Development 125, 713-723 (1998)
Printed in Great Britain © The Company of Biologists Limited 1998
DEV7623

The genetic control of the distinction between fat body and gonadal mesoderm in *Drosophila*

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Accepted 26 November 1997: published on WWW 22 January 1998

SUMMARY

The somatic muscles, the heart, the fat body, the somatic part of the gonad and most of the visceral muscles are derived from a series of segmentally repeated primordia in the *Drosophila* mesoderm. This work describes the early development of the fat body and its relationship to the gonadal mesoderm, as well as the genetic control of the development of these tissues.

Segmentation and dorsoventral patterning genes define three regions in each parasegment in which fat body precursors can develop. Fat body progenitors in these regions are specified by different genetic pathways. Two regions require *engrailed* and *hedgehog* for their development while the third is controlled by *wingless*. *decapentaplegic* and one or more unknown genes determine the dorsoventral extent of these regions. In each of parasegments 10-12 one of these regions generates somatic gonadal precursors instead of fat body. The balance between fat body and somatic gonadal fate in these serially homologous cell clusters is controlled by at least five genes. We suggest a model in which *tinman*, *engrailed* and *wingless* are necessary to permit somatic gonadal develoment, while *serpent* counteracts the effects of these genes and promotes fat body development. The homeotic gene *abdominalA* limits the region of *serpent* activity by interfering in a mutually repressive feed back loop between gonadal and fat body development.

Key words: *Drosophila*, Mesoderm, Fat body, Somatic gonads, *serpent*, *engrailed*, *wingless*, *decapentaplegic*, Fate map

INTRODUCTION

During the early stages of mesoderm differentiation the primordia of most mesodermal organs – visceral and somatic muscles, heart, fat body, gonads and mesodermal glia cells – become specified in defined, segmentally repeated positions of the *Drosophila* embryo (Fig.1). The allocation of the mesodermal cells to their different fates is controlled both by segmentation genes (Azpiazu et al., 1996; Riechmann et al., 1997) and by inductive signals from the dorsal ectoderm and the CNS midline (Staehling-Hampton et al., 1994; Frasch, 1995; Lüer et al., 1997). Along the anterior-posterior axis the mesoderm is divided into alternating domains by the function of *even-skipped* (*eve*) and *sloppy-paired* (*slp*). The progenitors of the visceral muscles, the fat body and the mesodermal glia cells arise from the *eve* domain, while the progenitors of the heart and most somatic muscles arise from the *slp* domain (Dunin Borkowski et al., 1995; Azpiazu et al., 1996; Riechmann et al., 1997). Besides *eve* and *slp* the segment polarity genes *engrailed* (*en*), *hedgehog* (*hh*) and *wingless* (*wg*) are involved in the spatial determination of the different mesodermal subpopulations (Bate and Rushton, 1993; Baylies et al., 1995; Lawrence et al., 1995; Wu et al., 1995; Azpiazu et al., 1996; Park et al., 1996; Ranganayakulu et al., 1996).

Along the dorsoventral axis inductive signals mediate the differentiation of the mesoderm (Staehling-Hampton et al., 1994; Frasch, 1995; Lüer et al., 1997). Dpp signalling from the ectoderm controls the specification of the dorsal mesodermal primordia (Staehling-Hampton et al., 1994; Frasch, 1995) in part via the regulation of *tinman* (*tin*), which is essential for the development of the visceral muscles, the heart and dorsal somatic muscles. *tin* is initially expressed in all mesodermal cells but its expression is only maintained in dorsal mesodermal cells receiving the Dpp signal (Frasch, 1995). Within the *eve* domain *tin* then activates expression of *bagpipe* (*bap*) which in turn is required for the formation of the visceral muscles (Azpiazu and Frasch, 1993). This activation is blocked in the *slp* domain and *tin*-expressing cells in this domain acquire cardiac and somatic muscle fates (Park et al., 1996; Riechmann et al., 1997). In the most ventrally located cells of the mesoderm, induction by CNS midline cells via the DER signaling pathway leads to the differentiation of mesodermal glia cells (Lüer et al., 1997).

Whereas the early subdivision of the mesoderm is now fairly well understood and the knowledge of gene activities responsible for the subdivision is sufficient to explain the specification of the visceral mesoderm, this is not the case for all of the other mesodermal organs. In particular, the specification of the primordia of the fat body is poorly understood, and that of the somatic part of the gonad is only partly explained. Rizki and Rizki (1978) suggested that the fat body has a segmental origin whose organisation is controlled by genes within the Bithorax

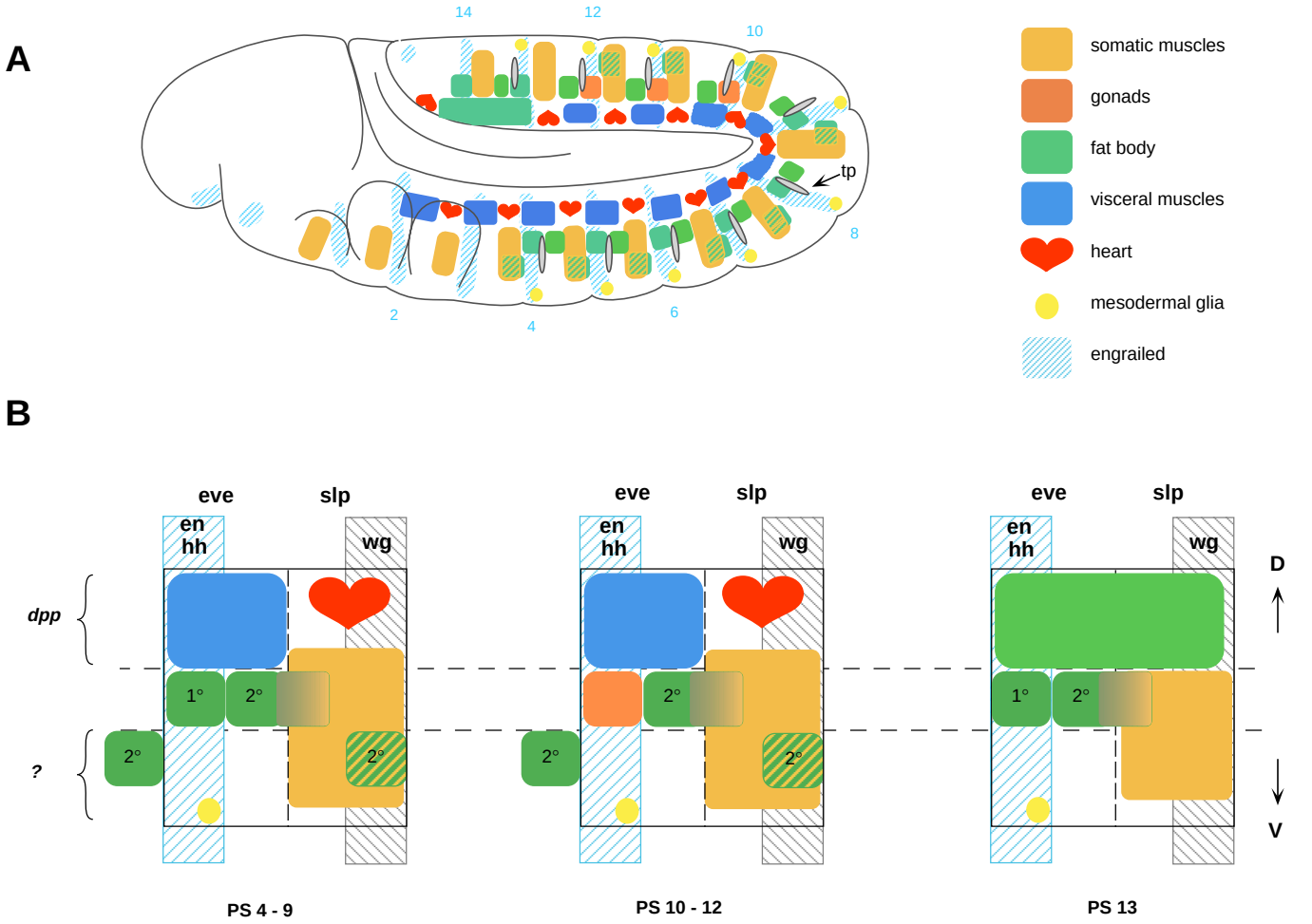

Fig. 1. A fate map of the trunk mesoderm. (A) Schematic fate map of the mesodermal cell layer, also showing the prospective ectodermal parasegment boundaries, tracheal pits (tp) and the outlines of the future head segments. *en* stripes at the anterior border of each even PS are numbered. The postulated map is based partly on early gene expression patterns (e.g. *bap* for the midgut visceral mesoderm) and partly extrapolated back from later gene expression patterns (*eve* for the heart, high levels of *twist* for the somatic muscles, *srp* for the fat body, 412 for the somatic gonads) taking into account the cell and tissue movements observed during mesoderm development. The SGP are placed ventral to the visceral mesoderm, as judged by their location relative to *bap* expression (Boyle et al. 1997). The fat body primordia are in the same relative dorsoventral position, firstly because they appear to be in serial homology to the SGP, and secondly because they lie outside the domain of *dpp* activity that directs visceral mesoderm development (this work). Positions of the mesodermal glia cells according to Dunin Borkowski et al. (1995) and Lüer et al. (1997). (B) Fate maps of three representative segments including genes that regulate the subdivision of the mesoderm. The posterior border of the secondary (2°) dorsolateral fat body cluster is shown as a gradient because we do not know whether this primordium is completely included in the *eve* domain or extends into the *slp* domain. The ventral secondary fat body cluster is shown as overlapping with part of the somatic mesoderm. We do not know whether fat body and somatic mesodermal cells in this region are initially intermingled and later sort out, or whether they arise from distinct regions within the *wg* domain.

complex. With the help of molecular markers it was confirmed that the fat body develops from repeated cell clusters, which later expand and coalesce to form the mature fat body (Abel et al., 1993; Hoshizaki et al., 1994).

The first sign of fat body development is the expression of *serpent* (*srp*) in small segmental cell clusters at stage 10. *srp* is not only the earliest marker for the fat body, but is also essential for its differentiation (Rehorn et al., 1996; Sam et al., 1996). These early *srp*-expressing clusters are located in the *eve* domain ventrally to the *bap*-expressing primordium of the midgut visceral mesoderm (Azpiazu et al., 1996; Riechmann et al., 1997). Like the visceral mesoderm primordium, the development of this part of the fat body requires the activity of *eve*, *en* and *hh* (Azpiazu et al., 1996). However, unlike the midgut visceral mesoderm, which is restricted in its extent by *slp* expression (Riechmann et al., 1997), the fat body primordium is limited by *wg* expression (Azpiazu et al., 1996). Here we show that the dorsoventral border between these two primordia is set by ectodermal *dpp* expression. Furthermore, we now find that the clusters of fat body prescursors that develop under the control of *eve* and arise from the *eve* domain do not make up the whole fat body primordium. We show that additional clusters of fat body cells appear to arise from the *slp* domain, and depend on *slp* and *wg* rather than *eve*, *en* and *hh* for their development. Surprisingly, the fat body is therefore made up from primordia determined by completely different genetic pathways that have opposing effects on the development of other mesodermal organs.

The somatic gonadal precursor cells (SGP cells) arise within parasegment (PS)10-12, in a region just ventral to the *bap*-expressing visceral mesoderm (Boyle et al., 1997). These cells can be visualized by the expression of the 412 retrotransposon and *clift* (Brookman et al., 1992; Boyle et al., 1997). Whereas *clift* is necessary for maintainance of gonadal cell fate (Boyle et al., 1997), 412 has no known function. In this study, we use 412 as a marker for somatic gonadal cells. At stage 11, 412 is expressed in PS2-14 in a region extending posteriorly from the ectodermal *wg* stripe to the tracheal pit (Boyle et al., 1997). During germband retraction 412 expression is maintained in these regions only in PS10-12 in the SGP cells (Brookman et al., 1992). We find that the position of the SGPs within PS10-12 corresponds exactly to the position of the early fat body clusters below the ectodermal *en* stripes in PS4-9. Thus, three findings connect the fat body precursors with the SGP cells: their identical relative positions within the PS, shared early expression of 412 RNA, and the fact that the development of the early fat body clusters is suppressed in regions of PS10-12 where the SGP cells originate. We suggest that both cell types belong to a mesodermal subpopulation which initially has the potential to take on either cell fate. Which of the two fates they will take on depends on their position along the anterior-posterior axis. In other words, the early fat body clusters and the SGP cells constitute serially homologous groups of cells.

We propose a model to explain how the genes *tin*, *en*, *wg* and *abdA* balance the activities of the fat body determining gene *srp* and a postulated SGP-competence factor resulting in the differential specification of fat body and SGP clusters along the anterior-posterior axis of the embryo.

MATERIALS AND METHODS

Fly stocks

We used the following mutants for this study: eve^{R13}, hh^{IJ35}, srp^{9L}, wg^{IIID23} (all from the Tübingen stock collection), en^{4} (from the Bloomington stock colletion), $abdA^{MX1}$ (Sánchez-Herrero et al., 1985), tin^{346} (Azpiazu and Frasch, 1993).

Antibody stainings and in situ hybridisation of embryos

The following primary antibodies were used: monoclonal mouse-anti En (4D9, provided by C. Klämbt; Patel et al., 1989), monoclonal anti-FasIII (provided by R. Smith; Brower et al., 1980), rabbit anti-Srp (provided by M. Brennan), rabbit anti-Twist (provided by S. Roth; Roth et al., 1989).

Embryos were fixed and stained following standard protocols. Double-labelling was performed as described by Riechmann et al. (1997).

mRNA was detected in situ as described by Tautz and Pfeifle (1989).

Microscopy

Embryos were mounted individually in Araldite. Pictures were taken with a ProgRes 3008 digital camera (Kontron Elektronik) on a Zeiss Axioplan. Figures were assembled in Adobe Photoshop.

UAS/GAL4 strains

For overexpression in the mesoderm we used *twi-GAL4* (*twi-GAL4* insertion on the second chromosome which was kindly provided by B. Giebel). Overexpression in the ectoderm of the *Krüppel* domain was achieved with the driver line *ZKrGAL8* (Frasch, 1995). We used the lines *UASen* (Guillén et al., 1995) for *en* and *UASdpp* (Staehling-Hampton and Hoffmann, 1994) for *dpp* overexpression.

