The embryonic origin of imaginal discs in *Drosophila*

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Summary

The thoracic imaginal discs of *Drosophila melanogaster* can be observed during embryogenesis as clusters of cells with particular shapes, sizes and behaviours. These structures can be detected soon after germ band shortening and their development appears to be tightly linked to that of the larval epidermis.

Key words: imaginal discs, embryos, *Drosophila*.

Introduction

The adult epidermis in *Drosophila* develops from precursor cells that are set aside during embryogenesis. While the abdominal segments are derived from histoblasts, clusters of cells that contribute to the larval epidermis and proliferate in the pupa, the rest of the adult epidermis is formed from the imaginal discs, groups of cells that do not contribute to the larval epidermis and proliferate during larval life (Nothiger, 1972).

It is known that the segregation of the imaginal disc precursors from the larval epidermis takes place during embryogenesis, and the imaginal discs can be identified in the first instar larva (Madhavan and Schneiderman, 1978). However, the embryonic events of segregation, as well as the number and position of the precursors in each primordium, have never been demonstrated directly, but inferred from the results of a variety of different experiments. Data from clonal analysis (Wieschaus and Gehring, 1976a) and gynandromorph (Garcia Bellido and Merriam, 1969; Wieschaus and Gehring, 1976b) or ablation fate mapping (Lohs Schardin et al. 1979) suggest that soon after cellular blastoderm, ectodermal cells are selected to become imaginal precursors and thus become determined as different from the larval cells.

Poulson (1950) observed some of the imaginal discs in the *Drosophila* embryo after cuticle deposition and the full complement has been described in the first instar larva shortly after hatching, when the imaginal cells start to proliferate (Madhavan and Schneiderman, 1978). Recently, the location of the primordia for the leg discs has been inferred from the pattern of expression of the gene *Distalless (Dll)* during early embryogenesis (Cohen, 1990). In the extended germ band, small patches of Dll expression on the lateral sides of the thoracic epidermis have been postulated to be the primordia of the leg discs (Cohen, 1990). This suggestion is difficult to confirm because there is, as yet, no independent description of the segregation and position of the imaginal primordia. The only relevant observation is the description of the segregation of the thoracic discs from the larval epidermis in embryos of the fly *Dacus tryoni* at lateral positions in the thoracic epidermis (Anderson, 1963).

In this paper, we describe the embryonic origin of the thoracic imaginal discs in *Drosophila* and place it in the context of the patterning of the larval epidermis. We have used flat preparations and antibody staining of *Drosophila* embryos at different stages of development to locate imaginal disc precursors and to follow their segregation from the larval epidermis. In addition, BUdR labelling at different stages of embryogenesis suggests that the definition and segregation of these primordia in the thorax are linked to the proliferation of the larval epidermis.

Materials and methods

Stocks

In these experiments we have used OregonR and Canton S as wild-type stocks. To produce mutants for genes of the bithorax complex, we have used two stocks: DpP5; Df(3R)P9, which produces embryos lacking the whole of the bithorax complex (Lewis, 1978) and *Ultrabithorax* gene, *Ubx*.

BUdR incorporation, antibody stains and histochemical stains

The incorporation and visualization of BUdR followed published protocols (Bodmer et al. 1989) with minor variations. The antibody DA.1B6 (Brower et al. 1980) also known as anti-fasciclin III (Patel et al. 1987) was kindly provided by M. Wilcox. All manipulations of embryos for and during antibody stains followed standard protocols (see Ashburner, 1990). Flat preparations of embryos, dissections and toluidine blue staining have been described before (Bate, 1990).
Results

The larval epidermis and the imaginal discs after embryonic proliferation

Between 12 and 14 h of embryogenesis, shortly before the onset of cuticle deposition, the larval epidermis consists of an ordered array of epithelial cells of about 10-12 cells long and 30 cells wide per hemisegment. In flat preparations stained with toluidine blue, cells appear slightly elongated in the dorsoventral axis and arranged in parallel lines at right angles to the anteroposterior axis (Fig. 1B). The epidermal cells have large nuclei and display different shapes and sizes at different levels of each segment. The overall features of a segment, in terms of number and arrangement of cells, are similar in the thoracic and the abdominal segments.

