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Observations on cell lineage of internal organs of *Drosophila*

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SUMMARY

Adult *Drosophila* mosaics can be used to study cell lineage and to map relative positions of primordia at the blastoderm stage. This information can define which germ layer an organ comes from and can help build models of genetic regulation of development. Here we use the *sdh* cell marker to map internal organs in mosaics made by nuclear transplantation. We confirm that oenocytes arise from the same progenitors as the adult epidermis, but that muscles and fat body have a separate (mesodermal) origin and that the precursors of epidermis and central neurones are closely intermingled in the ventral, but not dorsal, epidermis. We find that the malpighian tubules are more closely related to the hindgut than the midgut and are therefore ectodermal in origin. We find that each intersegmental muscle in the thorax arises from one specific parasegment in the embryo, but that very small numbers of myoblasts wander and contribute to muscles of inappropriate segments. We present evidence indicating that the visceral muscles of the midgut have a widely dispersed origin (over much of the embryo) while the somatic mesoderm of the female gonad comes from a small number of abdominal segments. The visceral mesoderm of the hindgut develops from a localized posterior region of the embryo.

INTRODUCTION

It may be possible to analyse *Drosophila* development in terms of independent units of cell lineage which have individual genetic instructions (Garcia-Bellido, Lawrence & Morata, 1979). Two types of evidence are needed for this analysis; a description of the cell lineage of developing organs and a definition of the realms of action of regulatory genes. Genetic mosaics (Lewis, 1963; Garcia-Bellido, Ripoll & Morata, 1973) and *in situ* hybridization to tissue sections (Akam, 1983; Hafen, Levine, Garber & Gehring, 1983) have contributed to this evidence but our picture of cell lineage in *Drosophila* is still incomplete; the epidermis is well described but the way the primordia of the embryo generate the internal organs of the larva and adult is not. In this paper we use nuclear transplantation and a general-purpose cell marker to answer a limited number of questions about the embryonic origin of internal organs.

MATERIALS AND METHODS

Nuclear transplantations were performed as previously described (Zalokar, 1971; Lawrence & Johnston, 1984*a*). Donors were late syncytial blastoderm and hosts were at early cleavage stages.

Key words: cell lineage, nuclear transplantation, *sdh*, *Drosophila* internal organs.

Our aim was to produce hosts that after heating would stain little or not at all for succinate dehydrogenase (Lawrence, 1981) so that the wild-type donor-derived cells could be identified wherever they were. In the cuticle, donor cells formed *yellow*$^+$ bristles which were easily distinguished in the *yellow* background (Fig. 1). About half the hosts carried a strong *Minute* allele which made the hosts grow slowly and gave donor cells a growth advantage (Morata & Ripoll, 1975). Hosts were produced by various crosses, most of which used *y; b pr cn* sdh^8 *bw* homozygous females which were crossed to *yellow* males that were either sdh^2, sdh^8 or sdh^2/sdh^8 on the second chromosome, and *Ki* Sb^{63b} *M(3)*$w^{124}/+$ on the third. (For genetic nomenclature see Lindsley & Grell, 1968 and Lawrence, 1981.)

The material was taken from experiments which also had other purposes – for example, to make mosaics that contained *engrailed*-lethal cells (Lawrence & Johnston, 1984*a*). Such experiments also produce mosaics where both donor and host cells develop as in wild-type animals (the donor genotypes are heterozygous for various mutations which have no significant dominant phenotype). We have also included mosaics which contain homozygous *Mcp* cells because this mutation produces only a local transformation of abdominal segment A4 into abdominal segment A5; *Mcp/Mcp* flies are viable and fertile (Lewis, 1978). It is difficult to imagine that the *Mcp* phenotype could materially affect the conclusions drawn here.

The amount of donor-derived tissue in the mosaics varies considerably from what are probably the descendents of only a few cells at the blastoderm stage (a portion of one segment in the dorsal epidermis) to more than half the fly. Typically mosaics contain donor tissue in three or four segments. Partly because nuclei were usually transplanted into the posterior half of the egg, most donor tissue was confined to the abdomen. However, there did seem to be a tendency for donor tissue to colonize the posterior segments – even when nuclei were intentionally placed in more anterior parts of the egg. The total number of mosaics used in this paper is 206, only a proportion of these being relevant for any particular question.

In nearly all the mosaics the donor territory forms coherent patches. For example, when there are cells of donor genotype in the epidermis they almost invariably colonize a small block of territory, with no outlying subpatches. Thus we assume, and the analysis depends on and supports this assumption, that at the blastoderm stage of each mosaic, the donor-derived cells were usually in the form of a single patch.

To produce 260 mosaics some 16 500 eggs were injected, approximately 2900 hatched and 1413 reached the adult stage. 54 of the mosaics contained patches of cells homozygous for lethal mutations, failed to emerge from the puparium or died before they could be stained. The viability of the different host genotypes was considerably reduced when compared to wild type and the viability of host eggs (even in the absence of any intervention) was never better than about 60 %. These factors explain the low efficiency of the procedures; we found that if we injected wild-type eggs with nuclei as many as one third of the eggs developed to adults.

All adults were aged for a few days, prepared as previously (Lawrence & Johnston, 1984*a*) and screened for donor-derived patches that stained blue for succinate dehydrogenase. All parts of the flies were kept, all the abdomens were dissected and mounted, and, in many cases, heads and thoraces were embedded in 1–2 % agar and then in Araldite and sectioned. All cell types could usually be scored for genotype, except the testis sheath of the male and, usually, the pericardial cells. The staining of the fat body was in large patches which lacked sharp boundaries. In the nervous system the neuropile and even the peripheral axons stained well, the cell bodies slightly less so. When the CNS was stained after sectioning, the cell bodies did not react (Fischbach & Technau, 1984) but when stained en bloc, as here, cell bodies could normally be allocated to host or donor (e.g. see Fig. 2).