RESULTS

The embryonic development of the fat body

The development of the fat body can be divided into three stages. The first sign of fat body development is the expression of *srp* in segmentally repeated clusters within the trunk mesoderm in PS4-9 at stage 10 (Abel et al., 1993; Rehorn et al., 1996; Sam et al., 1996). (We would like to point out that we use parasegmental nomenclature with reference to landmarks in the ectoderm. When we speak of ‘the mesoderm in PS X’ we mean the mesoderm underlying that particular parasegment at that stage. This may include mesodermal cells originating outside that region, and not corresponding genetically to the parasegment). These fat body clusters, which we will call the primary clusters, lie in a dorsolateral position directly underneath the ectodermal *en* stripes anterior to the center of the tracheal placode (see Fig. 3U,W in Riechmann et al., 1997). The locations of these primary clusters relative to the midgut visceral mesoderm primordium were determined by

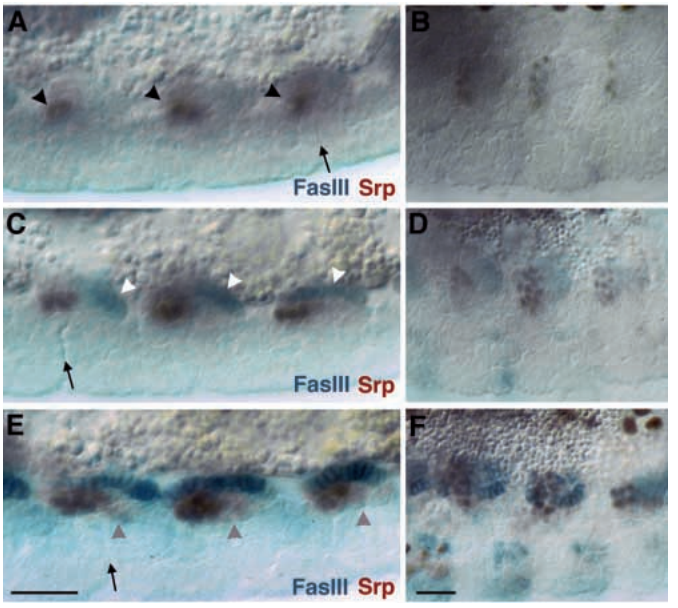

Fig. 2. Development of the fat body primordium relative to the midgut visceral mesoderm primordium.Three successively older embryos show the development of the dorsolateral fat body (brown) relative to the visceral mesoderm (blue). (A,C,E) Optical sections through mesoderm and ectoderm at the level of the tracheal pits (arrows) in PS4-6. (B,D,F) Dorsolateral surface views of the same embryos. (A,B) Stage 10. Mesodermal cells underneath the tracheal pits have invaginated and form bulges. Primary fat body clusters are located at the anterior margin of these bulges (black arrowheads). (C,D) Early stage 11. Fasciclin III- (FasIII) expressing visceral mesoderm becomes visible at the posterior margin of the mesodermal bulges (white arrowheads). (E,F) Late stage 11. Cells of the midgut visceral mesoderm spread anteriorly and posteriorly to form nearly a continuous row of cells. This well ordered file of cells lies internal to the fat body primordium which is recruiting posteriorly adjacent cells forming the dorsolateral secondary clusters (grey arrowheads). Scale bars = 20 μm.

staining for *Fasciclin III*. *Fasciclin III*-expressing clusters of cells are located posterior to the center of the tracheal pit and directly adjacent to the fat body progenitors in a more dorsal and more posterior position within the *eve* domain (Figs 2C-F, 3H,I). At this time, fat body and midgut visceral mesoderm form bulges overlying the invaginated tracheal pits, while the somatic mesoderm which expresses high levels of *twist* adheres to the ectoderm between the tracheal invaginations. The cells of the midgut visceral mesoderm then spread anteriorly and posteriorly to form a continuous band internal to the fat body progenitors, which are still present as separate clusters (Fig. 2E).

The appearance of fat body clusters varies along the anterior-posterior axis. In the first stage of fat body development there is no *srp* expression in PS10-12 (Fig. 3A,B). However, a large cluster is already present between *en* stripes 13 and 14. This cluster, which had not been described previously, differs from the other clusters in its size and its dorsoventral extent (Fig. 3A,B). In contrast to the anterior clusters it occupies the whole length of the PS and extends to the dorsal margin of the mesoderm (Fig. 3H). Heart and visceral mesoderm precursors, derived from the dorsal mesoderm in PS2-12 (Azpiazu and Frasch, 1993; Lawrence et al., 1995), do not appear in PS13. Thus, PS13 is unique, with fat body progenitors taking the place in the dorsal mesoderm normally occupied by heart and midgut visceral mesoderm precursors. There are also *srp*-expressing cells in what appear to be the primary clusters in these segments. However, since they are directly adjacent to the strongly stained large dorsal cluster, it is impossible to distinguish them unambiguously.

In the second stage of fat body development two secondary fat body clusters appear near each primary cluster. One secondary cluster lies directly posterior to the primary cluster (Fig. 3F). This cluster gradually increases in size by cell recruitment. The other secondary clusters arise ventrally and anterior to the primary clusters (Fig. 3F). They lie underneath the 'ventral prongs' of the somatic mesoderm which arise in the *slp* domain and move to their position underneath the *en* stripe by cell rearrangements (Dunin Borkowski et al., 1995). When these ventral clusters appear they are not connected to the dorsolateral fat body primordium and only after germband retraction do they start to fuse with the dorsolateral part (Fig. 5G) and both parts together form the lateral fat body. Simultaneously with the appearance of the secondary clusters in PS4-9, fat body progenitors now arise in PS10-12. Although no primary clusters exist in PS10-12, the secondary clusters arise in the same positions as described for PS4-9 (Fig. 3E).

At the onset of germband shortening the third stage of fat body development starts when mitoses become visible within the fat body primordium (Fig. 3G). These cell divisions are part of the previously described fourth wave of mesodermal cell divisions (Bate, 1993). This further growth of the fat body primordium leads to the loss of its segmental appearance and results in a continuous third mesodermal layer which lies between the midgut visceral mesoderm and the somatic mesoderm.

