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Gonadal mesoderm and fat body initially follow a common developmental path in *Drosophila*

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SUMMARY

During gastrulation, the *Drosophila* mesoderm invaginates and forms a single cell layer in close juxtaposition to the overlying ectoderm. Subsequently, particular cell types within the mesoderm are specified along the anteroposterior and dorsoventral axes. The exact developmental pathways that guide the specification of different cell types within the mesoderm are not well understood. We have analyzed the developmental relationship between two mesodermal tissues in the *Drosophila* embryo, the gonadal mesoderm and the fat body. Both tissues arise from lateral mesoderm within the *eve* domain. Whereas in the *eve* domain of parasegments 10-12 gonadal mesoderm develops from dorsolateral mesoderm and fat body from ventrolateral mesoderm, in parasegments 4-9 only fat body is specified. Our results demonstrate that the cell fate decision between gonadal mesoderm and fat body identity within dorsolateral mesoderm along the anteroposterior axis is determined by the combined actions of genes including *abdA*, *AbdB* and *srp*; while *srp* promotes fat body development, *abdA* allows gonadal mesoderm to develop by repressing *srp* function. Furthermore, we present evidence from genetic analysis suggesting that, before stage 10 of embryogenesis, gonadal mesoderm and the fat body have not yet been specified as different cell types, but exist as a common pool of precursor cells requiring the functions of the *tin*, *zfh-1* and *cli* genes for their development.

Key words: Gonadal mesoderm, Fat body, *Drosophila*, *abdominal A*, *Abdominal B*, *clift*, *serpent*, *tinman*, *zinc finger homeodomain protein-1*

INTRODUCTION

Developmental programs leading to the specification and differentiation of particular cell types often involve a combination of cell-autonomous factors, specific patterns of cell division and cell-cell interactions. How these combined elements lead to the specification of cell types within the developing *Drosophila* mesoderm has recently been the focus of intense study. During stage 8 and 9 of embryogenesis, the pair-rule genes *even-skipped (eve*) and *sloppy-paired (slp*) act in the mesoderm to allocate cells into two domains within each parasegment (PS) along the anteroposterior (A-P) axis (Azpiazu et al., 1996; Riechmann et al., 1997). Cells within each domain will give rise to a specific set of mesodermal tissues, such that midgut visceral mesoderm (gut musculature), fat body (see below) and gonadal mesoderm (somatic component of the gonad) are derived from the *eve* domain, whereas cardiac mesoderm (heart precursors) and somatic mesoderm (muscles) are specified within the *slp* domain. Concomitant with this early patterning of the mesoderm are two consecutive waves of cell division, as well as the migration of the mesoderm dorsally along the ectoderm (reviewed in Bate, 1993). This movement places the mesoderm in close contact with the overlying ectoderm, allowing signaling processes to occur between the two layers. Two such signals are the products of the segment polarity genes *hedgehog (hh*) and *wingless (wg*), which function downstream of *eve* and *slp*, respectively, to further define mesodermal subdomains along the A-P axis (Azpiazu et al., 1996).

Patterning of the mesoderm along the dorsoventral (D-V) axis also requires signaling events between the two germ layers. During stage 10 of embryogenesis, *decapentaplegic (dpp*) signaling is required in the ectoderm to maintain expression of the *tinman (tin*) gene exclusively in the dorsal region of the mesoderm (Frasch, 1995; Staehling-Hampton et al., 1994). Previous to this stage, *tin* is expressed throughout the mesoderm (Azpiazu and Frasch, 1993). Dorsally restricted *tin* expression is necessary for the specification of dorsal mesodermal derivatives, including the midgut visceral mesoderm, cardiac mesoderm and dorsal muscles (Azpiazu and Frasch, 1993; Bodmer, 1993). Another wave of cell division occurs during this stage, along with the segregation of the mesoderm into an inner and outer layer. The inner layer in part becomes visceral mesoderm, whereas the outer layer gives rise to the somatic musculature (Bate, 1993). By this time, specification of different mesodermal cell types has commenced, as is evident by the cell-type-specific expression of the *bagpipe (bap*) gene in the visceral mesoderm (Azpiazu

et al., 1996). Although a developmental and genetic pathway including *eve, hh, dpp, tin* and *bap* has been described for the specification of the midgut visceral mesoderm, similar pathways for other mesodermal derivatives such as gonadal mesoderm and the fat body have yet to be elucidated.

