Development 128, 2517-2524 (2001)
Printed in Great Britain © The Company of Biologists Limited 2001
DEV7887

A new approach reveals syncytia within the visceral musculature of *Drosophila melanogaster*

Robert Klapper*, Sandra Heuser, Thomas Strasser and Wilfried Janning

Institut für Allgemeine Zoologie und Genetik der Westfälischen Wilhelms-Universität, Schlossplatz 5, 48149 Münster, Germany
*Author for correspondence (e-mail: klapper@nwz.uni-muenster.de)

Accepted 17 April 2001

SUMMARY

In order to reveal syncytia within the visceral musculature of *Drosophila melanogaster*, we have combined the GAL4/UAS system with the single-cell transplantation technique. After transplantation of single cells from *UAS-GFP* donor embryos into ubiquitously GAL4-expressing recipients, the expression of the reporter gene was exclusively activated in syncytia containing both donor- and recipient-derived nuclei. In the first trial, we tested the system in the larval somatic musculature, which is already known to consist of syncytia. By this means we could show that most of the larval somatic muscles are generated by clonally non-related cells. Moreover, using this approach we were able to detect syncytia within the visceral musculature – a tissue that has previously been described as consisting of mononuclear cells. Both the longitudinal visceral musculature of the midgut and the circular musculature of the hindgut consist of syncytia and persist through metamorphosis. This novel application of the transplantation technique might be a powerful tool to trace syncytia in any organism using the GAL4/UAS system.

Key words: *Drosophila*, Visceral muscles, Somatic musculature, Syncytia, Cell lineage, Transplantation, GAL4/UAS system, Clonal analysis, GFP

INTRODUCTION

In *Drosophila*, the visceral musculature of the larval midgut forms a network consisting of two layers of fibres: an inner layer of circular muscles enveloping the midgut epithelium and the longitudinal musculature that covers them. Both sets generate the motive force for the peristaltic movements of digestion (Strasburger, 1932; Robertson, 1936; Bodenstein, 1950). Recent analyses provide evidence that circular and longitudinal muscles of the midgut are of different origin (Georgias et al., 1997; Broihier et al., 1998; Kusch and Reuter, 1999). While the circular musculature of the larval midgut derives from a mesodermal portion of the prospective trunk region (Tremml and Bienz, 1989; Azpiazu and Frasch, 1993), the longitudinal fibres originate from a region located at the posterior tip of the blastodermal mesoderm anlage (Tepass and Hartenstein, 1994b; Campos-Ortega and Hartenstein, 1997; Georgias et al., 1997; Kusch and Reuter, 1999). This primordium is defined by the expression of *bHLH54F* (Georgias et al., 1997) and represents the ventralmost part of the early expression domain of *brachyenteron*, the *Drosophila Brachyury* homologue (Singer et al., 1996; Kusch and Reuter, 1999). During embryonic development, cells from this anlage migrate anteriorly with the developing midgut and eventually adopt the stretched morphology that is characteristic of the longitudinal fibres (Tepass and Hartenstein, 1994b; Campos-Ortega and Hartenstein, 1997; Georgias et al., 1997; Kusch and Reuter, 1999). It has been shown that the longitudinal musculature of the larval midgut persists through metamorphosis (Klapper, 2000) and therefore represents the same tissue in adult flies. During metamorphosis, the muscle fibres contract and form morphologically detectable syncytia consisting of two to six nuclei each (Klapper, 2000). This was an unexpected observation, as the visceral musculature had previously been described as consisting of mononuclear cells (Elder, 1975). However, by morphological criteria alone we were unable to decide whether these muscles have a mononuclear or syncytial organisation prior to and after metamorphosis.

The visceral musculature of the midgut consists of two layers of fibres, whereas the hindgut of larvae and adult flies is solely coated with a single layer of circular muscles. Both larval and imaginal musculature of the hindgut originate from the caudal mesoderm anlage (Lawrence and Johnston, 1986a; Broihier et al., 1998; Klapper et al., 1998). Although progenitors of the imaginal muscles have not yet been identified, it has been shown that the cells giving rise to the larval muscles become distinguishable at stage 10 by the expression of *bagpipe* (*bap*) and high levels of Twist (San Martin and Bate stages according to Campos-Ortega and Hartenstein, 1997). During further embryonic development, these cells associate with the invaginated hindgut ectoderm and eventually move over the hindgut tube (San Martin and Bate, 2001) to give rise to the circular fibres. Our clonal analyses (data not shown) suggest that here, too, the tissue might be syncytial.

We now present a method that enables the detection of syncytia consisting of clonally non-related cells. For this purpose, we combined the GAL4/UAS system (Brand and Perrimon, 1993) with the single-cell transplantation technique (Meise and Janning, 1993). By this means it is possible to generate genetic mosaics of cells that carry either the *GAL4* gene under the control of a constitutive promoter or a *UAS* construct driving a reporter gene. An activation of the *UAS* construct and therefore the expression of the reporter gene is to be expected only when cells of the two genotypes fuse with each other. To test this approach, we first analysed the well-studied syncytia of the larval somatic musculature.

During embryogenesis, individual somatic muscles are formed by the successive fusion of separate cells until the final number of nuclei is reached. Each larval muscle can be traced back to one single founder cell (Bate, 1990; Dohrmann et al., 1990) that expresses *dumbfounded* (*duf*; *kirre* – FlyBase), a member of the immunoglobulin superfamily (Ruiz-Gómez et al., 2000). These founder cells fuse with so-called fusion-competent cells that are characterised by the expression of *sticks and stones* (*sns*), another member of the immunoglobulin superfamily (Bour et al., 2000). It appears that the founder cell determines the specification of the later muscle (reviewed by Baylies et al., 1998; Frasch, 1999) and simply fuses with fusion-competent cells located in its immediate vicinity, irrespective of their clonal relationships (Klapper et al., 1998; Frasch and Leptin, 2000). Hence, as has been shown for vertebrates (Mintz and Baker, 1967), it is very likely that in *Drosophila*, the cells that contribute to one muscle are also not necessarily clonally related. However, there is still no direct proof that clonally non-related cells are able to fuse with each other.