We have not succeeded in identifying the abdominal histoblasts, but, at this stage, dorsal (wing and haltere) and ventral (leg) thoracic discs are easily visible in flat preparations, or in wholemount embryos stained with the monoclonal antibody DA.1B6 (Brower et al. 1980), also known as anti-fasciclin III (Patel et al. 1987). The imaginal discs can be seen as subepidermal clusters of cells still connected to the outside layer of larval epidermis (Figs 1B,C and 3B). In T1, there is a prominent invagination associated with the anterior spiracle; this invagination is missing in T2 and T3 where in a more ventral and posterior position the wing and haltere discs can be found attached to the tracheal system (Fig. 2). The wing disc contains about 24 cells, has a fan-like appearance and is located posteriorly in the segment, hanging over T3. The haltere disc contains about 12 cells, lies in a homologous position in T3 and forms a smaller, rounder structure than the wing disc. The cells of these discs are highly elongated and their extended processes terminate as coherent groups in the epidermis of T2 and T3, where they form conspicuous clusters of end feet marking the sites of invagination among the larval epidermal cells (Figs 1 and 3). These clusters lie 3-4 cells anterior to the segment border (defined as the line of cells on which the larval longitudinal muscles insert) and at the level of the remnant of the tracheal pit in the dorsoventral axis. The leg discs appear as prominent spherical clusters on either side of the CNS towards the middle of each thoracic segment with the prothoracic discs located in a more medial position than the other two pairs (Fig. 1B,C). Unlike the wing and haltere discs, the leg discs remain close to the overlying epidermis, forming compact clusters of cells beneath the depressions that mark the sites of invagination and of the future Keilin's organs. In embryos treated with anti-fasciclin, the centre of each depression is marked by intense staining (Figs 1A and 4). We take it that this staining demarcates the anlagen of the Keilin's organs. An estimate of the number of epidermal precursors in the leg discs is difficult because, unlike the dorsal discs, these represent a mixed population of epidermal, nerve and mesodermal cells (Bate et al. 1991). We are confident that these groups of invaginated cells correspond to the thoracic imaginal discs because of their position, size, segmental specificity, attachments to the epidermal derivatives and behaviour in mutant backgrounds (see below). In addition, we have followed the cell clusters into the first instar larvae, where they coincide with the imaginal discs as described in earlier reports.

Segregation of imaginal discs

It is possible to trace back the imaginal discs to earlier embryonic stages. In the case of the wing and haltere discs, invagination is initiated at 9-10 h after egg laying (AEL) and is signalled by a local reduction in the apical diameter of cells and a basal location of their nuclei, as the cells elongate inwards (Fig. 3). This identifies groups of prospective wing and haltere cells located posteriorly in their respective segments (Fig. 1A) and at the level of the persistent remnants of the tracheal pits. We have not been able to identify the cells of the dorsal discs before this, and because there may have been extensive cell movements, we cannot say with certainty that the discs are derived from an earlier population of cells located at precisely this position in the developing segment. As for the leg discs, they too can be identified as invaginating cell groups in embryos 9-10 h AEL (Figs 3 and 4). However, they form part of a set of cells that clearly differs in its behaviour from the rest of the epidermis in a thorax-specific way from early on. Specifically, the cells of the prospective leg discs have a rounded mobile appearance at this stage and contrast with the more compact and closely packed cells of the abdomen (see Fig. 3A). Prior to invagination, and as far back as...
the onset of germ band shortening, this cell group, which lies just dorsal to the CNS and posterior in the segment, is conspicuous for its divergent cell shape and arrangement, properties that suggest that the cells already have distinctive adhesive characteristics, and/or that they are undergoing a specific programme of proliferation. One interesting behaviour of the prospective disc regions is a transient reduction in the levels of fasciclin III expression just prior to invagination, which could signal a change in adhesive properties (Fig. 4).

**Epidermal proliferation during embryogenesis**

After proliferation, the cells of the imaginal discs represent a substantial fraction of the embryonic epidermis (about 10%, i.e. some 40 cells out of a total of 300 cells per hemisegment). Assuming that during embryogenesis all epidermal cells divide the same number of times, the larval epidermis of the thoracic and abdominal segments after invagination of the disc cells, ought to consist of different numbers of cells. However, cell counts from embryos after germ band shortening and the segregation of the discs, indicate that, within the limits of observation, thoracic and abdominal segments have a similar number of epidermal cells. Since the separation of the imaginal cells from the larval epidermis takes place after the last epidermal mitosis, this suggests either that there are different numbers of mitoses in thoracic and abdominal segments, or that the discs initiate from very small primordia, which proliferate quickly after invagination.