Some of the genes required for specification and differentiation of gonadal mesoderm have been recently identified. It has been shown that the homeotic genes *abdominal A (abdA*) and *Abdominal B (AbdB*) are required for specifying somatic gonadal precursors (SGPs), those cells that become gonadal mesoderm, within PS10-12 (Boyle and DiNardo, 1995). The *clift (cli*, also known as *eyes-absent*) gene is required for gonadal mesoderm differentiation and is specifically expressed in SGPs beginning at stage 11 (Boyle and DiNardo, 1995). This restricted expression requires *abdA* and *AbdB* function (Boyle and DiNardo, 1995; our observations). Additional genes required for the development of gonadal mesoderm including *eve* and *hh* have been identified through screens for mutants affecting germ cell migration in *Drosophila* (Moore et al., 1998). These studies showed that gonadal mesoderm is virtually abolished in *eve* mutants, but present in *slp* mutants, demonstrating that gonadal mesoderm, like visceral mesoderm and fat body, is derived from the *eve* domain of the mesoderm (Azpiazu et al., 1996; Moore et al., 1998). It has been shown that gonadal mesoderm lies immediately ventral to the visceral mesoderm that requires *tin* function for its specification (Boyle et al., 1997). These and other studies found that *tin* is also required for gonadal mesoderm development (Boyle et al., 1997; Moore et al., 1998). However, this function for *tin* does not depend on a regulator of late *tin* expression, *dpp*, suggesting that the early, uniform expression of *tin* throughout the mesoderm is critical for the development of this tissue (Broihier et al., 1998). The *zinc-finger homeodomain protein-1 (zfh-1*) has been identified as another regulator of gonadal mesoderm development (Broihier et al., 1998; Moore et al., 1998). It has been demonstrated that, when both *tin* and *zfh-1* function are removed from embryos, gonadal mesoderm is abolished and virtually no fat body cells develop. This suggests a model by which *tin* and *zfh-1* function together in the determination of lateral mesoderm, from which gonadal mesoderm and fat body are derived (Broihier et al., 1998).

The *Drosophila* fat body is an organ composed of adipose tissue that is thought to function as the fly equivalent of the mammalian liver (Rizki, 1978). It is a mesodermally derived structure, which, like gonadal mesoderm, arises from the *eve* domain of the mesoderm (Azpiazu et al., 1996; Riechmann et al., 1997). Although a number of markers have been identified that are expressed in fat body precursors at various embryonic stages (Abel et al., 1993; Hoshizaki et al., 1994; Rehorn et al., 1996), little is known about the developmental and genetic steps leading toward the specification of this cell type. One fat body marker with a known developmental function is the *serpent* (*srp)* gene. *srp* encodes a GATA family member transcription factor that is expressed in fat body precursors from stage 10 throughout embryogenesis (Abel et al., 1993; Rehorn et al., 1996). In *srp* mutants, fat body precursors form, but fail to proliferate and differentiate (Rehorn et al., 1996).

Although these combined studies have provided valuable information toward understanding the developmental programs required for both gonadal mesoderm and fat body, many questions regarding the origin and specification of these two cell types remain unanswered. We present here an analysis of gonadal mesoderm development as it relates to the development of the embryonic fat body. Our studies indicate that both tissues are found at identical D-V positions within different parasegments and initially follow a common developmental path relying on the same subset of genes. Furthermore, we address the question of how cell fate decisions are made between gonadal mesoderm and fat body along the A-P axis. Our results show that *srp* promotes fat body development, while *abdA* allows gonadal mesoderm to develop by negatively regulating *srp* function.