After transplantation of single cells from *UAS-GFP* embryos into ubiquitously GAL4-expressing recipients, we frequently obtained clones contributing to the syncytial larval somatic muscles, whereas no labelling of the mononuclear fat body was detectable. Transplantation into the anlage of the longitudinal musculature revealed syncytia within this tissue in embryos, larvae and adult flies. Furthermore, we obtained labelled syncytia contributing to the circular musculature of the hindgut that persisted through all stages of postembryonic development.

MATERIALS AND METHODS

Fly strains

For the in vivo examination of syncytia, we used the *UAS-GFP.S65T* strain (B. Dickson, unpublished) as donor and either the *GAL4daG32* strain (Wodarz et al., 1995) or the P[GAL4] enhancer trap line 5053A as recipients for the transplantation experiments. The *GAL4daG32* strain ubiquitously expresses GAL4 under the control of the *daughterless* promoter (Wodarz et al., 1995). To highlight the cell lineage of the transplanted cell as well as the syncytial fraction of the resulting clones we used a *GAL4daG32; UAS-GFP.S65T* strain as the donor and the strain *UAS-lacZ*$^{4\text{-}1\text{-}2}$ (Brand and Perrimon, 1993) as the recipient. The strains *UAS-lacZ*$^{4\text{-}1\text{-}2}$, P[GAL4] 5053A and *UAS-GFP.S65T* were obtained from the Bloomington *Drosophila* Stock Center. The line *GAL4daG32* was a gift from Elisabeth Knust.

Single-cell transplantation

Single cells were transplanted at the cellular blastoderm stage by the transplantation technique of Meise and Janning (Meise and Janning, 1993). Living embryos and third-instar larvae were examined for GFP expression and raised to adulthood. For detailed examination of GFP expression larvae and adult flies were dissected in phosphate-buffered saline (PBS). An Olympus inverse microscope CK40 equipped with an EGFP filter set (AHF Analysentechnik) and a video enhancement system was used for fluorescence analysis. For simultaneous visualisation of the cell lineage and the syncytial fraction in embryos, donor embryos of the *UAS-GFP* strain were injected at the preblastoderm stage with 10% 2000S rhodamine b isothiocyanate dextran (RITC-dextran; 2000S; Sigma) in 0.2M KCl according to the injection technique of Technau (Technau, 1986).

Confocal microscopy

Stage 16 to 17 embryos with RITC-dextran GFP double labelling were heated for 10 seconds to 60ºC in a water bath. Subsequently the embryos were covered with 10S Voltalef fluorocarbon oil topped by a coverslip, and were examined with a Leica TCS NT confocal microscope. Images were processed with Adobe Photoshop 5.5 (Adobe Systems).

X-Gal staining

To analyse GFP and β-galactosidase expression in tissues of third-instar larvae, the specimens were dissected and first examined in PBS for GFP expression. Thereafter the tissues were fixed in 7.5% glutaraldehyde solution for 20 minutes and then washed several times in PBS. They were stained for histological demonstration of β-galactosidase by placing them in 1 ml of dye solution (Simon et al., 1985) plus 25 µl 8% X-Gal for about 2 hours at 37ºC. When the staining was sufficient, the tissues were washed again in PBS several times and transferred to 50% glycerine. In this solution the tissues were further dissected and flattened pieces were embedded in Faure's solution.

RESULTS

The GAL4/UAS transplantation system

In order to establish a system that exclusively labels syncytia, we combined the GAL4/UAS system with the single-cell transplantation technique. For this purpose we used a strain expressing the GAL4 protein ubiquitously under the control of the *daughterless* promoter *(da-GAL4*; Wodarz et al., 1995) and a strain carrying either the reporter gene *GFP* or *lacZ* under the control of the GAL4 responding element UAS (Brand and Perrimon, 1993). Genetic mosaics were generated by transplanting single cells from embryos of the *UAS-reporter gene* strain into the *da-GAL4* strain. Expression of the reporter gene should occur only if nuclei of both strains, *UAS-reporter gene* and *da-GAL4*, share a common cytoplasm (Fig. 1A).

Previous cell-lineage analyses within the trunk region of the mesoderm anlage using single-cell transplantation experiments reveal that the resulting clones frequently either label the syncytial somatic muscles or the mononuclear fat body alone or overlap the two tissues (Beer et al., 1987; Holz et al., 1997; Klapper et al., 1998). To show that only syncytia are labelled by the GAL4/UAS transplantation system we additionally tagged the entire cell lineage of the transplanted cell. In overlapping clones, the cell-lineage marker should label the mononuclear as well as the syncytial fraction of the clone. The GAL4-activated reporter gene, however, should be expressed only within the syncytial part of the clone.

The donor embryos of the *UAS-GFP* strain were injected with the fluorescent dye RITC-dextran at the preblastoderm

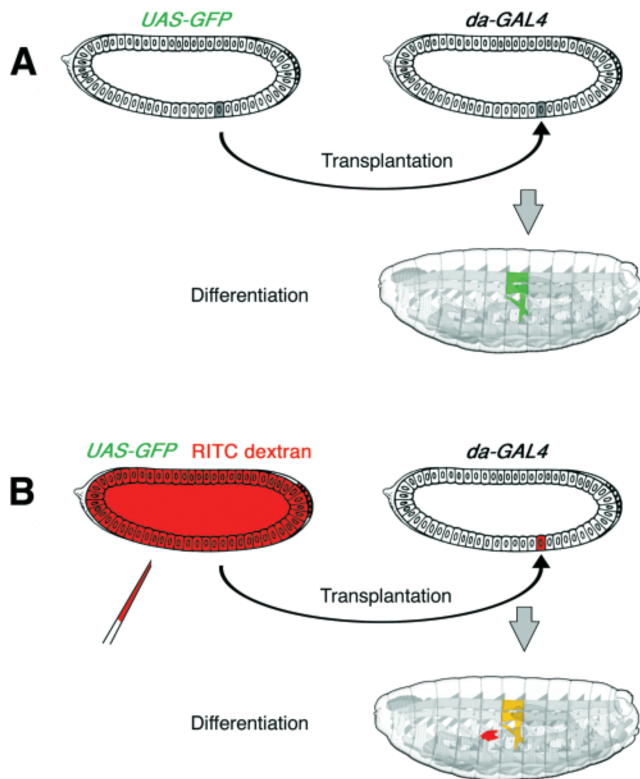

Fig. 1. The GAL4/UAS transplantation system. (A) Single cells are transplanted from *UAS-GFP* donors into *da-GAL4* recipients at the blastoderm stage. After differentiation, GFP expression (green) is expected only in those syncytia that contain both donor- and recipient-derived nuclei. (B) To detect all descendants of the transplanted cell, the *UAS-GFP* donor embryos were labelled with the fluorescent dye RITC-dextran (red) at the preblastoderm stage before the transplantation. In the differentiated *da-GAL4* recipients all mononuclear tissues, such as the fat body, generated by the descendants of the transplanted cell are solely labelled by RITC-dextran (red). Syncytia containing donor- and recipient-derived nuclei, like somatic muscles, exhibit an additional GFP expression (yellow indicates fluorescence superimposition of GFP and RITC-dextran).

stage. At the cellular blastoderm stage, single cells from these labelled donors were transplanted homotopically into *da-GAL4* recipient embryos at 40% EL (EL=egg length, 0% EL=posterior pole) and 0% VD (VD=ventrodorsal, 0% VD=ventral). RITC-dextran labels the entire progeny of the transplanted cell. Additional GFP expression is expected only within the clone fraction that participates in syncytia composed of donor- and recipient-derived cells (Fig. 1B).

Table 1. Distribution of mesodermal clones in embryos after transplantation of RITC-dextran labelled cells from *UAS-GFP* donors into *da-GAL4* recipients at 40% egg length

Tissue	RITC-dextran	GFP expression
Musculature	30	30
Fat body	2	None
Fat body and musculature	6	6 (only muscle fraction)

We performed 87 transplantations resulting in 38 clones labelling mesodermal tissues (Table 1). All 30 clones contributing to the somatic musculature were labelled by both the cell-lineage marker RITC-dextran and the syncytia marker GFP. Two clones exclusively labelled the fat body. In these cases, only the lineage marker RITC-dextran was detectable. In six further cases, the clone contributed to the fat body and somatic muscles at the same time. Strikingly, while the cell-lineage marker RITC-dextran labels fat body as well as somatic muscles (Fig. 2A,C), the GFP expression is restricted to the somatic musculature (Fig. 2B,C). Thus, the GFP expression does not simply reflect the cell lineage of the transplanted cell but is restricted to syncytia within this cell lineage. This limitation to syncytia indicates that the GAL4/UAS transplantation system is exclusively activated if nuclei of the donor and the recipient share a common cytoplasm. First signs of GFP expression within somatic muscles were detectable at stage 15 of embryogenesis.

Most clones (20 out of 30) that contribute to the larval somatic musculature exhibit a complete overlap of the two markers, demonstrating that these syncytia are generated by the fusion of donor- and recipient-derived cells. In 10 cases, however, one to three individual muscles of a given clone (up to 10 muscles, average 3.8 muscles per clone) are only labelled by the cell-lineage marker RITC dextran (Fig. 2C). This indicates that these muscles are exclusively generated by donor-derived cells.

We also tested the GAL4/UAS transplantation system in the third-instar larva. Owing to the decay of the cell-lineage marker RITC dextran during postembryonic development we had to modify the components of the system. Embryos from the strain *UAS-GFP; da-GAL4* were used as donors and *UAS-lacZ* embryos as recipients for transplantation experiments. Thus, here the cell-lineage is labelled by GFP, while syncytia consisting of donor- and recipient-derived nuclei additionally express β-galactosidase.

We carried out 90 homotopic single-cell transplantations at

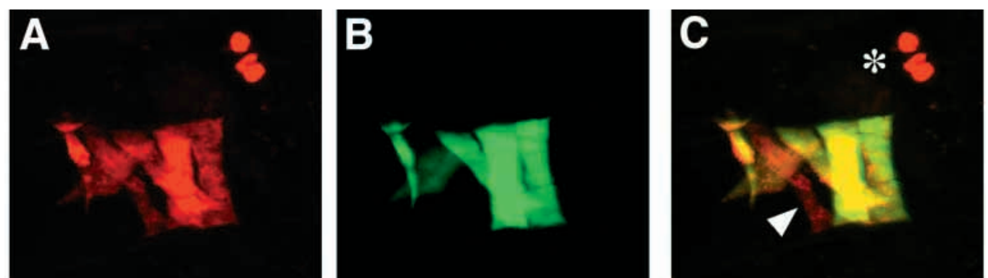

Fig. 2. The GAL4/UAS transplantation system exclusively labels syncytia. A clone in a stage 17 embryo that overlaps fat body and somatic musculature. All descendants of the transplanted cell are labelled red by the cell-lineage marker RITC-dextran (A), whereas syncytia containing both donor- and recipient-derived nuclei express GFP (B). The superimposition (C) reveals that the syncytia marker GFP is not expressed in the mononuclear fat body (asterisk). This tissue is solely labelled by the cell-lineage marker. Most somatic muscles are double labelled and therefore represent syncytia consisting of clonally non-related nuclei. The muscle indicated by the arrowhead is labelled only by the cell-lineage marker, indicating that this syncytium consists exclusively of donor-derived nuclei.

