Identification of fat-cell enhancer regions in *Drosophila melanogaster*

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Abstract

The insect fat body is a dynamic tissue involved in maintaining homeostasis. It functions not only in energy storage and intermediary metabolism but also in detoxification, communication and the immune response. Some of these functions are confined to distinct groups of fat body cells. In *Drosophila melanogaster*, discrete precursor-cell clusters populate the fat body [Hoshizaki, D.K., Blackburn, T., Price, C., Ghosh, M., Miles, K., Ragucci, M. and Sweis, R. (1994) Identification of embryonic fat-cell lineage in *Drosophila melanogaster*. Development 120: 2489–2499; Hoshizaki, D.K., Lunz, R., Ghosh, M. and Johnson, W. (1995) Identification of fat-cell enhancer activity in *Drosophila melanogaster* using P-element enhancer traps. Genome 38: 497–506; Riechmann, V., Rehorn, K.P., Reuter, R. and Leptin, M. (1998) The genetic control of the distinction between fat body and gonadal mesoderm in *Drosophila*. Development 125: 713–723]. Whether these clusters populate defined morphological regions or whether they represent the precursors to functionally similar groups of fat-body cells has not been formally demonstrated. We have identified a 2.1 kb enhancer region from *D. melanogaster*, discrete precursor-cell clusters populate the fat body. This enhancer region drives expression in specific groups of precursor-cell clusters, which we show give rise to defined regions of the mature embryonic fat body. We present evidence that *srp* expression in different precursor fat cells is controlled by independent cis-acting regulatory regions, and we have tested the role of trans-acting factors in the specification of some of these cells. We suggest that the different positional cues regulating *srp* expression, and therefore general fat-cell specification, might also be involved in the functional specialization of fat cells. This may be a common mechanism in insects to explain the origin of biochemically distinct regions of the larval/adult fat body.

Keywords: enhancers, *Drosophila*, fat body, fat-cell lineage, mesoderm.

Introduction

The insect fat body is a dynamic tissue that participates in multiple biochemical functions, including energy storage, intermediary metabolism, detoxification, communication and the immune response. The insect fat cell is the main site for the storage and production of proteins, lipids and carbohydrates. It produces a variety of stage-specific, amino acid-storage proteins, including calliphorin in blowflies (*Calliphora*) and drosophilin and hexamerins (e.g. the larval serum proteins) in *Drosophila* (Keeley, 1985 and references therein). The fat body is also the primary biosynthetic site of diacylglycerol and the major insect sugar, trehalose. Both diacylglycerol and trehalose are energy-storage molecules that are key to the survival of the animal and central for energy-intensive behaviour such as long-distance migration (e.g. *Locusta migratoria*; Becker et al., 1996; Vroemen et al., 1998). Fat-body cells produce a number of other significant proteins including vitellogenins for oocyte maturation in *Drosophila* and mosquitoes (*Anedes aegypti*) and diapause proteins and haemoglobin in midge fly larvae (*Chironomus thummi*; reviewed in Keeley, 1985). The fat body has been compared to the vertebrate liver in its role in trehalose biosynthesis and release and through its response to adipokinetic signalling, which is analogous to the hormone (insulin)-mediated synthesis and release of glucose by the liver (Becker et al., 1996). Interestingly, the fat body of the desert ant (*Cataglyphis niger*) is the major synthetic site of hydrocarbons involved in communication among colony members (Soroker & Hefetz, 2000). The fat body also plays a central role in the insect innate immune response (reviewed in Hoffmann et al., 1996; Engstrom, 1999).
The fat body is one of several tissues produced by the embryonic mesoderm (Hartenstein & Jan, 1992; Hoshizaki et al., 1994; Technau, 1987). In general, the primordia for each tissue type lie in defined positions determined by the anterior–posterior and dorsal–ventral patterning of the mesoderm. In Drosophila, pair-rule genes such as even-skipped help to establish intrinsic differences between mesodermal cells in a metameric pattern along the anterior–posterior axis (Azpiazu et al., 1996), while along the dorsal–ventral axis, decapentaplegic (dpp) expression in the dorsal embryonic ectoderm specifies the dorsal mesoderm through maintenance of tinman (tin) expression (Frasch, 1995; Staehling-Hampton et al., 1994). The establishment and maintenance of morphologically distinct cell types are controlled by homeotic genes that determine segment identity through the regional activation of target genes necessary for cell specification (Weatherbee & Carroll, 1999; Weatherbee et al., 1998). These patterning systems provide mesodermal cells with unique addresses that serve as positional cues to establish distinct populations of cells.