Individuals were usually isolated at the pupal stage and the pupal cases of mosaics mounted in Euparal. As the hosts were *yellow*, donor tissue (*yellow*$^+$) could be seen as darker regions in the ventral denticle belts (Hotta & Benzer, 1973). The pupal cases were scored without reference to the adult material and the correlation between the two was considerable. The resolution of scoring pupal cases was not very high; for example, one could allocate the left half of the third abdominal segment as at least partly *yellow*$^+$, but not determine exactly where the margin of the patch was.

Each mosaic was examined and all the donor-derived tissue marked on a standard diagram. For the cuticle, CNS and somatic muscles each half of the mosaic could be considered independently; but this was not possible for the fat body, gut or derivatives of the visceral

mesoderm. Because of the large amount of indigestible data we have decided to publish details of individual mosaics only in relation to specific questions. It is likely that other questions could be answered using these mosaics and, as they are permanent preparations, they and the records are available in Cambridge for anyone who wishes to inspect them.

One peculiarity of the results concerns the frequency of gynandromorphs amongst the mosaics which was significantly lower than expected (Table 1). It may be that gynandromorphs themselves are less viable than other mosaics or there may be some incompatability between blastoderm nuclei of one sex and the cytoplasm of the cleaving zygote in the other.

Table 1. *Numbers of mosaics of different types*

♂D♂H	♀D♀H	♂D♀H	♀D♂H
44	43	18	17

D = donor derived tissue; H = host. Only those cases where the sex of the donor tissue could be determined in the cuticle are included. The shortfall of gynandromorphs is significant ($P < 0{\cdot}001$).

RESULTS

In the following description host-derived tissue is sometimes referred to as *sdh* (which does not stain) and donor-derived tissue as sdh^+ (which stains blue). T1, T2 and T3 refer to the thoracic segments and A1–A7 the first seven abdominal segments.

The adult oenocytes arise from the histoblasts of the abdomen

Whenever cuticle and more than a few bristles are sdh^+ so are all or some of the underlying oenocytes, which stain blue. If the cuticle patch is confined to the ventral epidermis on one side (Fig. 1), then only the ipsilateral oenocytes in the same segments are sdh^+. These observations show that the oenocytes arise from the same precursor cells as those of the adult cuticle and that each nest of oenocytes, ventral and dorsal, arises locally – confirming previous findings (Ferrus & Kankel, 1981; Lawrence & Johnston, 1982).

The precursors of epidermis and central nervous system are closely interspersed in ventral (but not dorsal) blastoderm

In Poulson's picture (1950) of the blastoderm the ventral region is shown as neurogenic and the more dorsal part as giving rise to epidermal cells. Recently Hartenstein & Campos-Ortega (1984) have reexamined neurogenesis in the embryo and concluded from observations of sections that the ventral ectoderm contains both presumptive epidermal cells and neuroblasts, while the ectoderm dorsal to the tracheal pits consists of only presumptive epidermal cells. Our study confirms this latter picture; in every case where the adult ventral epidermis is marked there are also labelled cell bodies in the CNS (n = 45) (Fig. 2). These cell bodies are always marked in an appropriate region: for example, if the sdh^+ cuticle

is confined to ventral left abdominal segments A3–A5, then the sdh^+ cell bodies are on the middle of the left side of the abdominal neuromere.

There are only eight examples of mosaics where donor tissue is limited to adult dorsal epidermis, but in six of these eight cases, although sdh^+ input into the CNS can be detected, no sdh^+ cell bodies are seen. It therefore seems that there is a dorsal region of the blastoderm that gives rise to epidermis only (Hartenstein & Campos-Ortega, 1984; Technau & Campos-Ortega, 1985). This difference between the prospective fate of dorsal and ventral ectoderm is exemplified by one mosaic (BX-8). On the left side of this fly, T1 contains donor-derived cells in the cuticle of the leg (derived from ventral epidermis) and in the dorsal humerus, but in T2 and T3 only the dorsal structures (wing and haltere) are sdh^+, the legs being host-derived. In the CNS there are sdh^+ inputs from all three segments but cell bodies are restricted to the T1 neuromere.

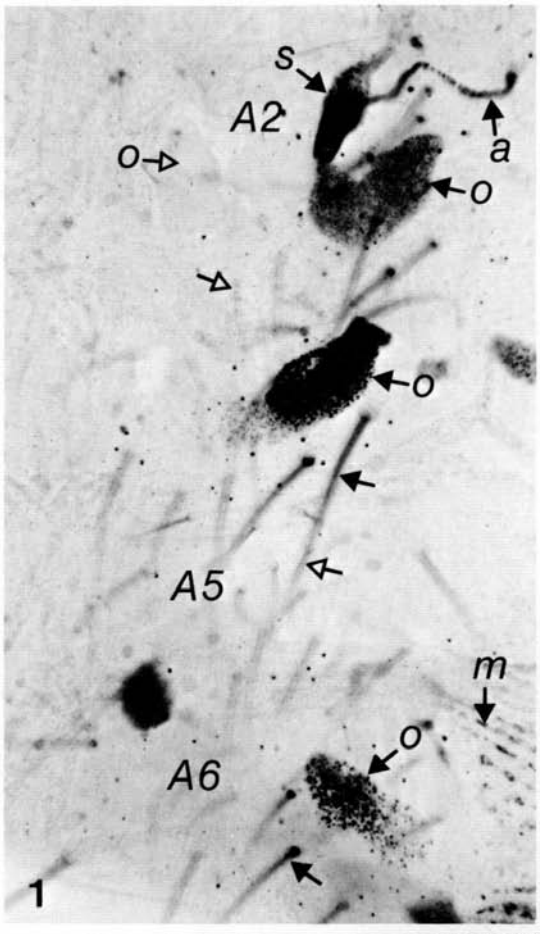
Precursor cells for hindgut and malpighian tubules are overlapping