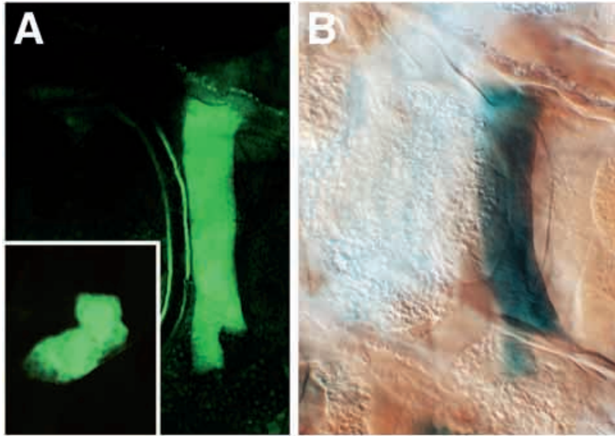

Fig. 3. The GAL4/UAS transplantation system highlights syncytia in third-instar larvae. (A) The expression of GFP, here used as a cell-lineage marker, demonstrates that the descendants of a single transplanted cell gave rise to two cells of the fat body (insert) and also contributed to a larval somatic muscle. (B) Only the muscle fraction of the clone expresses the syncytia marker β-galactosidase as revealed by X-Gal staining.

40% EL and 0% VD. 57 recipients reached the third larval instar and were examined subsequently for GFP expression (cell lineage) and β-galactosidase activity as revealed by X-Gal staining (syncytia). In 31 larvae, clones labelling mesodermal tissues were detected (Table 2). All clones contributing to the fat body (n=6) were exclusively labelled by the cell-lineage marker GFP (Fig. 3A). Expression of both GFP and β-galactosidase was detected only within the larval somatic musculature (Fig. 3A,B). Thus, the GAL4/UAS transplantation system also enables the selective detection of syncytia in larvae. Again, most of these syncytia are generated by the fusion of donor- and recipient-derived cells. In 10 of 29 clones, one to three muscles contributing to a given clone (up to seven muscles, on average 3.5 muscles per clone) express only the cell-lineage marker GFP. As observed in embryos, this again indicates that all nuclei of these muscles derived from descendants of the transplanted donor cell and therefore share a common cell lineage.

The longitudinal visceral musculature consists of syncytia

Recent analyses have revealed that the longitudinal visceral musculature of the midgut persists through metamorphosis and generates syncytia during this developmental stage (Klapper, 2000). In order to analyse whether these muscles are already generated as syncytia during embryogenesis, we again used the GAL4/UAS transplantation system. Single cells from *UAS-GFP* donor embryos were transplanted homotopically into *da-GAL4* recipients within the region of the respective anlage (5-10% EL; 0% VD). Thus, only syncytia consisting of donor and recipient nuclei are labelled by GFP. The use of GFP generally makes it possible to follow these syncytia in vivo throughout further development.

Table 2. Distribution of clones in third-instar larvae after transplantation of cells from *UAS-GFP; da-GAL4* donors into *UAS-lacZ* recipients at 40% egg length

Tissue	GFP expression	β-Galactosidase expression
Musculature	25	25
Fat body	2	None
Fat body and musculature	4	4 (only muscle fraction)

Table 3. Distribution of clones in embryos after transplantation of cells from *UAS-GFP* donors into *da-GAL4* recipients between 5 and 10% egg length

Tissue	Number of clones
Longitudinal musculature of midgut	27
Circular visceral musculature of hindgut	29
Circular visceral musculature of hindgut overlapping with somatic musculature	13
Somatic musculature	23
Total	92

We carried out 313 transplantations resulting in 27 embryos with labelling in the longitudinal muscles of the midgut (Table 3). We never observed overlapping with any other tissue. GFP expression within the longitudinal visceral musculature of stage 15 to 17 embryos clearly demonstrates the presence of syncytia at these early stages of development.

Although in most cases (17 of 21 surviving larvae) it was not possible to redetect the labelling in the longitudinal muscles at the third larval instar, in two cases we were able to follow syncytia from embryonic development through the third larval instar to the adult fly (Fig. 4). All specimens reaching the imaginal stage (n=11) again displayed a strong GFP expression. Hence, the longitudinal visceral musculature is organised as a syncytial tissue not only during metamorphosis (Klapper, 2000), but also prior to and after this stage of development.

The progeny of a crossing between the P[GAL4] enhancer trap line 5053A and the *UAS-GFP* strain shows a strong GFP expression within the longitudinal visceral musculature of the third larval instar (Klapper, 2000). The employment of the *UAS-GFP* strain as donor and the P[GAL4] 5053A line as recipient in our transplantation system resulted in a stronger GFP expression and the redetection of all embryonic labelling at the third larval instar (17 of 17). However, owing to the fact that the expression pattern of the enhancer trap line P[GAL4] 5053A is not ubiquitous but restricted to only few tissues, we could not use it for our cell-lineage analyses.

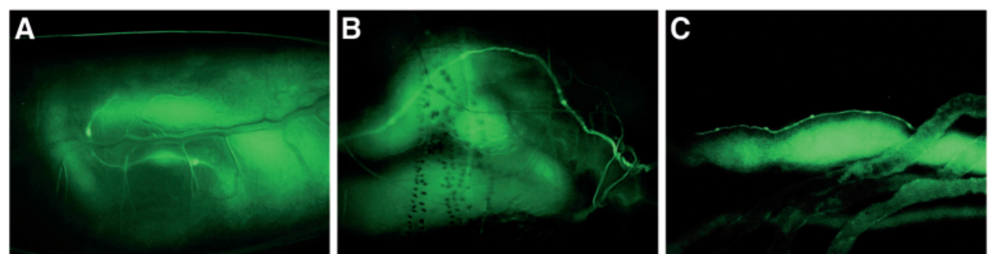

Fig. 4. GFP expression labels syncytia within the longitudinal visceral musculature at different stages of development. (A) Stage 17 embryo, (B) third-instar larva, (C) dissected midgut of an adult fly.

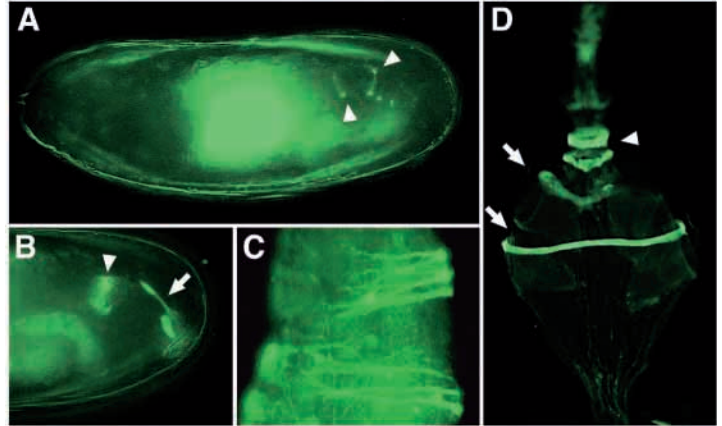

Fig. 5. The visceral musculature of the hindgut consists of syncytia throughout development. (A) First signs of GFP expression within the circular musculature of the hindgut (arrowheads) become visible in stage 15 embryos. (B) In some cases, labelling that overlapped visceral musculature of the hindgut (arrowhead) and larval somatic muscles (arrow) was detected. In the dissected hindgut of a third-instar larva (C), the reticulated structure of the individual muscles covering only one half of the gut tube becomes distinct. In adult flies (D), the circular muscles of the hindgut have lost their meshed structure and surround the gut tube as small bands, either entirely (arrowhead) or over about half its circumference (arrows).

The circular musculature of the hindgut persists through metamorphosis and consists of syncytia

The transplantation series performed at 5 to 10% EL additionally produced 42 embryos with labelling that contributed to the circular musculature of the hindgut (Table 3; 29 pure + 13 overlapping clones). Thus, like the longitudinal visceral musculature of the midgut, the circular muscles of the hindgut consist of syncytia (Fig. 5A). The number of labelled muscles per specimen varied from one to eight. Owing to the bent morphology of the hindgut and the superposition of other tissues, in some cases a detailed counting of GFP-expressing muscles was not feasible. The cytoplasm of each labelled muscle is organised in a reticular manner and covers about one half of the gut tube. We never detected more than two nuclei sharing a common cytoplasm. Of the 42 labelled embryos, 13 additionally displayed GFP expression within larval somatic muscles (Fig. 5B). The fact that this labelling overlapped different mesodermal tissues demonstrates that the precursors of the visceral musculature of the hindgut are not yet determined at the cellular blastoderm stage. Twenty-two of the 42 individuals reached the third larval instar. In all of them, the pure and the overlapping labelling was again detected. We never observed a GFP expression within the circular musculature of the hindgut of third-instar larvae when, embryonically, no labelling was apparent in this tissue. The movements of the living larvae sometimes made it impossible to specify the number of labelled muscles exactly. Nevertheless, the number of labelled visceral muscles roughly corresponds to that in the late embryonic stage. As in embryos, the reticulated cytoplasm of the individual muscles surrounds about one half of the gut tube (Fig. 5C).

Twelve of the 22 larvae survived metamorphosis and were dissected as adult flies. In each case, the labelling of the visceral hindgut musculature was redetected (Fig. 5D). The cytoplasm of every labelled muscle covers at least one half of the gut tube and again we never detected more than two nuclei per syncytium. In contrast to earlier developmental stages, the cytoplasm has lost its meshed structure and now forms small bands. The number of labelled muscles roughly corresponds to the number obtained at earlier stages. We never observed GFP expression within the circular musculature of the hindgut of adult flies when in the embryo and larva no labelling was detectable in this tissue. Taken together, these results indicate that the visceral muscles of the hindgut are generated as syncytia at the embryonic stage and persist throughout the entire development. Examination of the individuals that showed overlapping labelling of circular hindgut musculature and somatic muscles and survived metamorphosis (seven of 12) indicated that only the fraction labelling the visceral musculature was detectable in adult flies.

DISCUSSION

The GAL4/UAS transplantation system

The GAL4/UAS system of Brand and Perrimon is a powerful tool for the selective activation of any cloned gene in a wide variety of tissue- and cell-specific patterns (Brand and Perrimon, 1993; Phelps and Brand, 1998). In order to uncover and analyse syncytia within the musculature of *Drosophila*, we transplanted single cells from *UAS-GFP* donor embryos into *da-GAL4* recipients. By this means we produced genetic mosaics at defined positions within the mesoderm anlage at the blastoderm stage. The *daughterless*-promoter leads to a constitutive expression of the GAL4 protein within all recipient-derived cells. All donor-derived cells contain the reporter gene under the control of the GAL4-responsive promoter UAS. The reporter gene is silent in the absence of GAL4. An activation of the reporter gene transcription is expected only if an individual cell contains both components: GAL4 protein and *UAS-GFP*. This should exclusively occur when donor- and recipient-derived cells fuse to generate syncytia.

