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Larval and imaginal pathways in early development of *Drosophila*

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ABSTRACT In holometabolous development, higher insects have two different life forms, the larva and the imago. Both larval and imaginal cells are derived from cells of the blastoderm stage. After the final embryonic wave of mitosis, however, only the imaginal cells remain diploid, proliferate massively and do not differentiate until metamorphosis. The separation of these two pathways was described by many authors as a fundamental process that must take place at a very early stage of development, most probably the blastoderm stage. Mainly by using single cell transplantations at the blastoderm or early gastrula stages, respectively, we found common cell lineages between larval and imaginal structures by clones overlapping in the ectoderm (i.e. larval epidermal cells and imaginal discs within a segment, or larval and imaginal salivary gland cells), the mesoderm (i.e. larval somatic muscles and adepithelial cells), and the endoderm (i.e. larval and imaginal midgut cells). From these findings we conclude that it seems to be a principle in *Drosophila* embryogenesis that the separation of larval and imaginal pathways is postponed to a later developmental stage.

KEY WORDS: *cell lineage, fate map, mitotic recombination, HRP injection, single-cell transplantation*

Introduction

Our understanding of insect development, especially that of *Drosophila*, has increased considerably in the past 20 years on the basis of two lines of investigation. The first was the working out of the concept of developmental compartments (Garcia-Bellido, 1975). Clonal analysis, introduced by Becker (1957) as a tool to study *Drosophila* development, showed that blastoderm cells are restricted in developmental potentialities (Wieschaus and Gehring, 1976). By using the *Minute* technique the compartmental organization of the early embryo could be visualized (Garcia-Bellido *et al.*, 1976). The second line of evidence was the systematic search for genes responsible for early developmental processes which was initiated by Nüsslein-Volhard and Wieschaus (1980). Three sets of genes have been described to organize the fate map: i) maternal genes which act during oogenesis to establish the global coordinate values of the egg, ii) zygotically active segmentation genes which divide the developing embryo in metameres, and iii) homeotic genes that are responsible for segmental and/or compartmental identities (for reviews see Pankratz and Jäckle, 1990; St Johnston and Nüsslein-Volhard, 1992).

Besides the genetic analysis of early developmental processes, embryological observations and experimental procedures such as destroying cells by UV irradiation, injection of horseradish peroxidase into cells of the early gastrula, transplantation of marked cells or analyses of gynandromorphs revealed the fate map of the blastoderm embryo (for reviews see Janning, 1978; Campos-Ortega and Hartenstein, 1985; Technau, 1987). As in many other species the fate map of *Drosophila melanogaster* shows the positions of anlagen from which corresponding organs differentiate during normal development without including information on developmental potentialities of anlagen cells.

In this short review we will summarize some of the results we obtained on the cell lineage of blastoderm or early gastrula cells and on the localization of anlagen in the fate map.

Clonal analysis of the Malpighian tubules

Based on analyses of mitotic recombination clones and gynandromorph data, cell lineage relationships in the imaginal cuticle are now well known. As a model system to study the cell lineage in the developing anlage of a larval organ by clonal analysis, we have chosen the Malpighian tubules, which consist of an anterior and a posterior pair of tubules. Their larval cells have large nuclei and are arranged in a zigzag manner. A characteristic feature of the Malpighian tubules is that they are prob-

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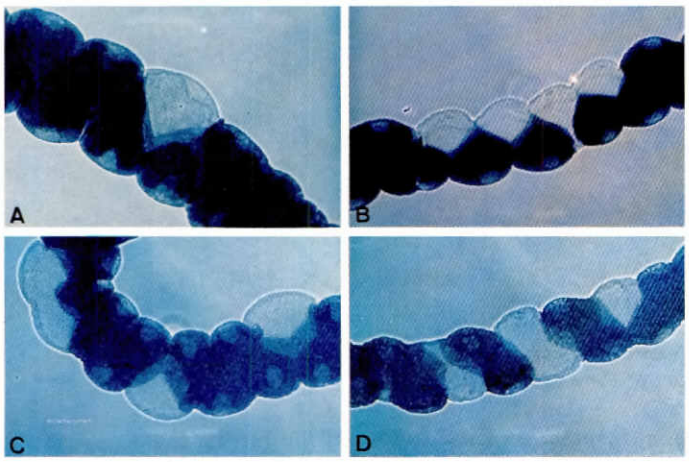

Fig. 1. Mitotic recombination clones in Malpighian tubules with wildtype cells (blue) and genetically marked *mal* cells. *These are unstained because of their lack of aldehyde oxidase activity. Clones containing one single* **(A)** *or four adjacent mal cells* **(B)** *as well as fragmented clones with four marked cells each* **(C,D)** *are shown. In the latter cases there is a high probability that all marked cells belong to a single clone.*

ably the only larval organ which remains essentially intact during pupation i.e., they are not histolysed during metamorphosis but rather undergo some structural changes (Bodenstein, 1950; Meise, unpublished results).

For these lineage experiments, genetically marked *maroon-like (mal)* clones were induced by mitotic recombination with X-rays at the blastoderm stage in *mal/mal*$^{+}$ heterozygotes and were analysed in differentiated Malpighian tubules. *mal* homozygous cell clones, which lack aldehyde oxidase activity, were not confined to single anterior or posterior tubules, but were distributed among the four tubules. Experiments using the *Minute* technique did not show any compartmental organization in the Malpighian tubules anlage. In both sets of data

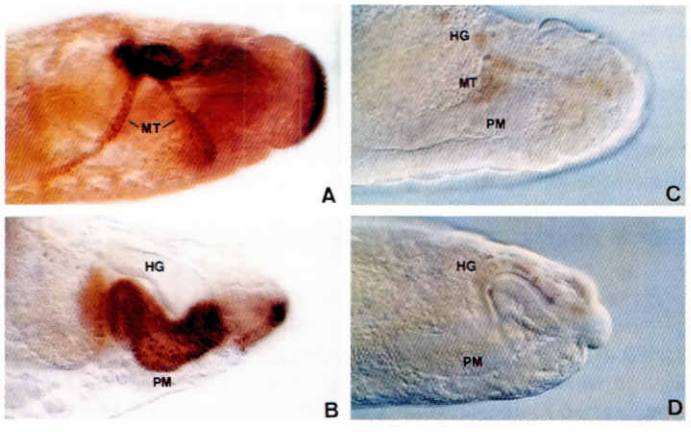

Fig. 2. Posterior of wildtype (A,C) and *Kr*^{1} homozygous embryos (B,D). *In the wildtype anti-caudal antibody labels posterior midgut and Malpighian tubules* **(A)**, *whereas in Kr*^{1} *the tubules are missing. Injection of horseradish peroxidase into dorsal cells at 20% egg length of the early gastrula results in marked cells of hindgut* **(C,D)** *and Malpighian tubules* **(C)**. *HG, hindgut; MT, Malpighian tubules; PM, posterior midgut.*

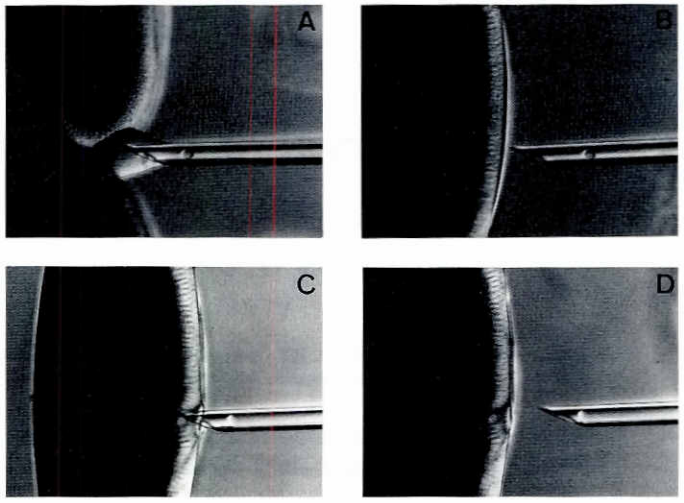

Fig. 3. Isolation and transplantation of a single cell at the early gastrula stage. *After a small number of cells is removed from the donor into the transplantation solution, the cells are repeatedly drawn into the capillary with some solution and ejected again until a single cell can be drawn out of the droplet into the capillary* **(A)**. *Now the capillary contains only the single cell and the clear, transparent transplantation solution* **(B)**. *Finally the isolated cell is transplanted between the cells of the host* **(C,D)**.

about 70% of the clones with two or more cells were fragmented, i.e. *mal* cells were separated by wildtype cells (Fig. 1). Since the clones contain, on average, 6 cells and the differentiated Malpighian tubules consist of 484 cells, we estimate that there are about 80 cells in the blastoderm anlage which on average pass through two to three mitoses. Only a small fraction of cells goes through a fourth or even a fifth mitosis (Janning *et al.*, 1986).

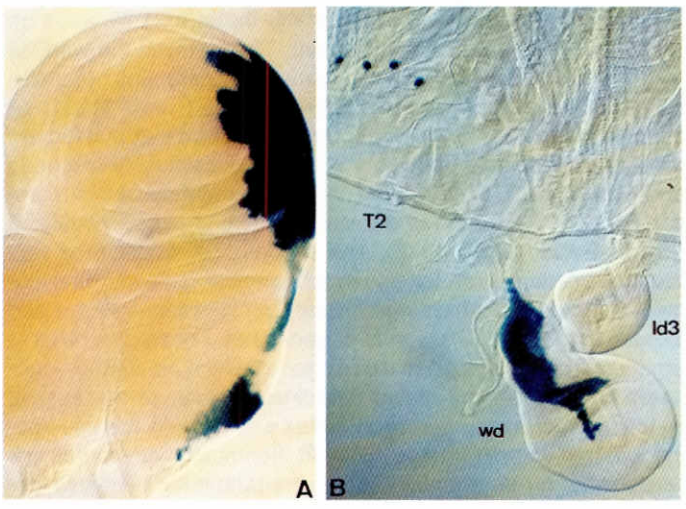

Fig. 4. Clones in larval as well as in imaginal tissues of the third instar larva after single cell transplantation at the early gastrula stage. *The nuclei of a clone express ß-galactosidase and are stained blue with X-gal.* **(A)** *Clone of a single transplanted cell in the posterior compartment of a wing imaginal disc.* **(B)** *A clone consisting of four cells in the second thoracic segment of the larva (T2) plus 1/6 of the cells in the wing imaginal disc (wd). ld3, third leg disc. Anterior is to the left.*

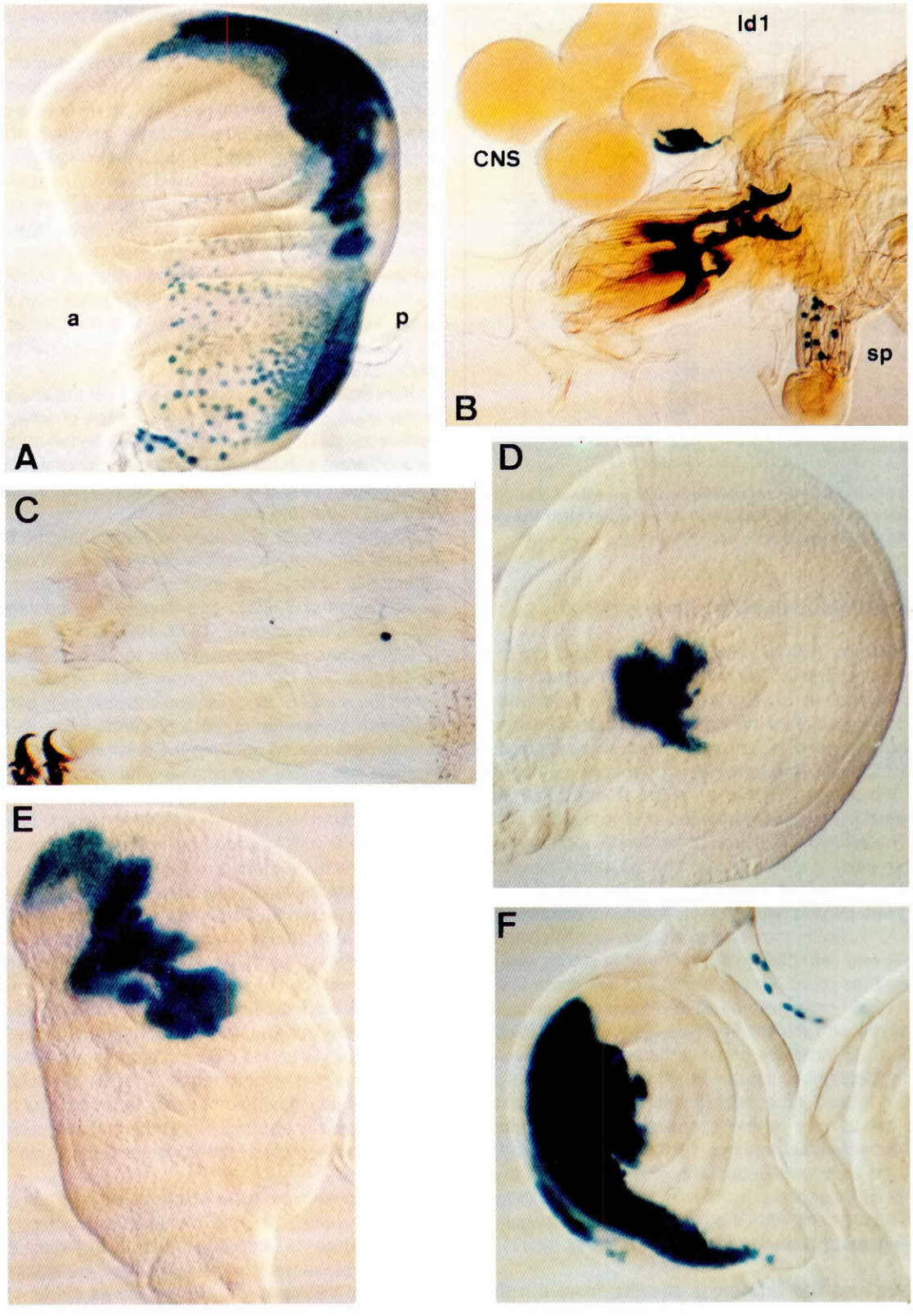

Fig. 5. Clones that overlap between imaginal discs and larval structures. (A) *A wing disc clone extending to the peripodial membrane. a, anterior; p, posterior.* **(B)** *Clonal overlap between the anterior spiracle (sp) of the first thoracic segment and the first leg imaginal disc (ld1). CNS, central nervous system.* **(C-E)** *A clone overlapping one cell in the third thoracic segment of the larva* **(C)** *and the third leg disc* **(D)** *and the haltere disc* **(E)**. **(F)** *Overlapping clone between a first leg imaginal disc and the nerve connecting the disc with the ventral ganglion.*