The origin of the malpighian tubules has been a controversial matter for a long time; some considered them to be endodermal and coming from the midgut rudiment, others concluded they are ectodermal and derive from the proctodaeum. Opinions have depended on varied guesses as to where the endodermal midgut joins to the ectodermal hindgut. Bodenstein (1950) concluded his perplexed summary of this subject: 'the point can best be decided by ascertaining the embryonic origin of the tubes' (p. 341). From the 206 total mosaics, 77 mark the malpighian tubules. Each can be detected not only from the autonomous sdh^+ staining of tubule cells, but also in some cases from the change in eye colour of the host, due to non-autonomy of cn^+ (Beadle & Ephrussi, 1936). In every one of the *cn bw* hosts where the eye colour changed from white to light brown, the malpighian tubules contained sdh^+ cells. Of 77 cases of sdh^+ malpighian tubules the ectodermal hindguts are also partly or entirely sdh^+ in all but 7 (Fig. 3), whereas the posterior midguts are sdh^+ in only 58/77. Of 71 cases where the hindguts are $sdh^+$the malpighian tubules are also marked in all but 8, while of 86

Fig. 1. Whole mount of ventral epidermis of the anterior abdomen. On the right the bristles (unlabelled arrows) are derived from the donor and are $yellow^+$ in colour, while the left shows *yellow* bristles derived from the host. Oenocytes (*o*) on only the right stain for succinate dehydrogenase; a complex sensory structure (*s*) in A2 is also genetically sdh^+, as well as the sensory axon (*a*) that comes from it. Mesodermal organs such as muscles (*m*) and fat body do not stain except in A6 right. Closed arrows mark donor tissue, open arrows host. ×210.

Fig. 2. (A) Longitudinal sections of the thoracic central nervous system. Only the abdominal neuromere contains sdh^+ donor axons and cell bodies (*n*), the rest being derived from the host. In this mosaic only the abdominal segments contained $yellow^+$ donor-derived parts, note the sdh^+ axon (*a*) extending into the T3 neuromere. Nomarski interference contrast. ×400. (B) Detail of another mosaic to show sdh^+ cell bodies (*n*) and axons in the abdominal neuromere. ×230.

Fig. 3. Whole mount of hindgut and malpighian tubules (*m*) to show areas of sdh^+ donor-derived tissue in both. The visceral mesoderm (*vm*) enwrapping the hindgut is also partially donor-derived. Closed arrows donor tissue, open arrows host. ×70.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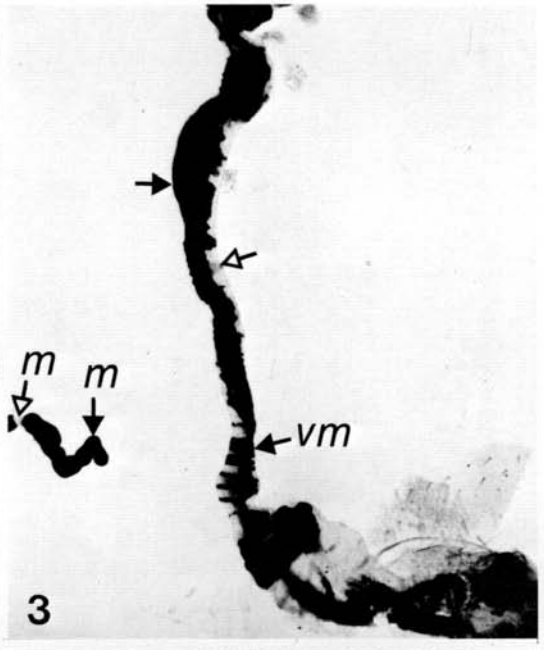
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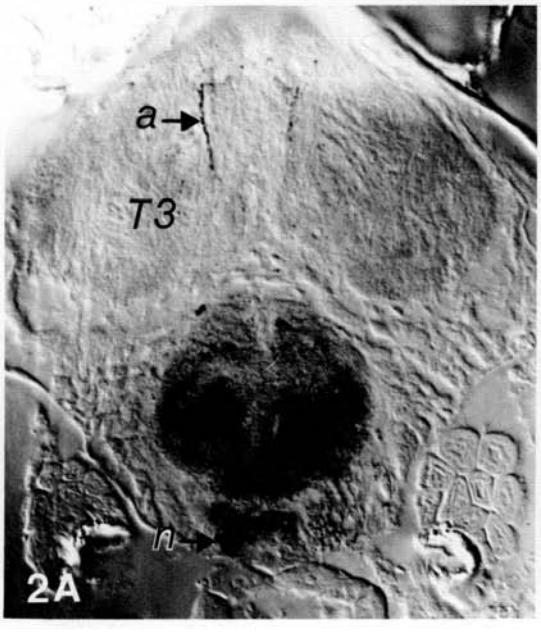
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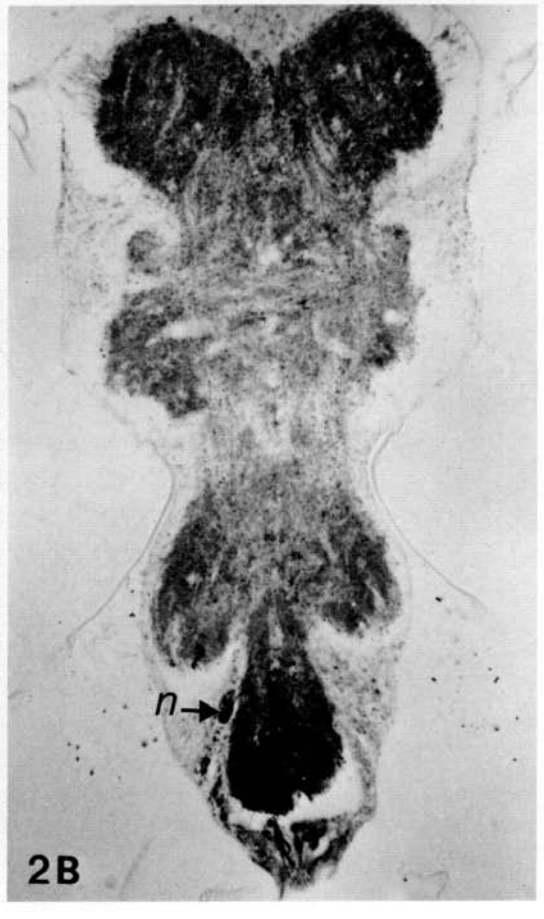
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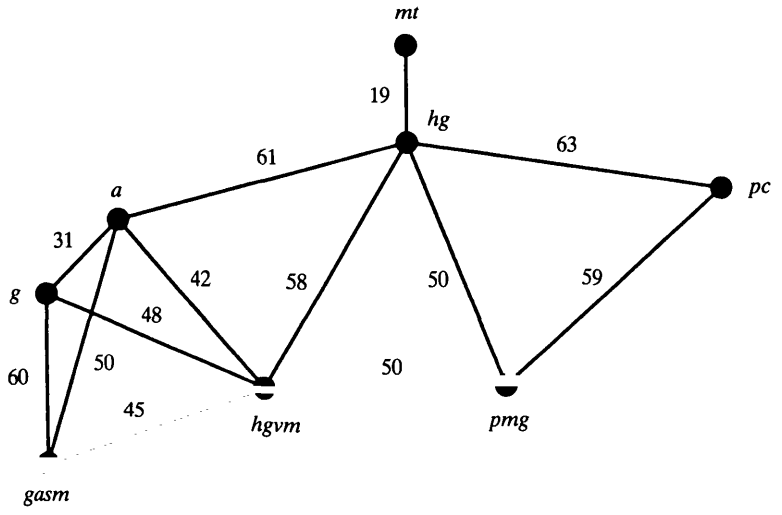


Fig. 4. A crude map of the centres of adult primordia in the posterior region of the egg. The map is based on 123 mosaics including all cases where at least one of the studied organs is partially or completely sdh^+, but excluding the 7 mosaics where all eight organs contain donor-derived cells. The map has been drawn to try and make the best fit; the distances between any two landmarks are given in sturtoids, that is the percentage of mosaics where only one landmark is sdh^+ from the total number of mosaics where either or both are marked (Hotta & Benzer, 1972; Gelbart, 1974). The map is imprecise but it does give an indication of the relative positions of primordia (compare Janning, 1978; Hartenstein, Technau & Campos-Ortega, 1985). *a*, anal plates; *g*, genitalia; *gasm*, somatic muscles of the terminalia; *hg*, hindgut; *hgvm*, visceral muscle of the hindgut; *mt*, malpighian tubules; *pc*, germ cells; *pmg*, posterior midgut. Dorsal to the top, posterior to the right.

cases where the posterior midguts are sdh^+ there are as many as 26 mosaics where malpighian tubules are unmarked. These data suggest that the precursors of the malpighian tubules are part of the proctodeum not of the posterior midgut rudiment, and therefore that the outpocketings which generate the malpighian tubules do indeed come from the hindgut rudiment (see Poulson, 1950, p. 199). This conclusion is supported by the behaviour of visceral muscles in mosaics. Frequently (17/81), the visceral muscles enwrapping the hindgut are completely donor-derived and, in these cases, the sdh^+ muscles also extend around the proximal regions of the malpighian tubules. It seems that the domain recognized by developing visceral mesoderm of the hindgut includes the malpighian tubules and a small region of the gut distal to their point of attachment. We therefore conjecture that the junction between the endodermal midgut and the ectodermal hindgut is just distal to the point of insertion of the malpighian tubules. This conjecture is consistent with mosaics where patches of midgut tissue are sdh^+ but, because of the small size of these patches, we cannot be quite certain of it.

There are enough cases which go to the posterior region of the adult to make a small map of primordia; the map is based on the simple and simplistic assumption that the closer two primordia are in the blastoderm the more frequently will they

both be sdh^+ in the same mosaic (Sturtevant, 1929; Garcia-Bellido & Merriam, 1969; Hotta & Benzer, 1972; Gelbart, 1974) (Fig. 4). It suggests that the analia and hindgut–malpighian tubules are closely related and that the pole cells are equidistant from the progenitors of the adult posterior midgut and adult hindgut.

The frequency of mosaicism might give an objective measure of the relative size of the primordia, but the estimates were devalued by two considerations. First, the primordia being measured are the number of founder cells for only the adult structures; while this would not matter for the pole cells it might result in an underestimate for such organs as the midgut, where the adult parts develop from anlagen that are localized in the larval gut. Second, the distribution of donor territory is biased with a clear tendency for nuclei to colonize the posterior end of the egg – but comparisons of mosaic frequency between nearby organs should not be much affected by this.

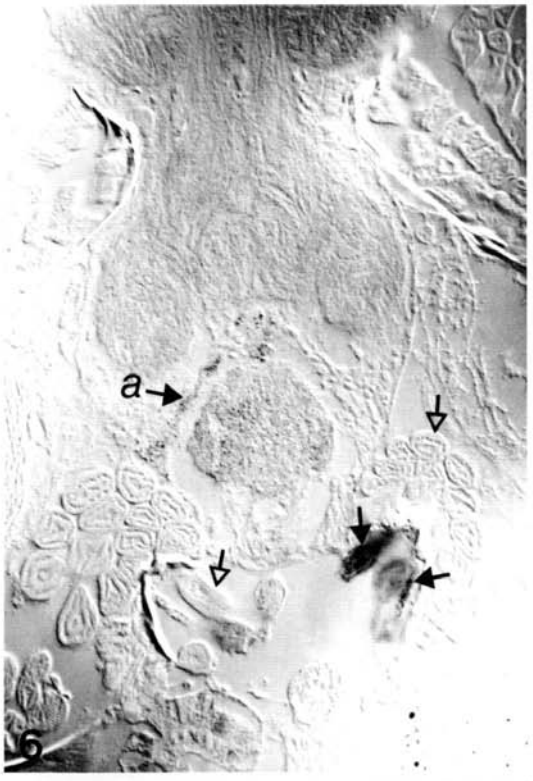
The adult musculature is mesodermal

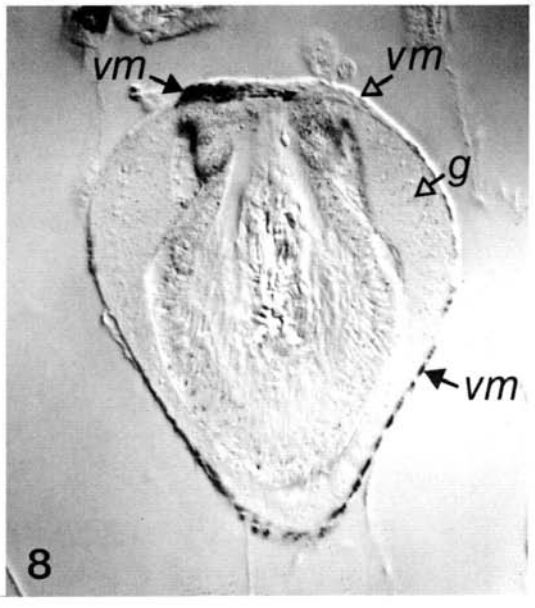
We have previously examined the possibility raised by Schneiderman (1979) that the somatic muscles of thorax and abdomen might develop from precursors in the imaginal discs or histoblasts; precursors which also form the ectoderm. No clones which overlapped between the cuticle and the muscles were found (Lawrence, 1982; Lawrence & Johnston, 1982). The present mosaics confirm this: considering only those cases ($n = 133$) where the cuticle and/or the muscles of A2–A7 are partly sdh^+, in 35 cases sdh^+ cells were confined to the abdominal cuticle and do not extend to any somatic muscles while in 53 cases there were sdh^+ cells present in the somatic muscles but not in the overlying cuticle. In most of these cases several segments were marked (Fig. 5). It seems absolutely clear that the classical view of the mesodermal origin of adult muscles is correct (Poulson, 1950).

The fat body is mesodermal

The orthodox view of the fat body is that it originates from mesoderm (Poulson, 1950; Kobayashi & Ando, 1984), but at least for the adult fat body of the notum this has been questioned (Ferrus & Kankel, 1981). Scoring of the fat body in our mosaics is not always easy, as staining is capricious. However, in sdh^+ controls the fat body is darkly stained while in *sdh* controls it is hardly stained at all and we therefore think that it is legitimate to make use of it. For study we chose those mosaics that include sdh^+ territory in A1–A7 and in which the fat body could be easily scored as stained or unstained. Of those in which only mesodermal organs are sdh^+, and do not include the pupal case, 7/8 mark the fat body; of those which mark the muscles and the pupal case but not the adult epidermis organs 12/12 include the fat body (see Fig. 5) and of those cases where the pupal case was not scored, but the sdh^+ cells are confined to the muscles, 6/6 mark the fat body. By contrast, of those 30 mosaics which are confined to the ectoderm, only 3 mark the fat body. It is obvious that the adult fat body is much more closely related in origin to the adult muscles than to the adult epidermis or even to the larval epidermis.

One mosaic (Mc42) with a small donor-derived patch is illustrative of the mesodermal orgin of the fat body; two cells of the heart, one hemisegment of the somatic muscle, some visceral muscle enwrapping the midgut, and some fat body are the only *sdh*$^+$ parts. The pupal case contains no donor-derived denticles.





Intersegmental muscles originate in one segment but not in both

In a study of cell lineage of the thoracic muscles nearly all muscles were allocated to one of eight sets, each set corresponding to a single imaginal disc or spiracular primordium (Lawrence, 1982). In the second thoracic segment (T2) there are a dorsal set of muscles (which include all the indirect flight muscles) that arise from adepithelial cells of the wing imaginal disc and a ventral set of muscles that are formed by adepithelial cells of the leg disc. In addition the muscles which close the thoracic spiracles probably have somewhat independent origins (Lawrence, 1982). Some muscles were not allocated and two possible explanations for the failure to allocate them were offered: First, the muscle compartment(s) they belong to might be so small that there would not be sufficient proliferation of clones to permit *sdh* cells to fill the muscle fibres and be detected as reduced staining. Second, intersegmental muscles might have an origin in two segments and form by the fusion of two separate sets of myoblasts; this would make it impossible for a single *sdh* clone to fill a muscle (Lawrence, 1982). We have now allocated these muscles using mosaics where the minority tissue is sdh^+. In all six cases of mosaics where the muscles of T3, but not of T1 or T2, are sdh^+ we find that the ipsilateral muscles #62, #77 and #79 (for numbers, see Miller, 1950) are sdh^+. There are two cases where the T2 muscles are sdh^+ but the T3 muscles as well as #62, #77 and #79 are derived from the host. These cases show that the three muscles belong to T3 and therefore originate in the mesoderm of parasegment 5 (Martinez-Arias & Lawrence, 1985; Akam & Martinez-Arias, 1985; Lawrence, 1985). In five other cases, where the muscles of A1 on one side are sdh^+ but the ipsilateral thoracic muscles are derived from the host, #80 and #81 are sdh^+ (Fig. 6), while in one case where T3 muscles are sdh^+ and A1 muscles are *sdh* #80 and #81 are *sdh*. Muscles #80 and #81 therefore derive from A1 and originate in the mesoderm of parasegment 6. Muscles #62, #80 and #81 seem intersegmental because they attach to cuticular parts belonging to two segments (Miller, 1950), however our findings show that they have only one segment of origin.

Fig. 5. A mosaic with extensive donor-derived tissue in the fat body and muscles on the right side but all the adult epidermis is derived from the host and is *yellow* (some bristles contain air and look dark). This mosaic points to the separate origin of epidermis from ectoderm and fat body and muscles from mesoderm. ×67.

Fig. 6. Longitudinal section through posterior region of the thorax to show that muscle 80 and 81 (closed arrows) are sdh^+ while the rest of the thoracic muscles are *sdh* and therefore entirely derived from the host. Nomarski interference contrast. ×270.

Fig. 7. Anterior end of the heart to show several cells that are sdh^+ (closed arrow) although the majority of the heart (and all of the right side) is host-derived (open arrow). On the left-hand side abdominal somatic muscles (*sm*) that are sdh^+ are shown somewhat out of focus. Nomarski interference contrast, detail of Fig. 5 (mirror image). ×270.

Fig. 8. Section of proventriculus to show visceral muscle (*vm*) that is sdh^+ and donor-derived. The endodermal cells of the gut (*g*) are *sdh* and do not stain. Nomarski interference contrast. ×370.

Spiracular muscles belong to the expected parasegments

The origin of the two spiracular closing muscles of the thorax (#76, #97, Miller, 1950) was not certain although *sdh* clones suggested a common origin for #76 and the muscles of T2 (Lawrence, 1982). It would now be expected that the anterior spiracle (which we believe to arise in the embryo from parasegment 4) should be most closely related to the somatic muscles of T2 which themselves probably come from parasegment 4 (Martinez-Arias & Lawrence, 1985; Akam & Martinez-Arias, 1985; Lawrence, 1985). The posterior thoracic spiracle should come from parasegment 5 and be related to the somatic muscles of T3. This is supported; of the two cases where T2 muscles are sdh^+ and T3 muscles *sdh*, #76 is sdh^+ and #97 *sdh*. Of six cases where T3 muscles are sdh^+ and T2 muscles *sdh*, #76 is *sdh* and, in five of them, #97 is sdh^+. In two cases where the muscles of T1 are sdh^+ but the other muscles of the thorax are *sdh*, #76 is *sdh*.

Muscles #6–8 belong to a separate lineage compartment

Raghavan & Pinto (1985) have made an analysis of lineage of muscles in the head and proboscis. They describe three separate lineage compartments in these muscles. One of these sets consists of muscles 5, 6, 7 and 8 in the distal proboscis (Miller, 1950). We have independent evidence for part of this set as in four cases #6–8 were of different genotype to the remaining muscles in the head and proboscis.

The primordia of the abdominal somatic muscles are scattered and their fate is not precisely defined

The somatic muscles of the adult abdomen (only the abdominal segments A2–A6 have been studied) consist of the dorsal and ventral longitudinal muscles, the sheet of lateral fibres closely apposed to the pleura, the spiracular closing muscles and the circular and alary muscles of the heart (Fig. 7). The evidence for this is that all these structures are frequently labelled together. For example, one might have guessed from their tenuous structure that the alary muscles might be visceral in origin. However, it is rare for these muscles to be labelled independently of the rest of the heart. The longitudinal muscles of the heart, a thin sheet of mainly ventral fibres (Miller, 1950), are sometimes separately labelled, suggesting that these muscles may be of visceral origin.

We have suggested that clones of *sdh* cells in the longitudinal ventral muscles of the abdomen sometimes cross between segments (Lawrence & Johnston, 1982) and our present mosaics confirm this. It is unusual for the sdh^+ patches to stop cleanly at segment boundaries or to respect other landmarks and the general impression gained from them is of some variation in development. For example, the pleura is often sdh^+ when the ventral muscles of the corresponding segments are not and sometimes the spiracular muscles are labelled with sdh^+ independently from other muscles. It is hard to present the data upon which this impression is based without including many drawings of cases. The simplest explanation for this

variation is that there are a number of scattered myoblasts which have diverse fates in different individuals. Although the pericardial cells stain unreliably, in those cases where they could be assigned as sdh^+ they are always associated with nearby sdh^+ cells of the heart. Their origin is therefore close to that of the heart.