The fat-body precursors are organized in a metameric pattern. Within a given range of parasegments, they are located in defined dorsal-ventral positions (reviewed in Riechmann et al., 1997). It is likely that the specification of precursor fat cells requires the integration of different anterior–posterior and dorsal–ventral positional cues and homeotic gene information. The maturation of the fat body requires the coordinated migration of cells from the fat-cell clusters to their final positions, where they are organized into a functional organ composed of three morphological domains: the lateral fat body, the dorsal fat-cell projections, and the ventral fat-cell commissure (Campos-Ortega & Hartenstein, 1997; Hoshizaki et al., 1994; Riechmann et al., 1998). A variety of studies have suggested that the fat body is also divided into different regions based on the diverse biochemical functions of this tissue (reviewed in Hauenerland & Shirk, 1995).

We describe here a 2.1 kb enhancer region from the srp (srp) promoter that drives expression in a subset of fat cells. The srp gene is one of three known D. melanogaster genes belonging to the GATA transcription-factor family (Brown & Castelli-Gair Hombria, 2000; Lin et al., 1995; Ramain et al., 1993; Winick et al., 1993) and was initially identified as a transcriptional activator of the alcohol dehydrogenase (Adh) gene in fat cells (Abel et al., 1993). SRP protein is present at embryonic stage 10/11 (embryonic stages are those of Campos-Ortega & Hartenstein, 1997) in all fat-cell precursors (Sam et al., 1996) and is the earliest known gene to be expressed in the fat-cell lineage (Abel et al., 1993; Rehorn et al., 1996; Sam et al., 1996). Genetic analysis has revealed that srp is necessary for the maintenance of the fat-cell lineage (Sam et al., 1996) and is sufficient to induce fat-cell formation within the mesoderm (Hayes et al., 2001). Thus, the activation of srp is a likely step in fat-cell specification. Because precursor fat cells arise from stereotopic anterior–posterior and dorsal–ventral positions within the mesoderm, it is possible that a combination of positional information directs srp expression through different enhancers located within the srp regulatory region.

The srp regulatory/promoter region spans at least 8 kb, based on the location of srp regulatory mutations (Rehorn et al., 1996). Sequences within this region were identified as an in vitro target of the homeodomain transcription factor, Ultrabithorax (UBX) and contain putative UBX response elements (Mastick et al., 1995). We describe enhancer activity that is associated with this region. By using this enhancer region, we have traced the origin of specific morphological regions of the fat body and tested factors that might trans-activate srp. We discuss the possibility that the positional information that directs the specification of precursor fat cells is also responsible for the eventual biochemical differences found in the larval fat body.

Results

Developmental studies of the embryonic fat body demonstrate that fat cells originate from specific precursor-cell clusters that lie in the lateral, ventral, and dorsal mesoderm (Fig. 1; Hoshizaki et al., 1994; Hoshizaki et al., 1994). Thus, the activation of srp is a likely step in fat-cell specification. Because precursor fat cells arise from stereotopic anterior–posterior and dorsal–ventral positions within the mesoderm, it is possible that a combination of positional information directs srp expression through different enhancers located within the srp regulatory region.

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Figure 1. Fat cell development. (A, C, E) Lateral views of whole mount embryos immunostained for SRP protein and (B, D, F) corresponding schematic drawings highlighting precursor fat-cell clusters and fat body domains. (A, B) Stage-10 embryos. The dorsal cell cluster is located in the dorsal mesoderm of PS 13 and the primary cell clusters are located in the lateral mesoderm of PS 4–9. (C, D) Stage-11/12 embryos. The secondary ventral cell clusters are located in the ventral mesoderm of PS 3–11. A second group of subsidiary precursor fat cells are located as small cell clusters immediately posterior to the primary cell clusters in PS 4–9 and in the equivalent position in PS 10–12. (E, F) Stage-16 embryos. The fat body is made up of three morphological domains: the dorsal fat-cell projections, which extend in the anterior direction from the posterior–dorsal region of the lateral fat body; the lateral fat body which spans the lateral region of the embryonic ventral midline commissure, which extends from the anterior-lateral fat body and spans the ventral midline.© 2002 Royal Entomological Society, Insect Molecular Biology, 11, 67–77
Fat-cell enhancer regions and fat body domains

et al., 1995; Riechmann et al., 1998). At stage 10/11, precursor fat cells lie in the lateral mesoderm in a metamERICLY repeating pattern (Fig. 1A). These cells make up the primary cell clusters and are organized as serially duplicated clusters positioned in the even-skipped domain of parasegments (PS) 4–9 (Fig. 1A,B). These cell clusters are likely to populate most of the lateral fat body (Hoshizaki et al., 1994; Riechmann et al., 1997).

Within the dorsal mesoderm of PS 13 lies a large cluster of cells (Fig. 1A,B); this particular group of cells is likely to make up the dorsal fat-cell projections (Fig. 1A,B; Riechmann et al., 1998). At stage 11, two secondary sets of cell clusters are identified that are serially duplicated along the anterior–posterior axis in the lateral and ventral mesoderm (Fig. 1C,D; Riechmann et al., 1998). Each morphological region of the fat body is thought to arise from spatially distinct precursor-cell clusters, but the lack of cell markers for specific precursor fat cells has made it difficult to confirm this idea.

Putative UBX response elements are associated with enhancer activity in a subset of srp-expressing cells

We have completed a genomic walk of the srp locus and have positioned the srp gene within this region (Fig. 2A and see Rehorn et al., 1996). Within this walk is the A7.1ES fragment, which contains three putative UBX protein-binding sites (Fig. 2B; Mastick et al., 1995). We have tested this region for enhancer activity in vivo. A7.1ES was subcloned into the P-element vector pCaSpeR-hs43-lacZ and transgenic animals were generated. The activity of the A7.1ES-lacZ reporter was studied in whole-mount embryos. We found that the A7.1ES-lacZ reporter was active in a subset of srp-expressing cells, including cells that make up specific regions of the fat body (Figs 3, 4).

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expressed in several other tissues: the primordia of the anterior and posterior midgut; the cephalic mesoderm and haemocytes; the amnioserosa primordium and amnio-serosa; and the lymph glands (see Fig. 2 in Sam et al., 1996). Within the fat body, we detected A7.1ES-lacZ reporter activity in only a subset of fat-body cells: the ventral- and posterior-most edge of the mature embryonic lateral fat body, the dorsal fat-cell projection, and a portion of the ventral commissure (Fig. 3). Using A7.1ES-lacZ as a cell-lineage marker, we have traced the origins of the aforementioned groups of fat body cells (Figs 4, 5). A7.1ES-lacZ reporter activity was detected at stage 10 in a single cluster of cells located within the dorsal mesoderm of parasegment (PS) 13 (Fig. 4A). The cells of this cluster populate the fat-cell projections and the posterior-most cells of the lateral fat body and are described in more detail in the following section. By stage 11, the number of A7.1ES-lacZ-expressing cells in the aforementioned dorsal cell cluster has increased and we began to detect expression in cells located in the ventral region of the mesoderm (Fig. 4B).

By stage 12, strong expression was detected in the ventral mesoderm in serially duplicated cell clusters composed of 4–6 cells located in PS 3–11 and in a smaller cluster in PS 12 (Fig. 4C). The ultimate fate of the small cluster in PS 12 is not known, because β-galactosidase activity ceased to be detected in its cells after stage 13. By stage 14, the cell clusters in PS 3–11 fused to form a 1–2 cell-wide row that defines the ventral edge of the lateral fat body (Fig. 4G,H). These cell clusters correspond to the secondary ventral fat-cell clusters.

The ventral secondary cell clusters in PS 3–5 also contribute to the ventral commissure, as further described in detail below (Riechmann et al., 1998). At stage 16, A7.1ES-

Figure 3. A7.1ES is active in a subset of fat body cells. (A) Wild-type, stage-15 embryo stained for A7.1ES-lacZ activity (brown) and Adh transcripts (blue). (B) Enlargement of (A). A7.1ES is active in the dorsal fat-cell projections (overline) and in the posterior-most and ventral-most cells (bracket) of the lateral fat body. A7.1ES is also active in portion of the ventral commissure (see Fig. 5 and text for more detail).

Figure 4. A7.1 ES- lacZ reporter recapitulates a portion of the srp expression pattern. (A) Lateral view of a stage-10 embryo. Reporter activity is first detected in the cephalic mesoderm and in PS13 in a dorsal cluster of cells, which eventually gives rise to the dorsal fat-cell projection and the posterior-most lateral fat body. (B) Lateral view of a late stage-11 embryo. Prohaemocytes are detected as they migrate from the cephalic mesoderm (underline). Transient expression is detected in single cells in the ventral mesoderm (arrow) and later persists as nine clusters of cells. (C) Lateral and (D) dorsal view of a stage-12 embryo. (E) Reporter gene activity persists in bilateral clusters of cells located in PS 3–11, which are the ventral secondary cell clusters, and is detected in a small bilateral cluster of cells in PS 12. (D) Another small bilateral cluster of cells is detected in the ventral mesoderm of PS 13, adjacent to the dorsal cell cluster. Reporter gene activity is detected in the amnioserosa cells abutted to the posterior edge of the retracting germ band (asterisk in (D)) and in the large dorsal cell cluster in PS 13. Reporter gene activity is also detected in cells likely to be the precursors to the longitudinal visceral muscle fibres (bracket in (D)). (E) Dorsal and (F) lateral view of a late stage-12 embryo. Reporter gene activity is detected in the posterior amnioserosa cells (asterisk in (E)) and in the putative precursor longitudinal visceral muscle cells (bracket in (F)). The ventral cell cluster in PS13 has fused across the midline (arrow in (E)) and the bilateral ventral cluster in PS 12 is absent. (G) Lateral view of a stage 14/15 embryo. Secondary ventral cell clusters have begun to fuse to form the ventral edge of the lateral fat body. (H) Lateral and (I) ventral and (J) dorsal view of early stage-16 embryos. Reporter gene activity is present (H) in the ventral (brackets) and posterior cells of the lateral fat body (I) in one of the two bridges that form the ventral commissure (bracket) (see text and Fig. 5 for details) and (J) in the dorsal fat-cell projections (arrowheads) and posterior amnioserosa cells (star).
was active in a portion of the ventral commissure that makes up the posterior bridge, including the bilateral horns, but was not active in the anterior bridge (Fig. 4I). The ventral commissure lies in the anterior region of the embryo and eventually spans the ventral midline (Fig. 5). It consists of at least two fat-cell bridges that arise from cells of the secondary ventral cell clusters in PS 3 and 4 and fuse across the ventral midline. The posterior bridge extends from cells in the bilateral ventral cell cluster in PS 5 and also fuse across the ventral midline. (C) The posterior bridge lies in a more peripheral position within the embryo than the anterior bridge and is a continuation of the ventral most cells of the lateral fat body. (D) Extending from the posterior bridge are two horns that extend in the anterior direction and crossover the anterior bridge (Second horn is out of the plane of focus).

The A7.1ES-lacZ reporter also was active in the cephalic mesoderm and in the prohaemocytes as they migrate from the cephalic mesoderm as they migrate from the cephalic mesoderm (Fig. 4B). Reporter gene activity, however, was absent in the majority of haemocytes by stage 14. A7.1ES-lacZ reporter expression was also detected in the amnioserosa, but only in the posterior-most cells (Fig. 4D,E). At stage-12, we detected the novel expression of A7.1ES-lacZ in cells that arose from the caudal pole (Fig. 4C). These cells subsequently appeared as rows of cells ensheathing the midgut (Fig. 4F). Based on the morphology and position of these cells (Campos-Ortega & Hartenstein, 1997), it is likely that A7.1ES is active in the longitudinal visceral muscle fibres and their precursors.

Expression of srp in the dorsal fat-cell cluster is controlled separately from the remaining fat body cells

A7.1ES has strong activity in posterior srp-expressing cells, i.e. the posterior amnioserosa and the dorsal cell cluster. We have restricted this posterior enhancer activity to the distal portion of A7.1ES, designated A7.1EB (Fig. 1A). Transgenic lines carrying the A7.1EB-lacZ reporter exhibited activity only in the posterior fat cells and their precursors and in the posterior amnioserosa cells (Fig. 6). Lying partially beneath the posterior precursor fat cells are srp-expressing cells that make up the posterior midgut primordium. The A7.1EB enhancer is not active in these cells. The simple expression pattern driven by A7.1EB has allowed us to carry out a detailed examination of the origin of the posterior fat cells, i.e. the dorsal fat-cell projections and the
posterior–lateral fat body, without the difficulties presented by the underlying SRP-expressing posterior midgut primordium. Using A7.1EB-lacZ, we mapped the dorsal cell cluster in the mesoderm relative to the engrailed (en) stripes in the ectoderm (Fig. 7). The majority of the dorsal cell cluster lies between en stripes 13 and 14, which mark the anterior portions of the parasegments in the ectoderm (Lawrence, 1992). A few dorsal cells lie beneath the en stripes, but are not positioned beyond the boundaries of these two stripes.

Using the A7.1EB-lacZ reporter, we detected strong expression at stage 11 in the dorsal cell cluster of PS 13 and in a single cell located immediately ventrally to this large cluster (Fig. 6A,B). By early stage 12, the single cell formed a small cluster of approximately 4–6 cells. Although this cell cluster is located in the ventral mesoderm, its behaviour at later stages revealed that it is distinct from the secondary ventral cell clusters. During germband retraction, the bilateral ventral PS 13 cell cluster fused across the ventral midline (Fig. 6D) and later contributed to the posterior-most cells of the lateral fat body (Fig. 6I,J). At late stage 12, the dorsal cell cluster began to separate, and by stage 13, two distinct subgroups of cells could be identified (Fig. 6E,G). The dorsal-most subgroup forms the dorsal fat-cell projection and the other subgroup coalesces with the fused ventral cluster to form the posterior region of the lateral fat body (Fig. 6J).

UBX affects the morphology of the fat body

The precursor fat-cell clusters are located in defined positions within the specific segments of the mesoderm. Because UBX-target sequences map to A7.1EB (Fig. 2), we tested whether the Ubx gene might play a role as a transcriptional regulator of srp. Because Ubx is expressed in the mesoderm of PS 6–12 but not of PS 13 (Akam & Martinez-Arias, 1985; White & Wilcox, 1984), Ubx might function to repress the formation of a dorsal cell cluster in anterior segments. We examined the fat-cell phenotypes caused by both the loss-of-function and mis-expression of Ubx. The mis-expression of Ubx throughout the mesoderm was achieved by employing the GAL4/UAS targeted gene expression system of Brand & Perrimon (1993).

We found that Ubx does not have a role in the specification of the dorsal cell cluster. Loss of Ubx function did not induce ectopic dorsal cell clusters in the anterior segments nor did mis-expression of Ubx throughout the mesoderm (including PS13) repress dorsal fat-cell specification (data not shown). We note, however, that mis-expression of Ubx in PS 13 leads to morphological alterations of the dorsal fat-cell projections. The fat-cell projection is normally 6–8 cells in width at its broadest region. In the Ubx mis-expression embryos, the dorsal fat-cell projections extended properly in the anterior direction, but they were composed of only a single row of cells (Fig. 8B). This change in cell number was also reflected in the number of the A7.1EB-lacZ positive cells (data not shown). On the other hand, the loss of Ubx occasionally caused a slight reduction in the number of cells present in the projections (Fig. 8C). Taken together these data suggest that Ubx does not play a direct role in

![Figure 7. The dorsal cell cluster is located between en stripes 13 and 14.](image)

(A) Lateral and (C) dorsal view of a stage 11 A7.1EB-lacZ embryo immunostained for ENGRAILED and β-galactosidase protein. (B) is a higher magnification of (A). The dorsal cell cluster lies in the mesoderm beneath the ectodermal en stripes 13 and 14. Cells of the cluster extend within the boundaries of the stripes but do not extend beyond them.

![Figure 8. Mis-expression of Ubx leads to a morphological alteration of the dorsal fat-cell projection.](image)

(A) Dorsal view of wild-type (B) twi-GAL4; UAS-Ubx, and (C) Ubx mutant stage-16 embryos immunostained for SRP protein. (A) wild-type dorsal fat-cell projection (arrow) (B) Mis-expression of Ubx reduces the width of the projections (arrow) to 1–2 cells compared to the normal 6–8-cell width and causes the loss of the lymph glands. (C) Loss of Ubx has little or no effect on the number of cells contributing to the projection (arrow). The lymph glands have hypertrophied (arrowhead) as previously described by Mastick et al. (Mastick et al., 1995).
the trans-activation of srp, but that it is involved in determining segment identity and can affect the differentiation of cells that contribute to the dorsal fat-cell projections.

Surprisingly, Ubx had a dramatic effect on the formation of the ventral commissure, even though the precursors to the commissure lie in PS 4–5 and not in the UBX domain. It has been previously shown that loss of Ubx activity results in strong derepression of srp within the secondary ventral cell clusters in PS 6–12 (Fig. 9B) and see Fig. 4 in (Mastick et al., 1995). We have followed up on this observation and have found that in Ubx mutants, a third bridge arises from PS 6 (Fig. 9E). Thus, it appears that UBX might be directly or indirectly involved in repressing the formation of fat-cell bridges. To further test this involvement, we asked whether UBX is sufficient to suppress the formation of the endogenous ventral commissure by employing the GAL4/UAS system of Brand & Perrimon (1993). In twi-GAL4; UAS-Ubx embryos, we found that both bridges of the ventral commissure were absent (Fig. 9F). These data suggest that Ubx is involved in suppressing the differentiation of the commissure in posterior segments through its role as a segment identity gene. Ubx might repress a hierarchy of genes that control whether the fat-cell bridges will arise from the ventral-most cells of the lateral fat body.

Role of tin and dpp in the specification of the secondary ventral and dorsal cell clusters

The dorsal cell cluster lies in the dorsal mesoderm. We hypothesize that only cells within the dorsal mesoderm are competent to acquire a dorsal fat-cell fate, and that srp, through the A7.1EB enhancer, might be responsive to factors that establish the dorsal mesoderm. Key to the formation of the dorsal mesoderm is the NK homeodomain protein tin (Kim & Nirenberg, 1989). tin is expressed in three phases during embryonic development. In the first phase, it is expressed in a pan-mesodermal fashion. In the second phase, tin expression is lost within the mesoderm except in the dorsal region where it is maintained by the action of dpp in the overlying ectoderm. In the third phase, tin expression is restricted to and marks the precursor to the heart (Azpiazu & Frasch, 1993; Bodmer, 1993; Yin & Frasch, 1998).

Based on the different characterized roles for tin, we predicted that the second phase of tin expression is important for the eventual establishment of the dorsal cell cluster. Moore et al. (1998), however, suggest that early tin expression is important in the specification of fat cells based upon a comparison of the phenotypes associated with the loss of tin and dpp activity. This comparison can distinguish between the first and second phase of tin expression because the second phase, but not the first phase of tin expression, is dependent upon dpp. In tin mutants, in addition to the loss of visceral, heart and dorsal somatic muscle (Azpiazu & Frasch, 1993; Bodmer, 1993), there is a loss of some lateral fat cells based upon the location and number of srp-expressing cells (see Fig. 4 in Moore et al., 1998). In a dpp mutant, more fat cells appear to be present than in a tin mutant, although because of the gross developmental defects associated with the loss of dpp, it is difficult to determine whether any fat cells are absent (see Fig. 4 in Moore et al., 1998). Based upon this comparison, it was suggested that early tin is involved in the specification of fat cells (Moore et al., 1998).

We have re-examined tin's role in fat-cell development using the A7.1EB-lacZ reporter gene as a cell marker for the dorsal cell cluster and the dorsal fat-cell projections. The reporter gene was introduced into both tin- and dpp-mutant backgrounds using standard genetic crosses. We found tin-mutant embryos had few, if any, cells in the dorsal cluster, and concomitantly, there was a loss of dorsal fat-cell projections (and the posterior-most cells of the lateral fat body) (Fig. 10C,D). To determine whether this loss is due

Figure 9. Mis-expression of Ubx leads to morphological alterations of the ventral commissure. (A) Ventral-lateral view of stage-13 wild-type (B) Ubx-mutant and (C) twi-GAL4; UAS-Ubx embryos immunostained for SRP protein. (B) Loss of Ubx results in the de-repression of srp in the ventral cell clusters in PS 6–12 while (C) mis-expression of UBX throughout the mesoderm has little or no effect on srp expression in the ventral cell clusters. (D) Dorsal view of stage-16 wild-type (B) Ubx-mutant and (C) twi-GAL4; UAS-Ubx embryos immunostained for SRP protein. (B) Loss of Ubx results in the formation of an ectopic fat-cell bridge from PS 6 (bracket) while (C) mis-expression of Ubx throughout the mesoderm represses the formation of the ventral commissure.

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to the first or second phase of tin expression, we examined dpp-mutant embryos. We found that reporter gene activity was nearly absent in the location of the dorsal cell cluster, and concomitantly the dorsal fat-cell projections were absent (Fig. 10F, G). These data suggest that the specification of the dorsal cell cluster requires formation of the dorsal mesoderm and depends upon the second phase of tin expression. To further examine the role of dpp-dependent expression of tin, we examined twi-GAL4; A7.1EB- lacZ; UAS-dpp embryos where the dpp was expressed throughout the mesoderm. In these experimental embryos, the dorsal cell cluster was expanded to fill most of PS 13, and the dorsal fat-cell projection was not formed (Fig. 10H–J).