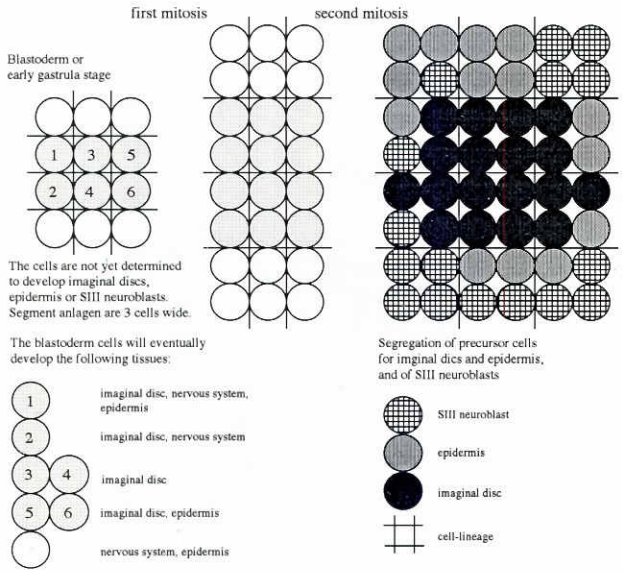

Fig. 6. Model explaining how determination of imaginal disc precursor cells is likely to occur by positional information after the second postblastodermal mitosis. *For further explanation see text.*

Krüppel and the fate map of the early embryo

Malpighian tubules, hindgut and posterior midgut develop from the posterior region of the blastoderm (Poulson, 1950; Janning, 1976; Hartenstein *et al.,* 1985). One of the genes which influences the differentiation of the Malpighian tubules is *Krüppel (Kr),* a segmentation gene of the gap class. *Kr* homozygous embryos lack thoracic and anterior abdominal segments; depending on the allele, there is a range of effects on the Malpighian tubules, from not differentiated at all to nearly normal (Fig. 2A,B). In the wildtype, injection of horseradish peroxidase into cells of the early gastrula at various posterior positions results in labelling of hindgut, Malpighian tubules and posterior midgut. Malpighian tubules were labelled only in combination with hindgut. In Kr^1 homozygous embryos which lack Malpighian tubules the label was restricted to hindgut and posterior midgut (Fig. 2C,D). Since we could not find significant cell death in the posterior region of Kr^1 embryos we counted the cell nuclei in the hindguts of wildtype and mutant embryos. The results indicate that the hindgut in Kr^1 embryos contains those cells that would differentiate into Malpighian tubules in wildtype. We therefore conclude that *Kr*, in addition to its function as a segmentation gene, plays a critical role in the specification of Malpighian tubules cells (Harbecke and Janning, 1989). This interpretation is supported by the results of Redemann *et al.* (1988) and Gaul and Weigel (1991). Besides its function in segregating hindgut and Malpighian tubule cells, *Kr* is also involved in the elongation process of the tubules (Harbecke and Janning, 1989).

Clonal analysis and the fate map: results from single-cell transplantations

In holometabolous development, higher insects have two different life forms, the larva and the imago. Both larval and imaginal cells are derived from the cells of the blastoderm stage. After the final embryonic wave of mitosis, however, only the imaginal cells remain diploid, proliferate massively and do not differentiate. The separation of the two pathways was described by many authors as a fundamental process that must take place at a very early stage of development, most probably the blastoderm stage. The development of the imaginal discs in *Drosophila melanogaster* has been followed back to the late embryo (Bate and Martinez-Arias, 1991). In this stage, very small groups of cells can be seen to separate from the larval cells. Postembryonic mitosis in the imaginal discs does not begin until the end of the first larval instar (Madhavan and Schneiderman, 1977; Garcia-Bellido and Ripoll, 1978), but then the cells proliferate exponentially, reaching a final number of as many as 50,000 cells by the end of pupation (Garcia-Bellido and Merriam, 1971; Steiner, 1976).

Very little is known about the origin of the imaginal structures in the blastoderm. Are the cell lineages of larval and imaginal cells separate? That is, are the cells of the larva already destined for a different ontogenetic fate than those of the fly, even at this early stage? Where are the anlagen of the imaginal disc cells localized in the fate map?

To answer these questions we have analyzed the cell lineage of larval and imaginal cells in the thoracic ectoderm of the early *Drosophila* embryo by homotopic transplantation of single cells in the region of 50-60% egg length (0%= posterior pole of the egg) (Meise and Janning, 1993, 1994).

Single cells were isolated prior to transplantation in an *in vitro* solution (Fig. 3). The donors were "enhancer-trap" lines in which the nuclei of all larval and imaginal cells exhibit a uniformly intense expression of the *lacZ* gene of *E. coli.* The transplantations were carried out from the blastoderm to the early gastrula stage, as a rule immediately after the onset of gastrulation (stage 6). Our results show that at this time the cells of the thoracic ectoderm are not yet committed to form larval or imaginal structures; this is indicated by the presence of clones overlapping all structures formed by the thoracic ectoderm, i.e. the nervous system, the larval epidermis, the tracheae and the imaginal discs (Figs. 4 and 5). The average size of pure epidermal clones was 5 cells. In clones overlapping either other larval tissues or imaginal discs, the average number of epidermal cells was between three and four. The mean relative clone size was 1/5 of the size of the total structure for leg imaginal discs and 1/7 for the wing imaginal disc. We therefore infer that the precursors for the three leg discs and the wing disc on one side together number 22 cells in the blastoderm or early gastrula stage. These cells eventually give rise not only to precursors of the imaginal discs but usually also to larval epidermal and/or nervous-system cells, because most of the imaginal disc clones (80%) overlap larval tissue.

We propose a model in which the segregation of the cells that are to differentiate into the imaginal tissues does not occur until the second postblastodermal mitosis. In Figure 6, the six cells that are precursors of an imaginal disc are assumed to be present in the blastoderm or early gastrula stage. Of the 24 cells present after the second mitosis, a certain number are exposed to the positional information for imaginal disc formation, which is confined to a small region. For example, if the descendants of one of the six blastoderm or early gastrula cells are situated

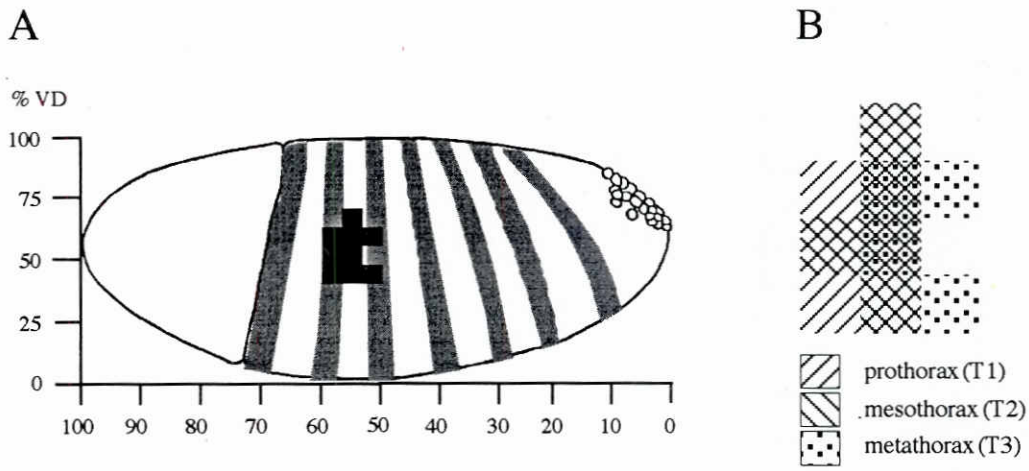

Fig. 7. Fate map derived from single-cell transplantations. (A) *Projection of the transplantation positions at which clones in the imaginal discs of the different segments were obtained onto the stage 6 embryo. The expression pattern of a ftz-lacZ fusion gene is indicated.* **(B)** *Magnification of the positions shown in A. Transplantations at the same site in different embryos can give rise to clones in different segments. However, clones were never observed to overlap segments in a single embryo.*

together at the periphery of the region supplied with the relevant positional information, it is easily conceivable that some but not all of them will become determined imaginal disc cells. The remaining cells can become epidermal cells or segregate as SIII neuroblasts. Thus, the model explains how overlapping clones can be derived from one blastoderm or early gastrula cell.

To localize the imaginal-disc primordia we transplanted cells into precisely defined positions along the anterior-posterior and dorsoventral axes. In about 90 clones, within the region of 50-60% egg length and 40-70% of the dorsoventral axis (0% corresponds to the most ventral part of the embryo), larval and/or imaginal epidermal tissue was labelled. From these data we concluded that the precursor cells of the thoracic imaginal discs are locally restricted to a small lateral area of the thoracic region (Fig. 7) (Meise and Janning, 1994).

Although the clones are often comprised of different ectodermal tissues and can include both imaginal discs in a given hemisegment, no clones were found that clearly crossed larval or imaginal segment boundaries. To follow the question whether clones in imaginal discs originating from the blastoderm are restricted to developmental compartments, we have combined single-cell transplantations with the *Minute* technique. Fast growing M^+ cells were transplanted into the region of the thoracic imaginal disc anlagen of *Minute* embryos. The results so far obtained are ambiguous: by comparison with the expression pattern of *engrailed* which marks the posterior compartments most of the clones seem to be restricted to anterior or posterior compartments, respectively (Fig. 8, unpublished results). But there are also clones that look like crossing the boundary, as it was observed for mitotic recombination clones (Brower *et al.*, 1981). To get a conclusive result it will be necessary to counterstain the discs with anti-*en* antibody.

Imaginal discs are composed of different cell types. In addition to the cells of the disc epithelium and the peripodial membrane (Figs. 4 and 5) there are also adepithelial cells which give rise to the imaginal muscles. These cells originate from the most ventral part of the embryo, the mesodermal anlage (for review see Bate, 1993). To localize the anlagen of the adepithelial cells we transplanted genetically marked single cells at the blastoderm stage homotopically into the mesodermal anlagen. From cells transplanted at about 50-60% egg length and near the ventral midline we found clones of adepithelial cells in all major thoracic imaginal discs (Fig. 9, Holz and Janning, 1993). All clones we found so far overlap with larval somatic muscles which shows

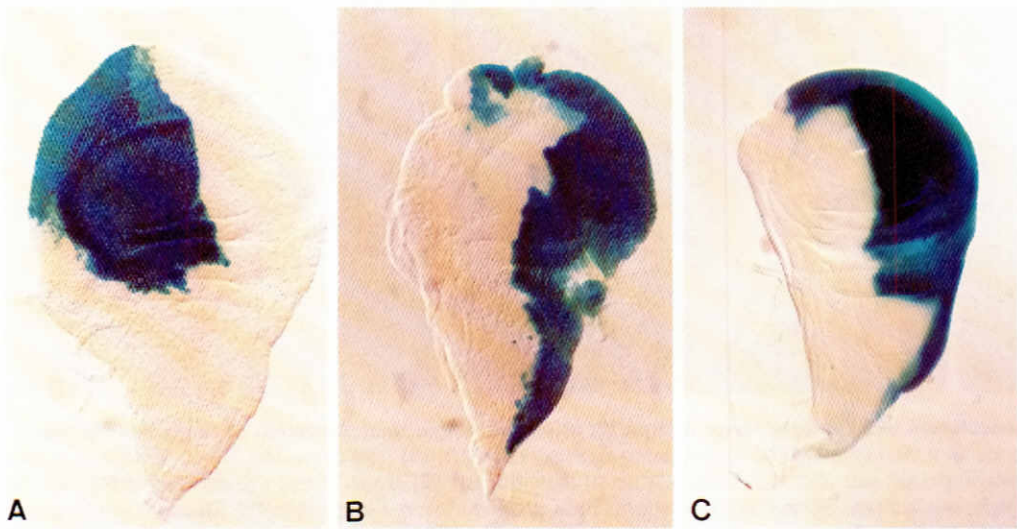

Fig. 8. *Minute*$^+$ clones in *Minute* imaginal discs after single-cell transplantation at blastoderm stage. *Donor: enhancer-trap line, recipient: y; Dp(1;3)sc^{J4}, y$^+$ sc^{J4} M(3)i^{55}/+. Clone in the anterior* **(A)** *and posterior* **(B)** *compartments of the wing imaginal disc.* **(C)** *engrailed expression in the posterior compartment for comparison.*

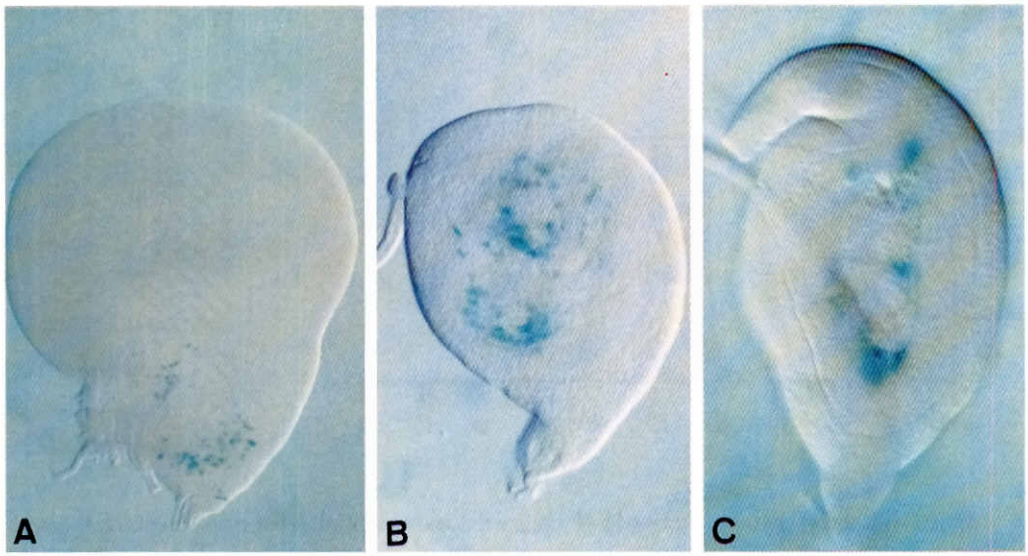

Fig. 9. Clones of adepithelial cells in wing (A) and leg (B,C) imaginal discs after single-cell transplantations into the mesoderm region of blastoderm embryos. *The clones reflect the distribution of adepithelial cells seen in histological sections: in the wing imaginal disc the adepithelial cells were only found in the region of the prospective notum, whereas in leg imaginal discs these cells are spread over the whole imaginal disc; they extend from a central point like concentric rings to the edge of the disc.*

that the transplantation site really was in the mesodermal region. Our results from these transplantations can be summarized as follows: i) mesodermal cells with larval and imaginal fate are not separated clonally at the blastoderm stage, the same finding as for the clonal relationships in the epidermis (see above), ii) while clones of adepithelial cells are restricted to one thoracic segment, the accompanying muscle clones can overlap two to three segments (as found for larval somatic muscle clones all over the mesodermal anlage; Fig. 10, unpublished results; see also Beer *et al.*, 1987).

We found some further clones that support the clonal relationship of larval and imaginal cells in the early embryo, i.e., the idea that a single cell can give rise to both larval and imaginal cells. Clones from single-cell transplantations at blastoderm stage can overlap larval and imaginal cells of the midgut (Fig. 10) as well as of the salivary glands (Fig. 11, unpublished results).