During later stages of embryogenesis cellular

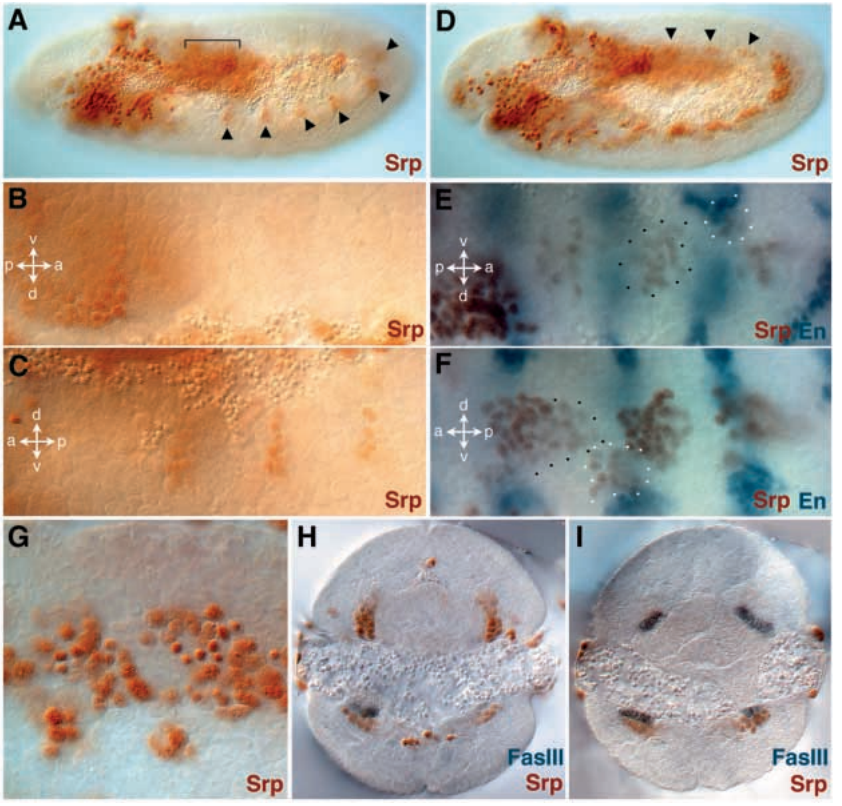

Fig. 3. Development of the fat body. Embryos were stained as indicated. (A-C) Stage 10. (A) Srp protein is detected in the primary fat body clusters in PS4-9 (black arrowheads) and in PS13-14 (bracket). *srp* is also expressed in the hemocytes on the ventral side of the head region and in the amnioserosa. (B,C) Same embryo as in A in dorsolateral views at higher magnification. Anterior-posterior and dorsal-ventral orientations are marked. (B) PS10-14. No Srp protein is detected in PS10-12, whereas a large cluster of fat body precursors is present in PS13-14. (C) PS3-6. Primary fat body clusters are present in PS4-6. (D-F) Late stage 11. The dorsolateral fat body primordium expands by recruitment of neighbouring cells. Fat body progenitors are also detected in PS10-12 (arrowheads in D). (E,F) Dorsolateral views of PS10-12 and 3-6 stained with antibodies against Srp (brown) and En (blue) at higher magnification. (E) PS10-13. In PS10-12 secondary fat body clusters appear in the dorsolateral (black dots) and ventral (white dots) mesoderm. Note that there are no primary fat body clusters underneath the *en* stripes. (F) PS3-6. In the dorsolateral mesoderm secondary fat body clusters arise immediately adjacent to the primary clusters (black dots). In the ventral mesoderm secondary fat body clusters appear anteriorly to the ectodermal *en* stripe (white dots). The most anteriorly located ventral cluster arises from PS3. (G) High magnification of a stage 12 embryo. Nuclear Srp staining reveals fat body cells in various stages of cell divisions. (H) Cross section of a stage 11 embryo. The lower half of the embryo is sectioned in the region of PS4-9 showing the dorsolateral position of the fat body progenitors (brown) and the midgut visceral mesoderm (blue) lying more internal at the dorsal margin of the mesoderm. In the upper half the embryo is sectioned through PS13. Here the fat body primordium extends to the dorsal margin of the mesoderm. (I) Same embryo as in H, upper half sectioned through PS10-12 where no fat body progenitors can be detected at this stage.

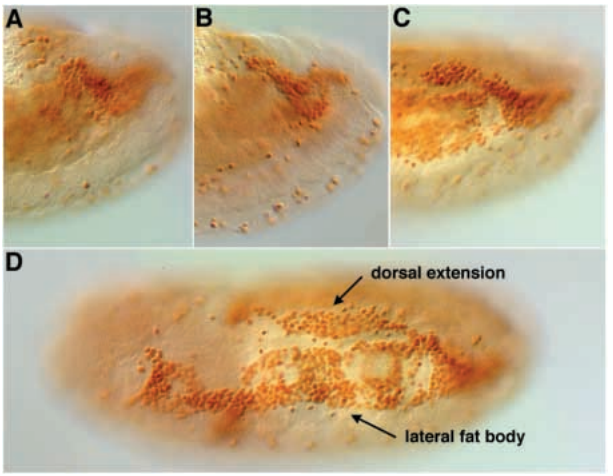

Fig. 4. Development of the dorsal fat body extension. Successively older Srp-stained embryos showing the formation of the dorsal extension of the fat body. (A) At stage 13, after germ band retraction, fat body progenitors from PS10-14 form a dorsally projecting protusion. (B,C) Fat body progenitors in this protusion migrate anteriorly. (D) Ventrolateral view of a stage 16 embryo showing the dorsal extension positioned between the dorsal midline and the lateral fat body.

rearrangements take place in the fat body primordium. The secondary ventral clusters in PS3-5 form the ventral commissure (anterior plate in Campos-Ortega and Hartenstein, 1997) that connects the two lateral halves of the fat body in the cleft between gut and CNS (not shown; Rizki, 1978; Campos-Ortega and Hartenstein, 1997). Cells in the posterior part of the fat body including those from the large cluster in PS13 form a protrusion at the dorsal margin of the fat body (Fig. 4A). Anterior migration of the cells in this protrusion results in the dorsal extension of the fat body that runs between the lateral fat body and the heart and extends to the head of the embryo (Fig. 4B-D).

en function during fat body development

The spatial overlap between the *en* stripes and primary fat body clusters suggests that the specification of the fat body might be directly regulated by *en*. Surprisingly, in embryos mutant for *en* the fat body is only slightly reduced (not shown). The same is true for embryos mutant for *hh*, which codes for a secreted protein expressed in the same cells as *en* (not shown). However, in *en hh* double mutants only a small number of fat body cells develop (Azpiazu et al., 1996).

To analyze further the influence of *en* on fat body development we overexpressed *en* in the mesoderm (see Materials and Methods). Ubiquitous mesodermal *en* expression leads to an expansion of the primary clusters into the *slp* domain resulting in a continuous band of *srp*-expressing cells in PS4-9 (Fig. 5B). We observed the same phenotype in *slp naked* double mutants where derepression of *en* leads to *en* expression throughout the PS (Cadigan et al., 1994; Fig. 5C). The observed effect of *en* on fat body development is seen not only upon mesodermal overexpression but also when *en* is overexpressed ectopically in the ectoderm, for example in the *Krüppel* domain (Fig. 5E).

Concomitant with the expansion of the dorsolateral fat body we also observe an expansion of other primordia normally occupying the *eve* domain, e.g. the visceral mesoderm (not shown) and the somatic gonads (Fig. 5N,Q). By contrast, the primordia that normally occupy the *slp* domain, those of the somatic muscles and the heart, are abolished (not shown). Significantly, the ventral fat body clusters respond to *en* overexpression in the same way as the primordia of the *slp* domain, i.e. their development is suppressed (Fig. 5F). Thus, *en* promotes the development of dorsolateral fat body, midgut visceral mesoderm and somatic gonads while it suppresses development of somatic muscles, heart and ventral fat body.

wg function during fat body development

The finding that *en* overexpression leads to an expansion of the dorsolateral fat body while it suppresses the ventral fat body shows that the two parts of the fat body primordium are specified by different mechanisms. The difference in determination of the two fat body primordia can be seen most clearly in *wg* mutants. Loss of *wg* leads to an expansion of the dorsolateral fat body primordium similar to the situation after *en* overexpression (Fig. 5D and Azpiazu et al., 1996). However, while *en* overexpression does not result in ectopic *srp* expression in PS10-12, the loss of *wg* does (Fig. 5B,D). While all dorsolateral mesodermal cells are specified as fat body progenitors in *wg* mutant embryos, the specification of the ventral secondary fat body clusters is abolished (Fig. 5H). Thus, the dorsolateral fat body is repressed by *wg* and the ventral fat body needs *wg* for its specification.

The findings that the ventral clusters are *wg* dependent, suppressed by *en* and that they underlie the ‘ventral prongs’ of high *twist* expression suggest that they arise not from the *eve* domain but from the anteriorly adjacent *slp* domain where *wg* is expressed (Fig. 1). Consistent with this we find that *eve* mutant embryos, which had been thought to have no fat body (Azpiazu et al., 1996; Riechmann et al., 1997), do not lack all of the fat body primordium but still have small groups of ventrally located *srp*-expressing cells (Fig. 5I). Thus, the cells of the ventral fat body clusters most likely arise in the *slp* domain under the control of *wg* and reach their final position at the border between the *eve* and the *slp* domain by reorganisation of cells in the ventral mesoderm. Taken together our results show that the fat body primordium consists of a dorsolateral and a ventral part and that these two parts are determined by different genetic pathways.

dpp determines the dorsal extent of the fat body in PS4-12

The dorsolateral fat body primordium lies in close proximity to the primordium of the midgut visceral mesoderm (Fig. 3H,I). Since the development of both primordia is activated by *en*, we asked which genes might be responsible for the distinction between these tissues. A good candidate is *dpp*, the expression of which in the dorsal ectoderm is essential for the specification of the midgut visceral mesoderm (Staehling-Hampton et al., 1994; Frasch, 1995). In contrast to the visceral mesoderm, the fat body is not abolished in *dpp* mutants (not shown). To assay further the influence of *dpp* on fat body development, we expressed *dpp* ectopically in the ectoderm of the *Krüppel* domain and found that fat body development was repressed (Fig. 5J). The positive effect of *dpp* on the visceral mesoderm and its repessive effect on fat body suggests that *dpp*

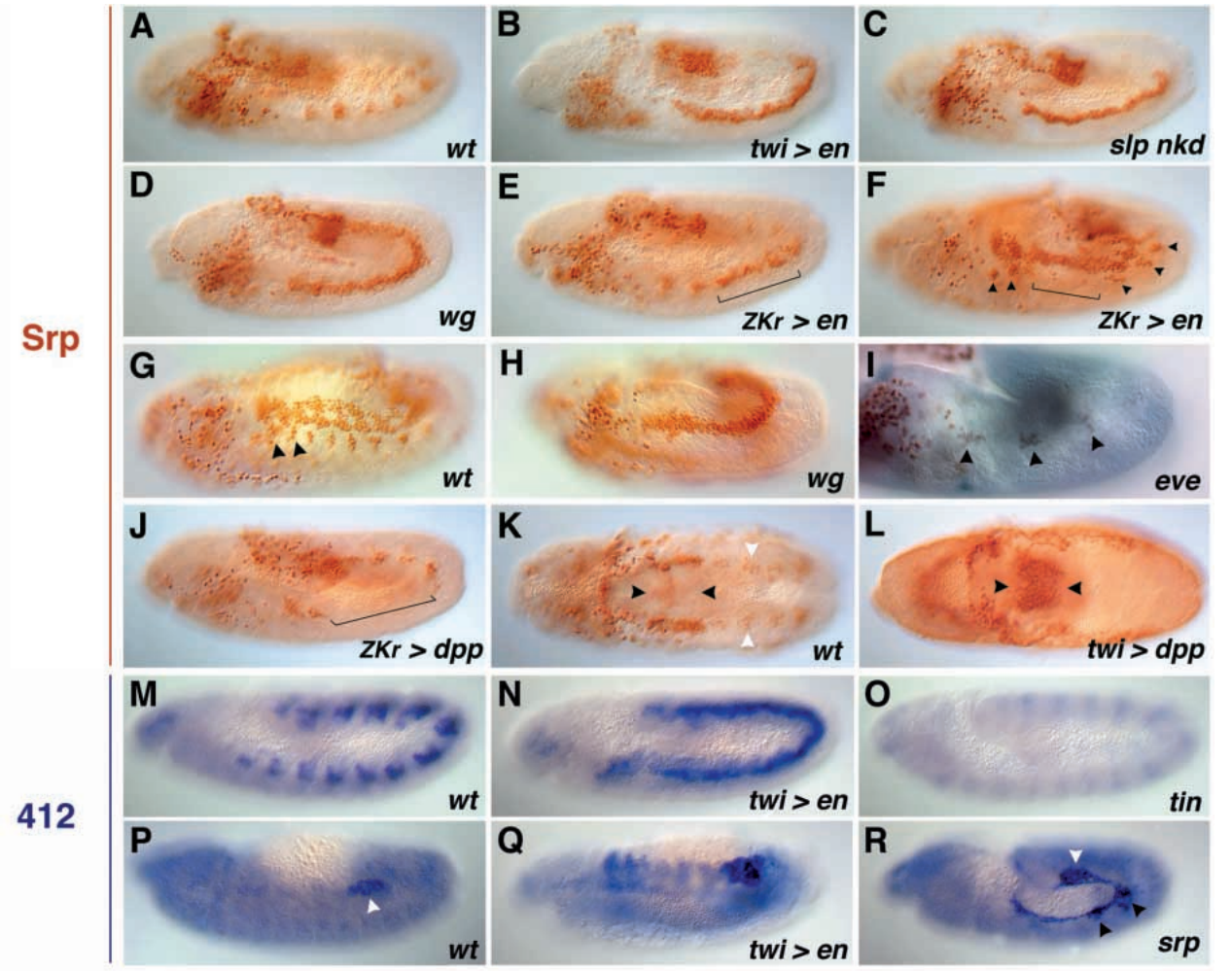

Fig. 5. Development of the fat body and somatic gonads in various mutants and after overexpression of *en* and *dpp*. (A-L) Embryos stained with an antibody against Srp. (A-D) Stage 10. (A) In wild-type embryos primary fat body clusters arise in PS4-9. (B) *en* overexpression in the mesoderm leads to an expansion of the primary clusters resulting in a continuous dorsolateral fat body primordium in PS4-9. (C) Derepression of *en* in *slp naked* double mutant also results in a continuous dorsolateral fat body primordium. Note the absence of fat body progenitors in PS10-12. (D) In *wg* mutant embryos *srp* is derepressed even in PS10-12 (see also Azpiazu et al., 1996). (E-F) Overexpression of *en* in the ectoderm of the *Krüppel* domain. (E) Stage 11. The dorsolateral fat body primordium in PS6-8 is expanded (bracket) (F) Stage 12. The secondary ventral fat body clusters are missing in PS6-8 (bracket) while they are clearly visible in the other PS (arrowheads). (G-I) Stage 12. (G) Wild-type embryo showing the secondary ventral clusters ventrally to the dorsolateral fat body primordium. The ventral clusters from PS4-5 (arrowheads) are already fused with the dorsolateral fat body. (H) No ventral fat body clusters develop in a *wg* mutant embryo. (I) *eve* mutant embryo at a higher magnification. Although the dorsolateral fat body is missing the ventral fat body clusters are still present (arrowhead) indicating their origin from the *slp* domain. As a consequence of the pair-rule phenotype the number of the ventral clusters is reduced. (J-L) Stage 11. (J) Overexpression of *dpp* in the ectoderm of the *Krüppel* domain (bracket) represses fat body development in PS6-8. (K) Dorsal view of a wild-type embryo. In PS13, fat body progenitors are only present in the dorsal mesoderm but not the in ventral region (ventral midline is marked by black arrowheads). Secondary ventral fat body clusters are visible in PS9-12 (white arrowheads) (L) Same view as K. Overexpression of *dpp* in the whole mesoderm represses fat body development in PS4-12 but promotes it in PS13 resulting in ectopic fat body precursors in the ventral mesoderm (arrowheads). These cells lie in the mesoderm above invaginated PMG. (M-R) Embryos hybridized with a 412 probe. (M-O) Stage 10. (M) In the wild type, 412 is expressed in stripes in PS2-14 (N) *en* overexpression in the mesoderm results in continuous 412 expression in PS2-14. (O) In *tin* mutants only faint expression is visible. (P-R) Stage 13. (P) In the wild type, 412 expression is maintained at high levels only in the SGP cells (white arrowhead). (Q) *en* overexpression in the mesoderm leads to an increase in SGP cells. (R) In *srp* mutants 412 expression is maintained in PS4-12 and ectopic SGP cells form lumps indicating abnormal gonad formation (black arrowheads). White arrowhead marks gonad formation in PS10-12. *srp* mutant embryos do not retract their germ band.

delimits the dorsal extent of the fat body primordium and sets the border between visceral mesoderm and fat body.

We also expressed *dpp* ectopically in the mesoderm, using a *twist*-GAL4 driver. Since this driver line drives expression throughout the whole mesoderm we could assess the effect of *dpp* overexpression along the whole anterior-posterior length of the mesoderm. As with ectodermal *dpp* expression, this resulted in repression of fat body development, in this case seen in PS4-12. Surprisingly, in PS13, we found additional, ectopic fat body cells (Fig. 5L). Thus, Dpp represses fat body

development in the dorsal mesoderm of PS4-12 and promotes fat body development in PS13. The different response of the fat body primordium to Dpp signalling in PS13 helps to explain why in this PS fat body progenitors develop instead of heart and midgut visceral mesoderm precursors. The difference is due to the activity of *AbdB* since in embryos lacking *AbdB* the dorsal mesoderm of PS13 does not develop into fat body precursors and heart cells and visceral mesoderm develop instead (not shown).

Control of the distinction between fat body and gonadal mesodermal cell fates

The mesodermal part of the gonads, the SGP cells, arise in PS10-12 and can be identified by the expression of the retrotransposon 412 (Brookman et al., 1992). 412 is expressed in stripes in PS2-14 at stage 11 in the dorsolateral mesoderm extending posteriorly from the ectodermal *wg* stripe to the tracheal pit (Boyle et al., 1997). This is the position where the primary fat body clusters arise in PS4-9. Thus, at stage 11, 412 is expressed in the SGP cells as well as in the primary fat body clusters. This shared 412 expression, the identical relative position of primary fat body clusters and SGP cells within the PS and the suppression of the primary fat body clusters in the region where the SGP cells arise in PS10-12 indicate that primary fat body clusters and SGP cells represent serially homologous primordia.

An obvious candidate gene responsible for the diversification of the initially equivalent cell types is the homeotic gene *abdA*. *abdA* defines the identity of the PS from which the SGP cells derive and is known to be required for somatic gonadal cell specification (Brookman et al., 1992; Cumberledge et al., 1992; Warrior, 1994; Boyle and DiNardo, 1995; Greig and Akam, 1995). We found that *abdA* is also necessary for repression of fat body development in the region where the SGP cells arise. In *abdA* mutant embryos *srp*-expressing clusters appear during the first stage of fat body development in the position just where SGP cells normally arise (Fig. 6B,D). The function of *abdA* in providing positional information for fat body suppression is further illustrated by the finding that ubiquitous mesodermal *en* expression is not able to specify additional fat body progenitors in PS10-12 (Fig. 5B).

abdA alone is not sufficient to control the decision between fat body and SGP development since transformation of SGP cells into fat body also occurs in other mutants. Boyle et al. (1997) found few or no SGP cells expressing the gonadal mesoderm marker *clift* in *tin* mutants. We found that the loss of gonadal mesodermal fate in *tin* mutant embryos is accompanied by a gain of fat body precursors in PS10-12 at the position where the SGP cells normally develop (Fig. 6C). Thus, the loss of *tin* function leads to the transformation of SGP cells into fat body progenitors. Since these effects are seen in a region outside the late, *dpp* dependent *tin* expression domain, they must be due to the earlier, ubiquitous mesodermal *tin* expression. This is further supported by the finding that while late dorsal *tin* expression depends on *dpp*, SGP development does not (not shown and Broihier et al., 1998). The view of an early function of *tin* is strengthened by the finding that early 412 expression seen in all PS is abolished in *tin* mutants (Fig. 5O). Thus, *tin* confers competence upon mesodermal cells to develop as SGP. There appears to be a similar, if redundant, role for *tin* function in setting up fat body competence, revealed in embryos in which the function of the gene *zfh-1* is removed in addition to *tin* (Broihier et al., 1998).

tin has additional functions for fat body development in PS3 and PS13. In *tin* mutants the large fat body cluster in the dorsal region of PS13 is lost and fat body cells can be detected only in the dorsolateral mesoderm (Fig. 6C). An additional fat body cluster appears late in PS3 (not shown). Thus, *tin* is involved in the regionalisation of the dorsolateral mesoderm by the homeotic genes.

In *wg* mutant embryos all dorsolateral mesodermal cells, including those in PS10-12, acquire fat body fate (Fig. 5D). This phenotype can be interpreted as the combined effects of two separate functions of *wg*. Firstly, *wg* is necessary to repress fat body development in the dorsolateral mesoderm underlying the *wg* domain in all PS. Second, *wg* is required in the primary cluster to permit SGP instead of fat body development in PS10-12. The lack of SGP cells (Boyle et al., 1997) and the failure of gonad assembly (Warrior, 1994) in *wg* mutants is therefore due to the transformation of all dorsolateral cells in PS10-12 into fat body progenitors.

en is also required for SGP development. Loss of *en* results in early disappearence of 412 expression and absence of demonstrable SGP (Warrior, 1994 and our own data, not shown), similar to the situation in *tin* mutants. Ubiquitous mesodermal *en* expression leads to the formation of additional SGP cells in PS10-12 (Fig. 5Q). Thus *en* is necessary, and in the presence of *abdA* sufficient to induce SGP development in the dorsolateral mesoderm.

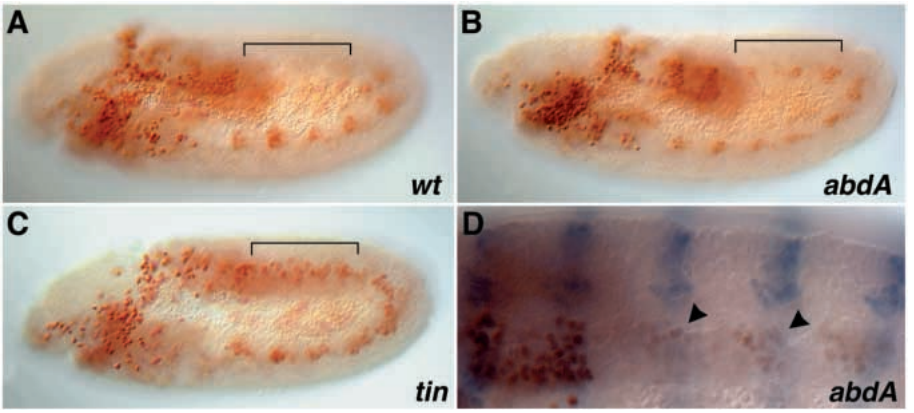

Fig. 6. Transformation of SGP cells into fat body precursors in *tin* and *abdA* mutants. (A-C) Srp protein in stage 10 embryos. (A) Wild type embryos showing the presence of primary fat body clusters in PS4-9 and absence of these clusters in PS10-12 (bracket) where SGP cells arise. Ectopic fat body precursors arise in PS10-12 (bracket) in *abdA* (B) and *tin* (C) mutants. (D) High magnification of stage 10 *abdA* mutant embryo in a dorsolateral view stained with antibodies against En (blue) and Srp (brown). Ectopic *srp*-expressing cells in *abdA* mutants are located underneath *en* stripes (arrowheads) indicating a transformation of SGP cells into primary fat body clusters.

Whereas somatic gonads are transformed into fat body in *tin*, *wg* and *abdA* mutants, the opposite transformation occurs in *srp* mutants. In *srp* mutant embryos the fat body fails to form (Rehorn et al., 1996; Sam et al., 1996) and the dorsolateral fat body is partly transformed into gonadal mesoderm (Fig. 5R). The loss of *srp* results in maintenance of 412 expression in PS4-9 indicating ectopic somatic gonadal differentiation. Thus, there is a balance between fat body and SGP develoment with *tin*, *wg* and *en* driving cells in the primary clusters towards SGP development and *srp* driving them towards fat body development. A model describing the interactions among these genes will be presented below.

DISCUSSION

Spatial organisation of the trunk mesoderm: the fate map for the fat body

The fat body primordium consists of different groups of segmentally repeated cell clusters which are distinguished by their locations within the segment, their time of appearance and their genetic control (Fig. 1).

In the first stage of fat body development the primary fat body clusters appear in the dorsolateral mesoderm underneath the *en* expression domain at the anterior margin of the PS. Like the primordium of the midgut visceral mesoderm this cluster belongs to the *eve* domain of the PS and lies in a bulge underneath the tracheal pits, moving towards the interior of the embryo together with the visceral mesoderm primordium (Dunin Borkowski et al., 1995). We find that visceral mesoderm and fat body then undergo different and independent further morphogenesis. While the cells of the midgut visceral mesoderm spread by cell rearrangements and form a continuous band of tissue covering the more externally located fat body primordium, the fat body primordium expands by recruiting neighbouring cells (second stage of fat body development).

Secondary clusters are recruited in the dorsolateral region as well as in the ventral mesoderm. Although ventral clusters of *srp*-expressing cells had been described previously they had not been recognized as a part of the fat body primordium (Rehorn et al., 1996; Sam et al., 1996). The ventral clusters are initially not connected to the dorsolateral fat body primordium but we found that they later fuse with it and become a part of the mature fat body. This finding is consistent with the demonstration by Lüer et al. (1997) that mesodermal cells adjacent to the ventral midline contribute to the fat body.

By the time the ventral clusters express Srp protein, they appear to lie underneath the *en* domain. However, a number of reasons lead us to believe that they originate in a position underlying the *wg* domain of the next anterior PS. Firstly, if these clusters are visualized at an earlier stage by in situ hybridisation with *srp* probes it is clear that they do not overlap with the primary clusters along the anterior-posterior axis, but are located anteriorly (not shown). Secondly, their regulation by segmentation genes such as *eve*, *slp*, *en* and *wg* shows that they are subject to the same control as the other mesodermal primordia in the *slp*/*wg* domain, rather than those in the *eve*/*en* domain. Finally, parts of the mesoderm are known to shift their positions relative to the overlying ectoderm. Specifically, a group of ventrally located somatic mesodermal cells expressing high levels of Twist, described as ‘ventral prongs’, arise in the *slp* domain but are later found underneath the ectodermal *en* stripe as a result of cellular rearrangements in the ventral mesoderm (Dunin Borkowski et al., 1995). The ventral secondary clusters of fat body precursors lie precisely underneath these ‘ventral prongs’ and these groups of cells therefore appear to move in concert.

The specification of the fat body primordium

Given the different locations of the clusters of fat body precursors along the anterior-posterior and dorsoventral axis it is not surprising that their specification is governed by different genes. We will discuss the specification of the different parts of the fat body primordium in turn.

Dorsolateral clusters

Like the other major mesodermal organ derived from the *eve* domain, the midgut visceral mesoderm, the specification of the dorsolateral fat body primordium depends on *en* and *hh* function. Although in single mutants these primordia are only slightly reduced, there is a severe reduction in *en hh* double mutants (Azpiazu et al., 1996). The border between these two expression domains is set by ectodermal Dpp signalling, which is essential for *bap* expression but represses *srp* expression (Staehling-Hampton et al., 1994; Frasch, 1995 and this work). Thus, in the wild type the *bap*-expressing midgut visceral mesoderm develops in the dorsal mesoderm, which receives positive inputs from *dpp*, *en* and *hh*, while the fat body arises in the dorsolateral mesoderm, which is influenced by *en* and *hh* but not reached by the Dpp signal.

The secondary dorsolateral clusters arise at the same dorsoventral level as the primary clusters and are therefore probably subject to the same dorsoventral control. Since there appears to be very little, if any, dorsolateral fat body in *en hh* mutants, these genes must be directly or indirectly responsible for the development of the secondary clusters as well. It is conceivable that Hh protein, which is expressed in the ectodermal cells above the primary clusters may reach the secondary clusters by diffusion. Alternatively, the secondary clusters might depend on other genes whose expression in turn depends on proper segmentation under control of *en* and *wg*.

Ventral secondary clusters

The secondary fat body clusters in the ventral mesoderm are specified by a different genetic pathway. This can be clearly seen in the opposite response of the ventral and dorsolateral fat body primordium to *wg* and *en* expression. While the dorsolateral fat body is repressed by *wg* and activated by *en*, the situation is reversed in the ventral fat body. This and the presence of the ventral fat body clusters in *eve* mutants and their absence in *slp* mutants (not shown) provides further support for our notion that the ventral fat body clusters are derived from the same anterior-posterior domain as heart and somatic mesoderm, the *slp* domain.

The different responses of the ventral and dorsolateral fat body primordia to *wg* and *en* are nevertheless surprising, since they reveal the existence of a dorsoventral border across which the effects of *en* and *wg* are switched, at least as far as control of *srp* expression is concerned. There must exist an unknown factor responsible for this switch which is either expressed only dorsally or only ventrally of the boundary. This factor would

be reponsible for setting the ventral boundary of fat body in the *eve* domain (by converting *en* from a fat body activator in the region dorsal of the boundary to a fat body repressor in the ventral part of the *eve* domain). Similarly, it would set the dorsal boundary for the fat body in the *wg* domain (Fig. 1B).

Dorsal cluster in PS13

The fat body progenitors in PS13 are subject to yet another set of regulatory inputs. In this PS fat body development is not repressed but activated by Dpp and as a result fat body is found in the dorsal mesoderm. The development of the visceral mesoderm and the heart which is induced by Dpp in the other PS is abolished in PS13. Thus, in this case it is the interpretation of the Dpp signal that is reversed across an anterior-posterior boundary, much like the interpretation of the Wg signal which is reversed below a certain dorsoventral boundary.

In summary, the fat body originates from two separate groups of primordia in PS4-12, one dorsolateral part that arises in the *eve* domain and is activated by *en* and *hh* and one ventral group that belongs to the *slp* domain and is activated by *wg*. An additional primordium in PS13 is specified by Dpp. Thus, the role of the segmentation and dorsoventral patterning genes is to set up areas in which certain groups of cells can take on specific fates, but interestingly, the same organ fate can be defined by different sets of patterning genes.

The distinction between fat body and somatic gonadal primordium: a model

The primary fat body clusters in PS4-9 and the SGP clusters in PS10-12 are serially homologous primordia as judged by their positions in the segment and the genetic circuitry that controls their development. The distinction between these two primordia is controlled by at least five genes. In *srp* mutants, the primary fat body clusters in PS4-9 are transformed into SGP cells. Thus *srp* promotes fat body development by suppressing SGP development. By contrast, in *wg*, *tin*, *en* and *abdA* mutant embryos the development of SGP cells is abolished and fat body precursors are specified instead (though not very efficiently in *en* mutants). We propose that three of these genes (*en*, *wg*, *tin*) are required to establish and maintain the competence of cells to develop as SGP, while *srp* has the capacity to block the SGP programme in these cells, and *abdA* limits the region where *srp* is active.

The roles of *wg*, *en* and *tin* appear puzzling at first sight. As there are no significant differences in the expression patterns of these genes between PS4-9 and PS10-12, it is surprising that they play a role in SGP development, but not in the development of fat body in the homologous patches in PS4-9.