To distinguish between these alternatives, we have used BUDR as a label to monitor DNA synthesis and proliferation in the embryonic epidermis (Truman and Bate, 1988; Bodmer et al. 1989). When embryos are
Fig. 4. Epidermis of embryos stained for fasciclin III expression. (A) 10–11 h embryo. The sites of the dorsal discs during their segregation from the larval epidermis can be seen as a local reduction in levels of fasciclin expression (arrowheads). The leg discs have a heavily stained central region corresponding to the location of the primordium for the Keilin’s organs (arrows). (B) After invagination, the expression of fasciclin III demarcates the endfeet of the wing (w) and haltere (h) discs and the position of the Keilin’s organs (arrows). Scale bar: 20 μm.

continuously exposed to BUdR from 5 to 12 h AEL, all epidermal cells are labelled (Fig. 5A). If a similar experiment is performed from 6 h AEL onwards (Fig. 5B), conspicuous label appears preferentially in the ventral region of the thoracic segments. In contrast, labelling from 7.5 h AEL shows no epidermal derivatives labelled other than the sense organs (Bodmer et al., 1989, and unpublished observations) and some cells of mesodermal origin associated with the imaginal discs. These results suggest that the extra proliferation in the thorax provides a pool of cells for the process of segregation.

Disc development in homoeotic mutants

The segmental specificity of the imaginal discs and their derivatives is under the control of the homoeotic genes and genetic evidence suggests that the assignation of imaginal primordia to particular segments occurs during embryogenesis (Morata and Kerridge, 1981). To confirm this, we have looked for the presence or absence of discs in embryos with mutations in Ubx, which transforms parasegment (PS) 5 and PS6 into PS4 and embryos homozygous for Df(3R)P9, which removes the complete bithorax complex and transforms PS5 through PS13 into PS4 (Lewis, 1978). Embryos homozygous for the Ubx92 allele have a set of extra leg discs, a rudimentary wing disc and two new sets of spiracles (Fig. 6). Embryos homozygous for Df(3R)P9 lack the whole bithorax complex and display a reiteration of a pattern of dorsal and ventral discs between T1 and ‘A8’. In these embryos, we identify dorsal disc primordia by the characteristic position, shape and clustering of their attenuated end feet, never seen in wild-type abdominal segments. Using this criterion, we see dorsal disc primordia variably in segments from T2–‘A7’, although we cannot say whether the primordia correspond to wings or halteres. Leg discs and spiracles are more obvious and are present in a reiterated pattern between T1 and ‘A8’. Posteriorly, it becomes progressively more difficult to identify the end feet clusters and, in the more
Fig. 5. Embryos treated with BUdR for different periods of time and stained with anti BUdR-antibody to reveal patterns of replication during the treatment. (A) Ventral view of an embryo that has been treated from 4.5-5 h AEL until 12-13 h AEL. All epidermal cells contain label, suggesting that during the period all cells replicated their DNA. The thoracic cells, however, show heavier labelling suggesting that they have undergone more replication and hence more proliferation. (B) Ventrolateral view of an embryo, which has been treated from 5.5-6h AEL until 11-12h AEL. The label is preferentially ventral and there is a clear thoracic label which can be correlated with the prominently labelled region in A. Scale bar: 20μm.

posterior segments, the number of end feet may be as few as two or three, indicating that only a small number of cells is taking part in the formation of individual primordia.

Discussion

During the second half of embryogenesis, the thoracic imaginal discs are conspicuous, tightly packed subepidermal clusters of cells with defined positions and shapes. They begin to separate from the epidermal sheet through characteristic cell movements after germ band shortening but remain connected to it by the ends of the invaginating cells. In the dorsal discs these connections become greatly elongated and attenuated. Our observations agree with those of Anderson (1963) for the discs of Dacus tryoni, in that the segregation of the discs from the larval epidermis takes place from positions about half way down the dorsoventral axis. Although at the time of invagination, the primordia for the ventral and dorsal thoracic discs are very close, about two cell diameters, we never see any connection between them as has been described for Dacus. However, cells that we have elsewhere identified as mesodermal (Bate et al. 1991) do form an obvious strand between them, once invaginated. The close spatial relationship between ventral and dorsal derivatives helps explain why it is that clones generated early in embryogenesis can cross between leg and wing or leg and haltere (Wieschaus and Gehring, 1976a). At about six and a half hours AEL, segment-specific patterns of DNA replication can be observed that prefigure the next round of divisions (unpublished observations). These patterns precede the segregation of the discs and highlight an extra division that will involve ventral thoracic cells, preferentially. This cellular behaviour may reflect some kind of programmed compensatory division of epidermal cells for the loss of the invaginating primordia. In this context, it is interesting that a major invagination event, which occurs at the site of the future anterior spiracle, is consistently preceded by a similarly segment-specific and prominent pattern of DNA replication. Such an increase is also visible in the ventral thorax and may be associated with the development of the leg discs. However, it is clear that the invagination of the wing and haltere discs is not associated with any obvious localised round of DNA synthesis and proliferation. We conclude that compensation for the loss of the invaginated cells must involve changes in cell shape and cell arrangement and that the extra ventral divisions in the thorax may compensate for the change in cell numbers associated with the invagination of both the dorsal and ventral discs. We estimate (simply by counting invaginating cells) that there are about 12 cells in the haltere disc and 24 cells in the wing. Clearly these could be under- or overestimating the actual number of cells in the disc. Counting cells in the first instar larva, Madhavan and Schneiderman (1978) found 37 cells in the wing disc and 20 cells in the haltere. Either there has been an additional round of cell division between the time of our observations and theirs, or they included cells in their estimate that we have shown (Bate et al. 1991) to be of mesodermal origin. Our observations on cell number are difficult to relate to the estimates for the disc anlagen obtained from clonal analysis (for review see Wieschaus, 1978), because these refer to the primordial cells in the blastoderm from which the discs are ultimately derived.

Clonal analysis has suggested that the imaginal discs are determined soon after the cellular blastoderm stage (Wieschaus, 1978). However, it is likely that at this early stage a population exists from which the specific imaginal discs will be drawn later in embryogenesis (Wieschaus and Gehring, 1976a,b). Our observations that specific cellular properties characteristics of disc cells are not visible until half way through embryogenesis agree with this suggestion. In addition, we believe that the development of the disc precursors is tightly integrated with the development of the pattern of the larval epidermis as a whole. In hemimetabolous insects, the development of limbs is a continuous process...
involving a sequence of determination, growth and differentiation. However, in holometabolous insects like Drosophila, there is an additional step in the development of structures such as legs and wings in that these cells are 'adult' and this adult state must be specified in some way so that the behaviour of these cells becomes different from their larval neighbours. These adult cells remain in a quasi-undifferentiated, proliferative state and invaginate as discrete clusters, while their neighbours differentiate as a characteristi-
cally patterned population of epithelial cells. It is these distinctive properties of the future adult cells in the embryo (different shape, size and movement) that we have described here and which have enabled us to identify the embryonic precursors of adult structures.

The imaginal disc precursors represent embryonic structures that appear as a response to the machinery that establishes cell states and thus positional information in the embryo (Cohen, 1990; Simcox et al. 1989). These structures will proliferate during larval development but during early embryogenesis the precursors are distinct from the future adult cells. For this reason, we believe that they participate in the generation of positional information but during early embryogenesis the precursors are distinct from the adult cells. Therefore, we believe that they are part of the epithelial sheet in which cell diversity is achieved, and the larval epidermis, are also required for proper patterning of the larval epidermis. This would agree with the fact that despite many attempts to find mutations that specifically affect the definition of the imaginal discs as larval viable mutations, all that has been found are mutations that affect the proliferation of the imaginal cells (Shearn and Garen, 1974; A. Shearn and A. Simcox, personal communication). Indeed, the elegant transplantation experiments of Simcox et al. (1990) show that some genes of the segment polarity class, which are necessary for the proper patterning of the larval epidermis, are also required for proper formation of imaginal disc precursors.

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References


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