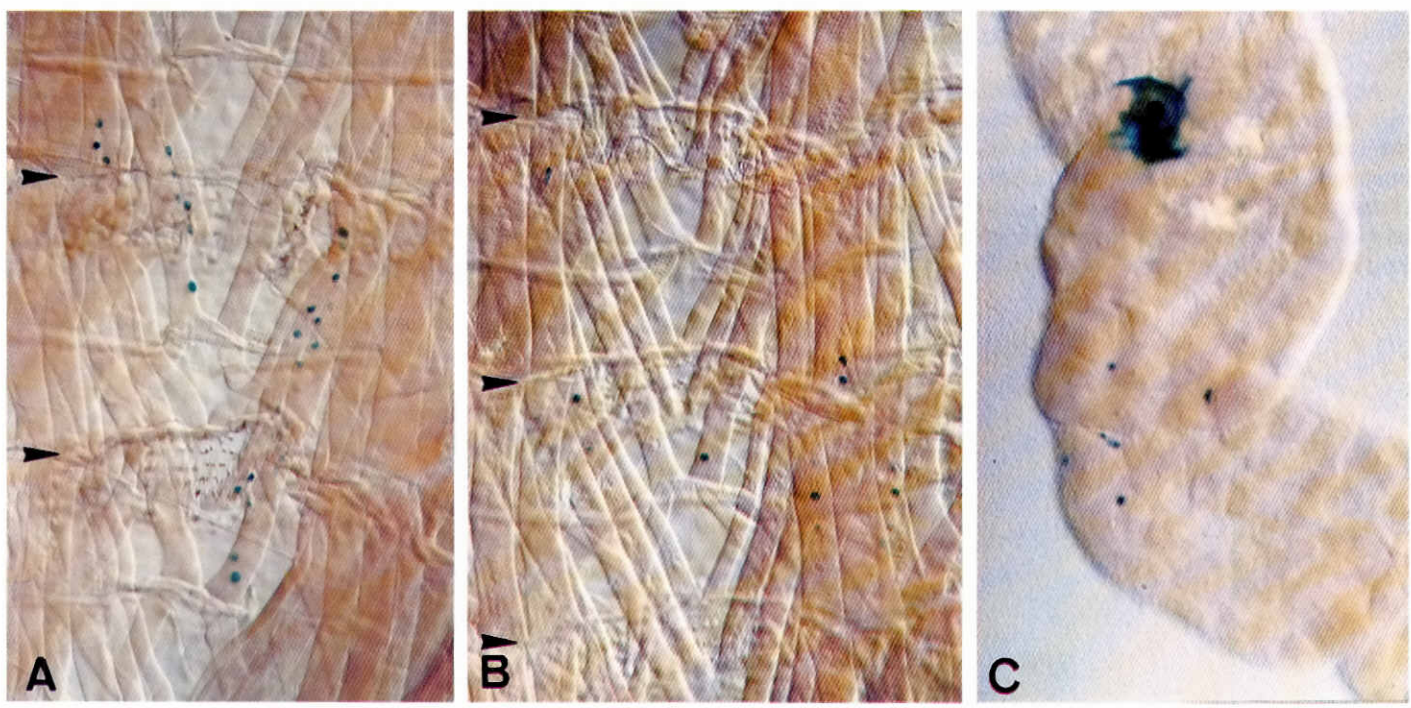

Fig. 10. Clones in the larval somatic musculature (A,B) and the larval midgut (C) after homotopic transplantation of single cells at blastoderm stage. *In the abdomen, muscle clones very frequently overlap neighbouring segments and left and right sides.* **(A)** *The clone comprises two muscles in the 4th abdominal segment (A4) on the left side and two muscles in A5 on the right side.* **(B)** *Nuclei of two muscles on the left (A3, A4) and of four muscles on the right (A3, A4) side, respectively, are stained. Arrowheads mark segment borders (denticle belts).* **(C)** *Clone in the midgut overlapping a large larval cell and some small imaginal cells.*

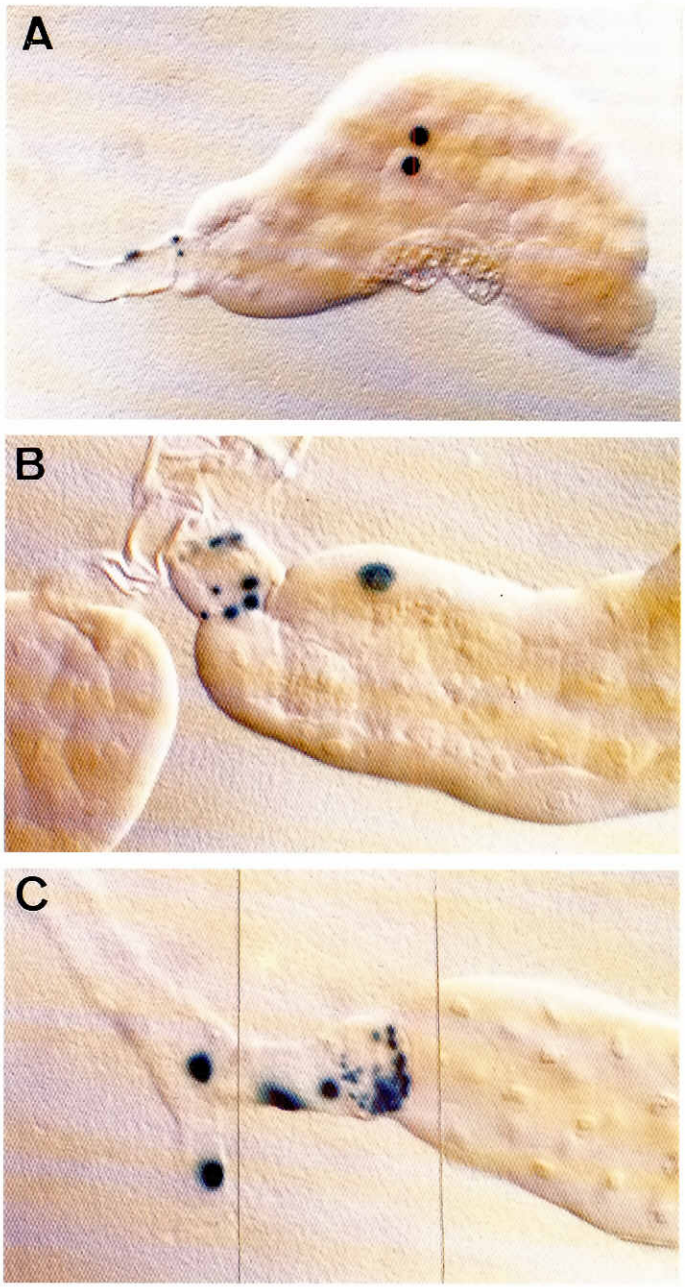

Fig. 11. Clones overlapping larval and imaginal cells in the salivary gland. *Single clones can comprise larval gland and duct cells as well as cells in the imaginal ring* **(A)**, *larval gland and imaginal cells* **(B)**, *or larval duct and imaginal cells* **(C)**. *These clones do not overlap cells of other tissues. This indicates that the anlage of the salivary glands is separate at blastoderm stage.*

Conclusion

The larval vs. imaginal cell fate has seemed to many investigators to be such a fundamental decision that it must occur very early, i.e. at the blastoderm stage when other critical decisions regarding the body plan are being made. Nevertheless, our results indicate that what people think to be a very important early decision in *Drosophila* development is apparently not so fundamental for the fly itself. Rather it seems to be a principle in *Drosophila* embryogenesis that the separation of larval and imaginal pathways is postponed to a later developmental stage.

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