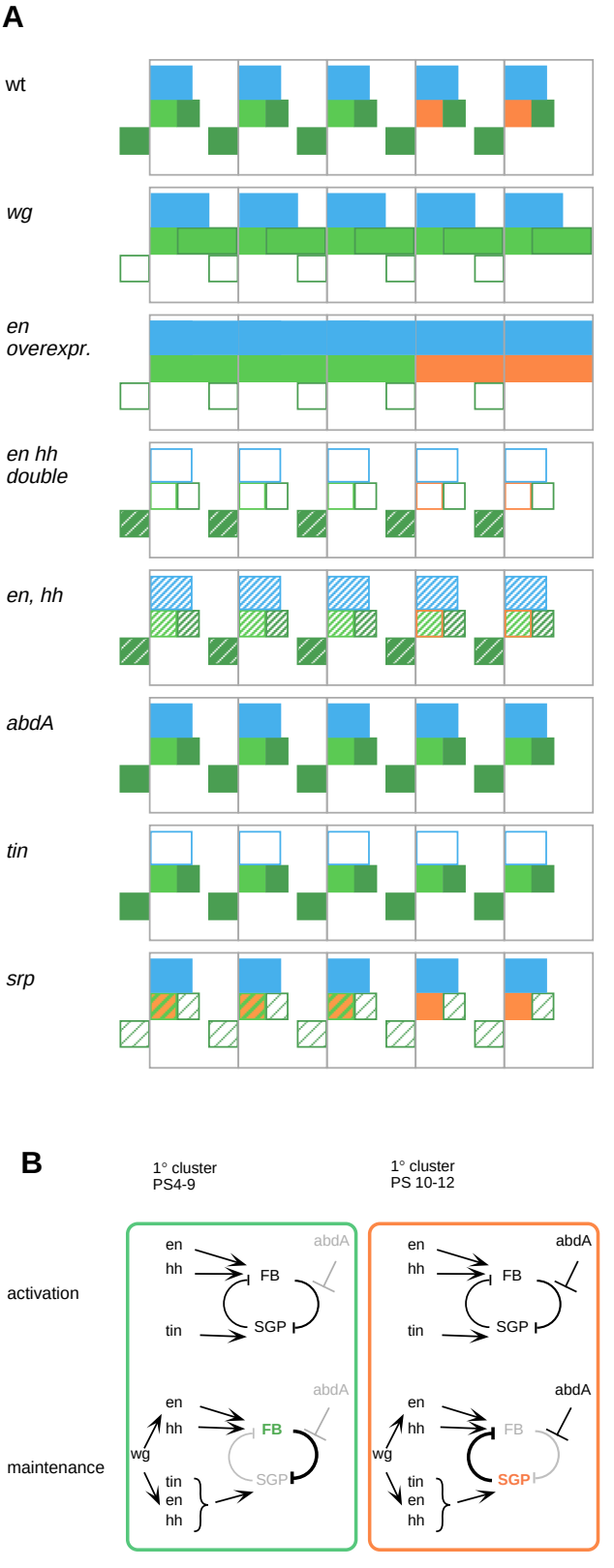

Fig. 7. A model for the distinction between fat body and somatic gonads. (A) Summary of the phenotypes of mutants discussed in this paper upon which the model in B is based. PS7-11 are diagrammed as fate maps for the fat body (green), visceral mesoderm (blue) and gonadal mesoderm (orange). For further details see Fig. 1. Filled boxes denote wild-type development of the primordium, hatched boxes reduction of the primordium, and empty boxes the failure of the primordium to develop. (B) Diagram of the interactions between genes regulating the development of the primary fat body clusters in PS4-9 and the SGP clusters in PS10-12. Inactive genes and interactions are shown in grey, active genes in black and the expression defining the final differentiated state in colour. FB = fat body gene expression (e.g. *srp*), SGP = somatic gonadal gene expression. The 'activation' state is not demonstrable in the embryo and is only shown for the purpose of explaining the model. An effect of *tin* on fat body development is discussed in the text but, for simplicity, left out of this diagram.

In addition, it is surprising that *wg* should have an effect on SGP development at all, since it is not expressed in the region in which the SGP develop, as has been discussed previously by Boyle et al. (1997). We propose a model to reconcile these and other findings (Fig. 7B).

Components of the model

1. The competence for mesodermal cells to develop as SGP is set up at an early stage in cells within the *eve* domain. This competence is expressed throughout PS4-12 as shown by the finding that removal of *srp* leads to SGP development in all primary clusters. Interestingly, gonadal mesodermal cell fate is not restricted to the posterior abdominal segments in all insects. For the Hemipteran *Pyrrhocoris apterus* and for the apterygote Thysanura segmental gonad primordia extending into more anterior PS were reported (cited in Greig and Akam, 1995). This further supports the idea that initially even in *Drosophila* more than three PS are competent to contribute to the gonads, but this competence is later not realized or is overridden by other inputs. SGP competence is set up by *tin* during the time of its early ubiquitous mesodermal expression.

2. The competence for SGP development is maintained in a subset of these cells in each PS by *en* and *hh*. (We will not discuss the genes responsible for narrowing the zone of competence along the dorsoventral axis). Unlike in the case of fat body development, *en* and *hh* are essential for this maintenance, and loss of either of their functions leads to loss of SGP development. *wg* has two functions. First it represses SGP competence in the *wg* expression domain in the same way as it represses fat body development in this domain. (An indication for this role of *wg* is an early expansion of the 412 expression pattern in *wg* mutants; not shown). Second, *wg* is required for the specification of SGP clusters underneath the *en* stripes. Thus *wg* appears to act in the SGP primordium itself, i.e. in the *en* domain. We propose that this function of *wg* is indirect, and is due only to the requirement of *wg* for the maintenance of *en* (DiNardo et al., 1988; Martinez-Arias et al., 1988). By stage 10 and before SGP differentiation is apparent, *en* expression is completely lost in *wg* mutants.

If either the initial establishment or the maintenance of SGP competence fails, cells that would otherwise develop as SGP develop as fat body instead. We conclude that the state of SGP competence must be expressed partly in the form of a factor that suppresses *srp* expression and thereby fat body development (called here ‘SGP competence factor’).

3. *srp* can repress SGP development. Thus, not only is the state of SGP competence correlated with a repression of fat body development, but also vice versa. This suggests that there must be a feed back loop between the fat body and SGP developmental pathways, because if cells in the primary cluster lose SGP competence they automatically express *srp* and develop as fat body, and conversely, if *srp* function is lost, primary clusters develop as SGP. The SGP ‘competence factor’ and *srp* appear to have mutually repressing effects.

4. In PS10-12 *abdA* is responsible for the loss of *srp* expression and the resulting SGP development in the primary clusters. This could be achieved by direct repression of *srp* transcription by *abdA*. However, in *wg* and *tin* mutants, *srp* is expressed in these groups of cells although *abdA* is still active. Thus it is unlikely that *abdA* represses *srp* directly. We propose instead that *abdA* blocks *srp* expression via the feed back loop between the postulated SGP competence factor and *srp*. If *abdA* prevents *srp* from inhibiting the competence factor, then this factor can in turn maintain *srp* repression.

5. Paradoxically, with the exception of *abdA*, the genes discussed here whose loss leads to transformation of SGP to fat body, are also required for fat body development. This common requirement is fully consistent with the apparent evolutionary relationship of the two primordia. However, the requirement for fat body is much less stringent, becoming fully apparent only when more than one gene function is abolished (e.g. *en* and *hh*, or *zfh-1* and *tin*). Thus there are lower thresholds of input for the fat body. The molecular basis for this difference in sensitivity is obscure. Perhaps an autoregulatory feed back loop ensures the maintenance of fat body fate once development along that pathway has been triggered.

Summary of the model

Under the control of *tin* a region of competence for SGP development is set up within the *eve* domain of each PS. *en* and *hh* are needed to maintain the competence, while they simultaneously activate *srp* expression. *wg* is required for the maintenance of *en*, and therefore indirectly for the maintenance of SGP competence. The state of SGP competence entails the repression of *srp* transcription, possibly via the expression of an ‘SGP competence factor’. *srp* in turn blocks the capacity for further SGP development. The pathways of SGP and fat body development, though dependent on the same upstream factors, mutually exclude each other by virtue of a feed back loop. This feed back loop is mediated directly or indirectly by the repressive effects of *srp* and the competence factor. *abdA* interferes with the repressive effect of *srp* on SGP development, and in those segments where *abdA* is expressed, the ‘SGP competence factor’ can therefore shut down *srp* expression, thus allowing further development of SGP. This also explains why *abdA* blocks *srp* only in the primary clusters, i.e. the region where SGP competence has been established, but not in the secondary clusters.

This model is consistent with the known data and an evolutionary picture in which homeotic genes allowed SGP fate to be switched to fat body fate in certain parts of the body. However, we realize that it must necessarily be incomplete, and probably oversimplified, and will have to be tested against new findings. The discovery of a gene or genes coding for the postulated ‘competence factor’ may lead to completely new insights and disprove the model, or, as we hope, confirm and refine it.

We thank the stock keepers in Bloomington and Tübingen for providing fly stocks. Information provided by the European and US Fly genome programs (FlyBase) was used as a source of information throughout this work. Antibodies and DNA were kindly provided by M. Brennan, J. J. Brookman, C. Klämbt, M. Frasch and S. Roth. We thank Ruth Lehmann for discussions and communication of results prior to publication and Edan Foley for discussions and comments on the manuscript. We especially thank Ruth Grosskortenhaus for providing and staining embryos. This work was supported by the Deutsche Forschungsgemeinschaft.

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