To test the system, we performed single-cell transplantations within the mesoderm anlage at 40% EL. It has been shown by previous cell-lineage analyses that this region most frequently gives rise to somatic muscles and fat body (Beer et al., 1987; Klapper et al, 1998). The expression of the reporter gene in somatic muscles, as well as the absence of labelling in mononuclear tissues such as the fat body, indicates that solely syncytia consisting of donor- and recipient-derived nuclei are detected by this technique.

It could also be possible that the proteins used in our experiments (GAL4, β-galactosidase and GFP) are exchanged between donor- and recipient-derived cells through cell-cell junctions. However, owing to the size of the proteins (GAL4, about 100 kDa (Laughon and Gesteland, 1984); β-galactosidase, 116 kDa (Fowler and Zabin, 1978); GFP, 27 kDa

(Prasher et al., 1992)), as well as to the size of known gap junctions (diameter 1.5 nm; passive diffusion possible up to 1.5 kDa; Weir and Lo, 1984), in *Drosophila* a free exchange of the components between individual cells seems to be very unlikely. Nevertheless, if such an exchange actually occurred, each of these cells would in any case be part of a functional syncytium.

The simultaneous use of the GAL4/UAS transplantation system and a cell-lineage marker reveals that most larval somatic muscles are generated by the fusion of clonally non-related cells. In some cases, however, we also obtained muscles exhibiting only the cell-lineage marker, demonstrating that here all nuclei are donor-derived and therefore clonally related. Thus, a somatic muscle can be generated by the fusion of either clonally related or non-related cells, and therefore clonal relationships generally seem to play no crucial role in the selection of the participating cells. Our findings support the inference of Frasch and Leptin (Frasch and Leptin, 2000) that the founder cell simply fuses with fusion-competent cells located in their immediate vincinity, regardless of their clonal relationships.

Longitudinal visceral muscles of the midgut consist of syncytia

Up to now the visceral musculature of *Drosophila* has been described as consisting of separate spindle-like mononuclear cells (Goldstein and Burdette, 1971; Elder, 1975; Tepass and Hartenstein, 1994a). Recent analyses reveal the existence of syncytia within the longitudinal visceral musculature during metamorphosis (Klapper, 2000). However, by morphological criteria alone it was not possible to determine whether the organisation of these muscles is mononuclear or syncytial prior to and after metamorphosis.

Because the GAL4/UAS transplantation system turned out to be a reliable tool to highlight syncytia, we used this approach to analyse the longitudinal visceral musculature at different developmental stages. By this means we were able to detect syncytia within this tissue and could follow individual labelling in vivo from the end of embryogenesis through larval stages to the adult fly. Hence, the longitudinal visceral musculature is not only organised as a syncytial tissue during metamorphosis, but also consists of multinucleate cells prior to and after this developmental process.

Using the P[GAL4] enhancer trap strain 5053A we were able to follow all syncytia detected within the embryo throughout larval development. However, employing the *da-GAL4* strain, most of the labelling observed in embryos could not be redetected in third-instar larvae, whereas all of them were found again in adult flies. It has previously been shown that the *daGAL4* strain drives a strong UAS-GFP expression within longitudinal visceral muscles of third-instar larvae if both constructs are located in the same nuclei (Klapper, 2000). If this strain is used as recipient of the GAL4/UAS transplantation system, it might be possible that the GAL4 expression of the *da-GAL4* strain is not sufficient to induce an appreciable GFP-expression in neighbouring nuclei of third-instar larvae. At this stage of development the nuclei of the longitudinal musculature are separated by cytoplasm over long distances, ranging from 200 to 400 μm (Klapper, 2000), owing to the elongation of the midgut during larval development. These distances might produce a diffusion gradient of the GAL4 protein within the stretched cytoplasm that is steep enough to prevent detectable GFP expression in neighbouring nuclei. The imaginal midgut is about half as long as the larval gut, while the number of nuclei contributing to the longitudinal musculature is the same at both developmental stages (Klapper, 2000). The distance between nuclei within a longitudinal muscle of the adult fly ranges from 100 to 150 μm (R. K., unpublished). Therefore, more GAL4 protein might again reach the neighbouring nuclei, so that GFP expression is increased.

The circular musculature of the hindgut persists through metamorphosis and consists of syncytia

It has been described previously that the circular visceral musculature of the hindgut consists of mononuclear cells (Tepass and Hartenstein, 1994a) and that the entire tissue is broken down and replaced by a newly formed imaginal musculature during metamorphosis (Robertson, 1936). In contrast to these observations, not only did we detect syncytia within this tissue but we were also able to follow individual clones throughout development. This clearly demonstrates that the circular visceral muscles of the hindgut persist through metamorphosis. In embryos, third-instar larvae and adult flies the muscles form syncytia, presumably comprising two nuclei each.

Owing to the fact that single cells were transplanted, this labelling also represents a fraction of the cell lineage. In embryos, as well as in third-instar larvae, we observed GFP expression overlapping between visceral and somatic muscles. The common cell lineage of the two tissues indicates that there exists no separate primordium for the circular musculature of the hindgut at the blastoderm stage. However, overlapping labelling between somatic and visceral musculature was never observed in adult flies. Only the fractions that contribute to the visceral musculature were redetected. Similar results regarding the hindgut musculature of adult flies were also obtained by clonal analyses of Lawrence and Johnston (Lawrence and Johnston, 1986a). Because they did not observe overlapping clones between the circular musculature and other mesodermal tissues in adult flies, a separate primordium at the blastoderm stage for the imaginal hindgut musculature was postulated.

In view of our finding that larval and imaginal visceral musculature of the hindgut represent the same tissue, there seems to be a contradiction concerning the state of determination of the respective primordium. We think this discrepancy can be resolved by taking into account that during metamorphosis the larval somatic musculature is replaced by newly formed imaginal muscles generated by only a few adult myoblasts (Crossley, 1978; Bate et al., 1991; Currie and Bate, 1991; Fernandes et al., 1991). Overlapping labelling of somatic and visceral musculature in larvae, as well as adult flies, is possible only if the descendants of the transplanted single cell contribute to visceral muscles of the hindgut and larval somatic muscles, as well as to the precursors of the imaginal somatic musculature. The occurrence of such clones is very unlikely, as clones that overlap larval somatic musculature and imaginal muscle precursors have seldom been previously observed (Holz et al., 1997; Klapper et al., 1998).

Formation of syncytia

The presence of syncytia within the visceral musculature now raises the question of whether the formation process might be

similar to that of the somatic muscles. First signs of GFP expression within the visceral and in the somatic musculature were detectable at stage 15 of embryogenesis. As there is a considerable delay, about 2-4 hours, between the activation of the *UAS-GFP* construct and the formation of the fluorescent product (Heim et al., 1994; Brand, 1995; Hazelrigg et al., 1998), we assume that the formation of syncytia begins at stage 12. It is also at this time that the first fusions within the somatic musculature have been observed (Bate, 1990). Thus, the initiation of fusion processes within both types of musculature might be triggered by the same signalling pathway. As it has been shown that *duf* and *sns* are also expressed within the visceral mesoderm (Bour et al., 2000; Ruiz-Gómez et al., 2000), there may also exist founders and fusion-competent cells that are specified by the same genetic mechanisms.

Conclusions

The formation of syncytia is an interesting aspect of cellular biology. Here, cells lose their individual identity to take part in a higher-level functional structure. Using the components of the GAL4/UAS system for single-cell transplantations, we were able to detect and follow syncytia within the visceral musculature. On the basis of morphological studies, these muscles have been thought to consist of mononuclear cells (Elder, 1975). It therefore appears to us that this approach might be very useful to discover further syncytia, not only in *Drosophila* but also in any other organism using the GAL4/UAS system.

We thank Elke Naffin for excellent technical support, and Anne Holz and Barbara Bossinger for valuable comments on the manuscript. The fly strain *GAL4daG32* was a generous gift from Elisabeth Knust. This work was supported by the Deutsche Forschungsgemeinschaft (Ja 199/12-4 to W. J.).

REFERENCES

Azpiazu, N. and Frasch, M. (1993). *tinman* and *bagpipe*: two homeobox genes that determine cell fates in the dorsal mesoderm of *Drosophila*. *Genes Dev.* **7**, 1325-1340.

Bate, M. (1990). The embryonic development of larval muscles in *Drosophila*. *Development* **110**, 791-804.

Bate, M., Rushton, E. and Currie, D. A. (1991). Cells with persistent *twist* expression are the embryonic precursors of adult muscles in *Drosophila*. *Development* **113**, 79-89.

Baylies, M. K., Bate, M. and Ruiz-Gómez, M. (1998). Myogenesis: a view from *Drosophila*. *Cell* **93**, 921-927.

Beer, J., Technau, G. M. and Campos-Ortega, J. A. (1987). Lineage analysis of transplanted individual cells in embryos of *Drosophila melanogaster*. *Roux Arch. Dev. Biol.* **196**, 222-230.

Bodenstein, D. (1950). The postembryonic development of *Drosophila*. In *Biology of* Drosophila (ed. M. Demerec), pp. 275-367. New York: John Wiley and Sons.

Bour, B. A., Chakravarti, M., West, J. M. and Abmayr, S. M. (2000). *Drosophila SNS*, a member of the immunoglobulin superfamily that is essential for myoblast fusion. *Genes Dev.* **14**, 1498-1511.

Brand, A. H. (1995). GFP in *Drosophila*. *Trends Genet.* **11**, 324-325.

Brand, A. H. and Perrimon, N. (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* **118**, 401-415.

Broihier, H. T., Moore, L. A., Van Doren, M., Newman, S. and Lehmann, R. (1998). *zfh-1* is required for germ cell migration and gonadal mesoderm development in *Drosophila*. *Development* **125**, 655-666.

Campos-Ortega, J. A. and Hartenstein, V. (1997). *The Embryonic Development of* Drosophila melanogaster. Berlin Heidelberg: Springer Verlag.

Crossley, A. C. (1978). The morphology and development of the *Drosophila* muscular system. In *The Genetics and Biology of* Drosophila, Vol. 2b (ed. M. Ashburner and T. R. F. Wright), pp. 499-560. New York: Academic Press.

Currie, D. A. and Bate, M. (1991). The development of adult abdominal muscles in *Drosophila*: myoblasts express twist and are associated with nerves. *Development* **113**, 91-102.

Dohrmann, C., Azpiazu, N. and Frasch, M. (1990). A new *Drosophila* homeobox gene is expressed in mesodermal precursor cells of distinct muscles during embryogenesis. *Genes Dev.* **4**, 2098-2111.

Elder, H. Y. (1975). Muscle structure. In *Insect muscle* (ed. P. N. R. Usherwood), pp. 1-74. New York: Academic Press.

Fernandes, J., Bate, M. and VijayRaghavan, K. (1991). Development of the indirect flight muscles of *Drosophila*. *Development* **113**, 67-77.

Fowler, A. V. and Zabin, I. (1978). Amino acid sequence of beta-galactosidase. XI. Peptide ordering procedures and the complete sequence. *J. Biol. Chem.* **253**, 5521-5525.

Frasch, M. (1999). Controls in patterning and diversification of somatic muscles during *Drosophila* embryogenesis. *Curr. Opin. Genet. Dev.* **9**, 522-529.

Frasch, M. and Leptin, M. (2000). Mergers and acquisitions: unequal partnerships in *Drosophila* myoblast fusion. *Cell* **102**, 127-129.

Georgias, C., Wasser, M. and Hinz, U. (1997). A basic-helix-loop-helix protein expressed in precursors of *Drosophila* longitudinal visceral muscles. *Mech. Dev.* **69**, 115-124.

Goldstein, M. A. and Burdette, W. J. (1971). Striated visceral muscle of *Drosophila melanogaster*. *J. Morphol.* **134**, 315-334.

Hazelrigg, T., Liu, L., Hong, Y. and Wang, S. (1998). GFP expression in *Drosophila* tissues: time requirements for formation of a fluorescent product. *Dev. Biol.* **199**, 245-249.

Heim, R., Prasher, D. C. and Tsien, R. Y. (1994). Wavelength mutations and post-translational autooxidation of green fluorescent protein. *Proc. Natl. Acad. Sci. USA* **91**, 12501-12504.

Holz, A., Meise, M. and Janning, W. (1997). Adepithelial cells in *Drosophila melanogaster*: origin and cell lineage. *Mech. Dev.* **62**, 93-101.

Klapper, R. (2000). The longitudinal visceral musculature of *Drosophila melanogaste*r persists through metamorphosis. *Mech. Dev.* **95**, 47-54.

Klapper, R., Holz, A. and Janning, W. (1998). Fate map and cell lineage relationships of thoracic and abdominal mesodermal anlagen in *Drosophila melanogaster*. *Mech. Dev.* **71**, 77-87.

Kusch, T. and Reuter, R. (1999). Functions for *Drosophila brachyenteron* and *forkhead* in mesoderm specification and cell signalling. *Development* **126**, 3991-4003.

Laughon, A. and Gesteland, R. F. (1984). Primary structure of the *Saccharomyces cerevisiae GAL4* gene. *Mol. Cell. Biol.* **4**, 260-267.

Lawrence, P. A. and Johnston, P. (1986a). Observations on the cell lineage of internal organs of *Drosophila*. *J. Embryol. Exp. Morphol*. **91**, 251-266.

Meise, M. and Janning, W. (1993). Cell lineage of larval and imaginal thoracic anlagen cells of *Drosophila melanogaster* as revealed by single-cell transplantations. *Development* **118**, 1107-1121.

Mintz, B. and Baker, W. W. (1967). Normal mammalian muscle differentiation and gene control of isocitrate dehydrogenase synthesis. *Proc. Natl. Acad. Sci. USA* **58**, 592-598.

Phelps, C. B. and Brand, A. H. (1998). Ectopic expression in *Drosophila* using the GAL4 system. *Methods* **14**, 367-379.

Prasher, D. C., Eckenrode, V. K., Ward, W. W., Prendergast, F. G. and Cormier, M. J. (1992). Primary structure of the *Aequorea victoria* green-fluorescent protein. *Gene* **111**, 229-233.

Robertson, C. W. (1936). The metamorphosis of *Drosophila melanogaster*, including an accurately timed account of the principal morphological changes. *J. Morphol.* **59**, 351-399.

Ruiz-Gómez, M., Coutts, N., Price, A., Taylor, M. V. and Bate, M. (2000). *Drosophila dumbfounded*: A myoblast attractant essential for fusion. *Cell* **102**, 189-198.

San Martin, B. and Bate, M. (2001). Hindgut visceral mesoderm requires an ectodermal template for normal development in *Drosophila*. *Development* **128**, 233-242.

Simon, J. A., Sutton, C. A., Lobell, R. B., Glaser, R. L. and Lis, J. T. (1985). Determinants of heat shock-induced chromosome puffing. *Cell* **40**, 805-817.

Singer, J. B., Harbecke, R., Kusch, T., Reuter, R. and Lengyel, J. A. (1996). *Drosophila brachyenteron* regulates gene activity and morphogenesis in the gut. *Development* **122**, 3707-3718.

Strasburger, M. (1932). Bau, Funktion und Variabilität des Darmtractus von *Drosophila melanogaster* Meigen. *Z. wiss. Zool.* **140**, 539-649.

Technau, G. M. (1986). Lineage analysis of transplanted individual cells in embryos of *Drosophila melanogaster*. *Roux Arch. Dev. Biol.* **195**, 389-398.
Tepass, U. and Hartenstein, V. (1994a). The development of cellular junctions in the *Drosophila* embryo. *Dev. Biol.* **161**, 563-596.
Tepass, U. and Hartenstein, V. (1994b). Epithelium formation in the *Drosophila* midgut depends on the interaction of endoderm and mesoderm. *Development* **120**, 579-590.
Tremml, G. and Bienz, M. (1989). Homeotic gene expression in the visceral mesoderm of *Drosophila* embryos. *EMBO J.* **8**, 2677-2685.
Weir, M. P. and Lo, C. W. (1984). Gap-junctional communication compartments in the *Drosophila* wing imaginal disk. *Dev. Biol*. **102**, 130-146.
Wodarz, A., Hinz, U., Engelbert, M. and Knust, E. (1995). Expression of *crumbs* confers apical character on plasma membrane domains of ectodermal epithelia of *Drosophila*. *Cell* **82**, 67-76.