The patterns seem to depend little on whether the host is carrying a *Minute* mutation. In the ectoderm and mesoderm of the thorax $Minute^+$ cells have a strong competitive advantage and the marked clones become very large (Morata & Ripoll, 1975; Lawrence, 1982) this is apparently not so in the abdomen, either in the epidermis (Morata & Ripoll, 1975) or in the mesoderm.

In the thorax small numbers of myoblasts may wander from their normal location

In an earlier clonal analysis of thoracic muscle development (Lawrence, 1982) it was found that homozygous $Minute^+$ *sdh* clones in a heterozygous *Minute* background could fill up the lineage compartments. Usually there were small blue flecks in the clone, especially in the fibrillar muscles, which could have been relicts of *Minute* cells from the same compartment or they could have been produced by wayward myoblasts from elsewhere. These two hypotheses can now be discriminated; in cases where the donor-derived tissue has a growth advantage, any sdh^+ cells that are present when the muscle compartments are founded should compete out the *sdh Minute* cells of the host and make the muscle sets stain blue. However, if the blue flecks are due to errant myoblasts entering the developing muscles after the main period of growth then it will be immaterial whether or not they have a growth advantage. We have found these blue flecks are present even in four cases where the somatic mesoderm of the thorax is mosaic in a *Minute*/$Minute^+$ host. The flecks are small which suggests to us that they are caused by the late truancy of a few myoblasts.

The primordia of the visceral and somatic mesoderm are somewhat separate at the blastoderm stage

In the embryo of about 4–6 h one can see the formation of the mesodermal parasegments as partly separated clumps of cells (Martinez-Arias & Lawrence, 1985). At about this time the visceral or splanchnic mesoderm appears as an epithelial layer lining the inner surface of these clumps. It is not known where the visceral mesoderm comes from – it could arise from cells which are intermingled with those that will generate the somatic mesoderm, or it could arise from a discrete mass of primordial cells that are close to, but not overlapping, the somatic primordia. In the former case sdh^+ tissue in the mosaics would rarely mark one type of mesoderm without the other, while in the latter case this could occur frequently. The results follow; of 128 cases where the mesoderm contains sdh^+ cells, the visceral but not the somatic mesoderm is sdh^+ in 16, and the somatic but not the visceral in 14. These figures can be contrasted with the ventral ectoderm discussed earlier where the presumptive epidermal and nerve cells are intermingled and, in all 45 mosaics that were examined, both the epidermis and the central nervous system contained donor-derived cells. We conclude that the

somatic and visceral mesoderm arise from completely separate or only partially overlapping groups of progenitor cells that are not intermingled at the blastoderm stage of development, or later on.

From the small number of mosaics in the head, there is some evidence that muscle #16 (Miller, 1950) is more closely related to the visceral muscles ensheathing the oesophagus than to the other somatic muscles of the head. Likewise the scutellar pulsatile organ (Thomsen, 1938) does not belong to the somatic muscles of T2 or T3 (Lawrence, 1982 and present study) nor does it always label with the somatic muscles of A1 (present study). Probably, therefore, these muscles are part of the blood circulatory system and arise from the visceral mesoderm. In some mosaics the longitudinal muscles of the heart and the pulsatile organs are coincidentally marked which might suggest a common origin.

The visceral muscle of the midgut and the follicular epithelium of the female gonad probably arise from widely dispersed primordia, while the hindgut visceral mesoderm has a local posterior origin

Usually, when the muscular lining of the posterior midgut contains sdh^+ patches there are also sdh^+ somatic muscles (51/53). In those cases where donor-derived territory is small, occupying only one or two segments of the abdomen, it is a reasonable assumption that the visceral cells had their origin nearby. The visceral muscles can be sdh^+ in association with the somatic muscles of A1 and A2 (n = 3), A4 and A5 (n = 5) or the terminalia (n = 4) which suggests that cells from a large part of the abdomen contribute to the lining of the abdominal midgut.

What is the origin of the muscular lining of the anterior part of the midgut? Although the number of cases where donor tissue colonizes anterior thoracic and/or head somatic mesoderm is small, there are ten instances where this mesoderm is associated with sdh^+ muscular lining of the proventriculus and nearby (Fig. 8). This tissue is host-derived in all other mosaics, which suggests that anterior visceral mesoderm has an origin near the anterior part of the embryo – where the anterior midgut itself originates (Poulson, 1950).

The same criteria can be used to determine the origin of the visceral mesoderm of the hindgut; there are 82 cases where this tissue contains sdh^+ cells of which 18 also mark only one or two segments of the somatic mesoderm. In all these 18 cases the territory is confined to A6, A7 and/or the terminalia, which points to a restricted posterior origin for the visceral mesoderm of the hindgut.

The gonadal mesoderm of the ovary, which forms the follicle cells, has been found to be sdh^+ in mosaics where both the donor and hosts are female (n = 15). Taking only those cases where the somatic mesoderm is confined to a small part of the body, the evidence suggests a dispersed origin for the follicle cells. Of mosaics where some of the follicle cells are sdh^+, one is confined to the somatic mesoderm of A3, four to parts of the middle region (A4–A6) and four to the posterior end of the abdomen (A6–terminalia). The evidence that the gonadal mesoderm has its origin in several segments is consistent with the high frequency of mosaicism found in this tissue in gynandromorphs (Gehring, Wieschaus & Holliger, 1967).

There is another argument that underwrites the above, and this depends on the sizes of the sdh^+ patches. In the midgut visceral muscle and in the gonadal mesoderm, the sdh^+ patches are always small, never filling the entire tissue. However the hindgut visceral mesoderm is occasionally (17/81) completely sdh^+.

DISCUSSION

Our aim is to contribute to an objective description of the cell lineage of *Drosophila*. We hope that this information can be incorporated with the results of developmental genetics and *in situ* hybridization of specific probes to tissues (Akam, 1983; Hafen *et al.* 1983). Together these facts can tell us how genes involved in the formation of pattern are deployed during development. For example, we decide here that the malpighian tubules share a primordium with the hindgut. If this is so the malpighian tubules are ectodermal and might therefore share the genetic address (the combination of active and inactive selector genes, Garcia-Bellido *et al.* 1979; Struhl, 1982) of the hindgut. More recent evidence provides independent support for this conclusion, the *engrailed* gene, which is crucially involved in compartition of the ectoderm, is expressed in parts of both the hindgut and the malpighian tubules (Ingham, Martinez-Arias, Lawrence & Howard, 1985).

We report that the precursors of the adult oenocytes and neurons are overlapping or closely intermingled with presumptive epidermis. This means that the genetic address should be largely or completely shared by these three ectodermal derivatives. Certainly the expression of Ubx^+, an element of the bithorax complex (Lewis, 1978), appears to correspond in the epidermis and central nervous system (Akam, 1983; White & Wilcox, 1984; Beachy, Helfand & Hogness, 1985). There seems to be both transcription and a requirement for $engrailed^+$ function in parts of the central nervous system (Lawrence & Johnston, 1984*a*; Ingham *et al.* 1985); presumably therefore both the epidermis and nervous system are divided up into anterior and posterior compartments.

There are maps of the blastoderm stage showing the arrangement of some internal primordia of the posterior end of the egg now available, and there are differences between them. Poulson's map (1950) is based on observations of embryos and this was modified by Wieschaus (see Janning, 1978) and Hartenstein & Campos-Ortega (1984). The map of Technau & Campos-Ortega (1985) and Hartenstein, Technau & Campos-Ortega (1985) depends on marking cells by dye injection and following their fate directly. There are also maps based on quantitative analyses of gynandromorphs (Gehring, Wieschaus & Holliger, 1976; Janning, 1974, 1978; Nissani, 1977). Our map is largely consistent with the gynandromorph maps but none of these located the primordia for the hindgut, the visceral muscles of the hindgut or the somatic muscles of the terminalia.

The traditional view that adult muscles and fat body arise from the mesoderm is confirmed here. It is difficult to understand how Ferrus & Kankel (1981) could have concluded from an analysis of clones made in the larval stages, that the fat

body and adult epidermis share precursor cells. Possibly, the cell marking method they used is not reliable – perhaps because of non-autonomy in the *Pgd* mutation. We also have to admit that the *sdh* marking method does not work perfectly in the fat body.

In the embryo the mesoderm is divided up into parasegments, and in the thorax these show differential expression of selector genes (Akam & Martinez-Arias, 1985; Martinez-Arias, 1985). Normally, each mesodermal parasegment forms the muscles of one segment (Lawrence, 1985; Martinez-Arias, 1985). If myoblasts from two parasegments with different selector gene activities (different genetic addresses) were to fuse there could be disorganization. For this reason one would expect, *a priori*, intersegmental muscles not to form by fusion of myoblasts from two parasegments, but to originate in only one. Our finding that the intersegmental muscles #62, #80 and #81 have a local origin in one segment only may therefore be typical of all intersegmental muscles. It seems likely that, in general, muscles originate in one location and either or both ends migrate to their final sites of attachment (Williams, Shivers & Caveney, 1984; Lawrence, 1982).

If, occasionally, an errant cell from one parasegment fused with muscles from another this might not be damaging, for the nucleus of that errant cell could become entrained by the cytoplasm of the majority (see Lawrence & Brower, 1982, reinterpreted in Lawrence & Johnston, 1984*b*). Indeed, in the thorax, we find evidence for a small number of wayward myoblasts which fuse with muscles from other segments. One should remember that these errant myoblasts are crossing from one muscle set to another only late in development; throughout most of development the muscle sets of the thorax (and also probably of the abdomen, Lawrence & Johnston, 1982) have independent cell lineages.

In the main part of the abdomen, the development of myoblasts appears to be more disorderly than in the thorax and there is evidence that myoblasts originating in one segment do contribute to muscles of another (Lawrence & Johnston, 1982; this study). This laxity might suggest that differences between the muscle pattern of the abdominal segments would depend more on the associated ectoderm than on different genetic addresses of the myoblasts themselves: a view consistent with the expression of Ubx^+ which is evenly spread along parasegments 6–12 of the embryo (Akam & Martinez-Arias, 1985). It will be important to find out whether other selector genes are differentially expressed in the mesoderm of parasegments 6–12.

The lineage information suggests that the origin of visceral and somatic mesoderm can be traced to adjacent, perhaps non-overlapping, primordia in the blastoderm. Poulson (1950) thought that perhaps the visceral mesoderm comes from that part of the presumptive mesoderm that is close to the midline and therefore implied that the primordia for the two types of mesoderm are distinct. The visceral and somatic mesoderm do seem very different, they have separate lineages, they look different as soon as they can be detected in the embryo and they give rise to different kinds of muscles. The somatic mesoderm, but not the visceral mesoderm, is obviously segmented. Even more important than these

subjective impressions is the observation that Ubx^+ and $Antp^+$ functions are quite different in the two primordia, each being confined to one parasegment in the visceral mesoderm (Akam & Martinez-Arias, 1985; Martinez-Arias, 1985). All these observations emphasize that the distinction between the two types of mesoderm is a fundamental one; it would not be rash to consider them as two different germ layers. Unfortunately our data are not good enough to determine whether the fat body and gonadal epithelium belong to the visceral or somatic mesoderm.

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