Discussion

Over 20 years ago, Rizki & Rizki (1978) suggested that the larval fat body has a segmental origin. Recent studies have firmly established the segmental nature of the precursor fat cells and have identified different classes or groups of metamERICALLY repeating cell clusters that contribute to the embryonic fat body (Abel et al., 1993; Hoshizaki et al., 1994; Riechmann et al., 1998). In our efforts to understand the molecular mechanism underlying the commitment to and specification of a fat-cell fate, we have focused our attention on srp, a transcription factor gene involved in the specification (Hayes et al., 2001) and differentiation of fat cells (Sam et al., 1996). We present here the first analysis of srp regulatory regions active in the secondary ventral cell clusters and the dorsal cell cluster. Because the precursor fat cells arise from unique anterior–posterior and dorsal–ventral positions within the mesoderm, it is likely that a combination of positional information and input from different homeotic genes are involved in the establishment and maintenance of fat cells that populate the different regions of the fat body. The capability of srp to induce fat-cell formation (Hayes et al., 2001) suggests that this patterning information is integrated at the srp locus through the use of the different srp fat-cell enhancers. We have examined Ubx as a possible direct activator of srp, but find no evidence that Ubx plays a role in establishing the dorsal cell cluster or the secondary ventral cell clusters. Although Ubx-mutant embryos displayed morphologically altered fat bodies, Ubx does not appear to directly control srp's activation or repression, rather that Ubx appears to function in maintaining the segment-specific characteristics of fat-cell clusters. In the dorsal–ventral axis, we find that the dorsal cell cluster and secondary ventral cell clusters are sensitive to dorsal–ventral patterning cues. The persistent expression of tin in the dorsal region of the mesoderm subdivides the mesoderm. The secondary ventral cell clusters are located in the ventral mesoderm in PS 3–11, and thus lie outside this tin domain. The loss of tin activity has no effect on the specification of these cells (data not shown); however, the specification of these cells can be repressed by the expansion of the dorsal mesoderm into the ventral region by the mis-expression of dpp through the mesoderm (data not shown).

The dorsal cell cluster lies within the dorsal mesoderm. Loss of the dorsal mesoderm (through the loss of tin or dpp expression) results in the loss of the dorsal cell cluster; conversely, extension of the dorsal mesoderm results in the expansion of the dorsal cell cluster. tin clearly plays a genetic role in the specification of the dorsal cell cluster. At this time, we cannot distinguish between a direct or indirect role of tin in this process. TIN might be a direct transcriptional activator of srp or TIN might indirectly activate srp.
through downstream factors intrinsic to the dorsal mesoderm. It is likely, however, that the activation of srp in the dorsal cell cluster will require both TIN and other dorsal mesoderm-intrinsic factors. We find that within the A7.1EB sequence there are TIN-binding sites at positions 53–36, 200–181 and 257–274 that contain single base-pair differences from the consensus TIN binding site 5'(TCAAGTGQ)3' (Gajewski et al., 1997). The presence of these sites raises the possibility that specification, at least on the dorsal–ventral axis, requires TIN for activating srp and specifying the dorsal mesoderm.

Our analysis of srp regulatory regions reveals that different enhancers control srp expression in specific precursor-cell clusters, and that these precursor cells give rise to fat cells that populate specific regions of the fat body. Additional factors intrinsic to the region of the mesoderm from which the precursors arise are likely to help define the overall morphology of the fat body. It has not escaped our attention that our observations also provide a model for the origin of functionally distinct regions in the fat body. The fat body is a diverse and extremely complex tissue which is involved in many metabolic processes; it is not a functionally homogeneous tissue and some of its functions are restricted to cells lying within specific regions of this tissue (reviewed in Haunerland & Shirik, 1995). These so-called functional domains have mainly been characterized within the orders diptera and lepidoptera. For several Drosophila species, eye pigment biosynthesis is compartmentalized within the fat body. The tryptophan pyrrolase metabolite kynurenic acid is localized in the anterior region of the larval fat body, whereas the pteridine-precursor isoxanthopterin is restricted to the posterior regions (Rizki, 1961). Additionally, in Drosophila melanogaster, cells in the posterior larval fat body store more protein granules than do the cells of the anterior fat body (Tysell & Butterworth, 1978). In the flesh fly (Sarcophaga peregrina), anterior fat body protein persists in the mesoectoderm and in the mesoderm until early stage 10 and late stage 11, respectively. In Drosophila, twi-driven expression is first detected in the mesoderm and in the mesoectoderm at gastrulation. Uniform expression persists in the mesoectoderm and in the mesoderm until early stage 11, respectively. twi-driven expression also persists in a subset of muscle-cell progenitors until at least stage 12 (Baylies & Bate, 1996).

**Experimental procedures**

Genomic walk
A cDNA clone of srp (a generous gift from T. Abel) was used to screen an EMBL-3 SP6/T7 Drosophila melanogaster genomic library (Clontech) using standard molecular procedures (Maniatis et al., 1982). The λ clones were restriction mapped and aligned. Our map is in agreement with the published map of Rehorn and co-workers (Rehorn et al., 1996).

Drosophila stocks
The transgenic lines carrying reporter gene constructs of A7.1EB or A7.1ES were generated by standard microinjection techniques using pCSpSR-his43-lacZ, a vector specifically designed to test for enhancer and tissue-specific regulatory elements in transgenic flies (Thummel & Pirrotta, 1992). A7.1EB is a 1.2 kb EcoRI–SalI fragment and A7.1EB is an EcoRI–BamHI 0.47 kb subclone of A7.1ES. The Ubxpp1792 mutant has a 1587 bp deletion that removes the final 1.4 kb of the 50 kb intron, the splice acceptor site of the 3’ exon, and 48 codons of the homeobox, and has a sequence of six nucleotides inserted between the deletion breakpoints (Weinzierl et al., 1987). The twi–mutant (generously provided by M. Frasch) has a 103 bp deletion of the transcription unit (Azpiazu & Frasch, 1993). dppppp is a loss of function allele (Andrew et al., 1997; Held & Heup, 1996; Mason et al., 1997). Ubx and dpp were ectopically expressed using the GAL4-UAS system derived from yeast (Brand & Perrimon, 1993). The stocks used in ectopic expression experiments were: twi-GAL4 (Baylies & Bate, 1996; Greig & Akam, 1993), UAS-Ubx (Akam, 1996), and UAS-dpp (Staehling-Hampton & Hoffmann, 1994). These three lines were provided by the Bloomington Stock Center. The twi-GAL4 driver contains the entire regulatory region of the twi promoter and directs ectopic expression in a pattern similar to that of the endogenous twi gene. twi-driven expression is first detected in the mesoderm and in the mesoectoderm at gastrulation. Uniform expression persists in the mesoectoderm and in the mesoderm until early stage 10 and late stage 11, respectively. Ectopic expression also occurs in a subset of muscle-cell progenitors until at least stage 12 (Baylies & Bate, 1996).

**Immunohistochemical staining and in situ hybridization to whole-mount embryos**

The generation of SPP antibody and immunohistochemistry to whole-mount embryos has been previously described (Hoshizaki et al., 1994; Sam et al., 1996). Goat anti-rabbit IgG alkaline phosphatase conjugate, anti-β-galactosidase monoclonal antibody, and goat anti-mouse IgG alkaline phosphatase conjugate were obtained from Promega. The anti-enlarged monoclonal antibody developed by C. Goodman was obtained from the Developmental Studies Hybridoma Bank (University of Iowa, Department of
Biological Sciences, Iowa City, IA 52242) (Patel et al., 1989). Double labelling with anti-ß-galactosidase and Adh-anti-sense RNA was carried out as described by Lloyd and Sankonju (Lloyd & Sankonju, 1991). Adh-anti-sense RNA was prepared from a 1.3 kb region of Adh-coding sequence subcloned from P13E-3 (Herberlein et al., 1985) and inserted into pGEM1 at the SatI-BamHI sites (kindly provided by K. Hales). The RNA probe was synthesized using a digoxigenin-labelled uracil triphosphate as described by the manufacturer (Boehringer Mannheim). Chromogenic substrates were X-phosphate and NBT (Boehringer Mannheim).

Microscopy

Embryos were equilibrated in mounting solution (50% glycerol, 150 mM NaCl, 10 mM Tris-HCl pH 8.0) and inserted into pGEM1 at the SalI–Bam HI sites. Microscopic analyses were performed on a Zeiss Axioplan2 microscope using Nomarski optics. Whole-mount embryos were photographed onto Kodak tungsten slide film with a 35 mm camera attached to the microscope and digitized by scanning at 600 dpi using a U-max S-12 scanner. Digital images of whole-mount embryos were taken with a Kodak MDS120 digital camera attached to the microscope. Both types of images were assembled into figures using CorelDraw8 software.

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References


Fat-cell enhancer regions and fat body domains


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