MATERIALS AND METHODS

Fly stocks

The following alleles were used for all phenotypic analyses. $abdA^{MX1}$ and $AbdB^{D101.3}$ were both provided by Welcome Bender. $abdA^{MX1}$ contains a deletion within the locus and is therefore thought to approximate the null phenotype (Karch et al., 1990). $AbdB^{D101.3}$ is a point mutation that is phenotypically null (I. Duncan, personal communication). cli^{2D} is a strong allele obtained from Bloomington stock center (Boyle et al., 1997). dpp^{H46} was provided by Vern Twombly and Bill Gelbart. This allele contains a deletion within the locus and is thought to approximate the null phenotype (St. Johnston et al., 1990). srp^{9L} was obtained from the Bloomington stock center and behaves as a phenotypic null (Reuter, 1994). $tin^{\Delta GC14}$ [Df(3R)GC14] was obtained from Manfred Frasch and is a deletion removing the entire locus (Azpiazu and Frasch, 1993). For analysis of lack of *zfh-1* function, embryos transheterozygous for $zfh\text{-}1^{75.26/65.34}$ were used and, in the *tin,zfh-1* and *cli;zfh-1* double mutant strains, $zfh\text{-}1^{75.26}$ was used. Both *zfh-1* alleles show no detectable protein in embryos and behave as phenotypic nulls (Broihier et al., 1998). The *abdA srp* double mutant was constructed using the $abdA^{D24}$ and srp^{9L} alleles; the $abdA^{D24}$ allele behaves as a phenotypic null (Hopmann et al., 1995).

The *hsp70-abdA* line was obtained from Gines Morata through Monica Boyle. Ectopic *abdA* function was induced by heat shocking embryos at 4 and 6 hours of development according to the method of Boyle and DiNardo (1995). Embryos were fixed and antibody stained as described below.

Antibody staining

The following antibodies were used in immunostaining of embryos: rabbit polyclonal anti-β-galactosidase (Cappel), mouse monoclonal anti-Cli (provided by Nancy Bonini), rabbit polyclonal anti-Srp (provided by Rolf Reuter) and mouse polyclonal anti-Zfh-1 (provided by Zichun Lai). Prior to use, the anti-β-galactosidase and secondary antibodies (see below) were preabsorbed against an overnight collection of wild-type embryos.

Antibody detection was performed with either horseradish peroxidase using a biotinylated secondary antibody (Jackson ImmunoResearch) and the Elite Kit (Vector Labs), or with a directly conjugated alkaline phosphatase secondary antibody (Jackson ImmunoResearch). Embryos were fixed and devitellinized according to the method described in Gavis and Lehmann (1992), with the modification that 1× PBS and 50 mM EDTA were used in place of PEMS during the fixation. Embryos were rehydrated and subjected to antibody staining as described in Eldon and Pirrotta (1991). For whole-mount analysis, embryos were mounted onto slides in PolyBed812 (Polysciences) according to Ephrussi et al. (1991), then analyzed with a Zeiss Axiophot microscope using Nomarski optics.

RESULTS

Gonadal mesoderm and fat body precursors share identical positions in different parasegments

It has been recently shown that both gonadal mesoderm and fat body are derived from within the *eve* domain of the mesoderm (Moore et al., 1998; Riechmann et al., 1997). Moreover, in this domain, both tissues arise from clusters of lateral-mesodermal cells as defined by expression of Zfh-1 protein at stage 10 of embryogenesis (Broihier et al., 1998). Since gonadal mesoderm is derived only from PS10-12 (Boyle et al., 1997 and Fig. 1A,B), we wanted to investigate in more detail the spatial relationship between gonadal mesoderm and fat body within different parasegments. We found that, in PS4-9 and PS13, precursors of the embryonic fat body, as visualized by expression of the Srp protein (Abel et al., 1993; