parallel-sided pipette and, after extrusion, cut into disc-sized pieces. These were then cultured in adult female hosts for 7–8 days at 25°C. Recovered implants had often grown appreciably and were cut into an appropriate number of pieces before reimplantation into mature 3rd instar larvae for metamorphosis and differentiation. Both adult and larval hosts were of the Barton wild-type strain. The techniques were essentially as described by others (Garcia-Bellido and Nöthiger 1976; Strub 1977). The collagenase preparation was used instead of trypsin as it appeared to be less harmful to the cells (larger implants were recovered from adult hosts); results were not affected qualitatively. As controls, some undissociated fragments of each type were implanted directly into larvae to determine the presumptive fate of each. Differentiated implants recovered from eclosed hosts were mounted in Hydromount aqueous mounting medium and analyzed at 400 x magnification using a Zeiss RA compound microscope.

Results

The wing disc fragments a–f used in this study are shown in Fig. 1. On metamorphosis, such peripheral fragments differentiate to give the more proximal adult cuticular structures, such as notum and wing hinge. Fragment f includes only tissue which will give rise to adult structures of the anterior compartment, while fragment d is similarly restricted to the posterior compartment. The other fragments contain varying proportions of anterior and posterior cells. When intact, each of these presumptive proximal fragments often regulated, during culture in adult hosts, by duplicating its existing structures (see Bryant 1975), but, with a single exception, none regenerated any new structures (a 0/27; b 0/23; c 0/18; d 0/9; e 0/12; f 1/13). In contrast, after dissociation, most were able to regenerate to some extent. This regeneration was almost entirely of the more distal structures, that is, those derived from central disc tissue (see legend to Fig. 1).

Regenerates of dissociated fragments from the presumptive ventral and dorsal regions of the disc, a and b, respectively, both showed evidence of such distal outgrowth (Table 1). In the case of fragment a, the regenerated tissue often made up the bulk of the implant and was limited to the most distal wing blade structures – large areas of wing blade hairs with marginal bristles and hairs, often including the junction between the double and posterior rows which lies at the distal wing tip. We have used estimates of wing area as a measure of the extent of distal outgrowth. This enabled us to determine this extent even in cases where small amounts of wing blade appear in the fate map of the fragment. Thus, while traces of wing blade appeared in the non-dissociated controls of fragment a in some cases, 12.5 times as much, on average, was found in recovered reaggregates (see Table 1).

Regeneration occurred much less frequently after dissociation of fragment b, but again it was almost exclusively distal. The appearance at low frequency of more proximal non-fate structures like costa and alar lobe, which lie close to the cut edge of the fragment, suggested that limited circumferential regeneration may be taking place.

These results demonstrate that distal outgrowth in the wing disc does not require the presence of a complete circumference of proximal disc tissue. The same conclusion was reached by Schübiger and Schübiger (1978) from their studies of regeneration in intact leg disc fragments.

To define further the requirements for distal out-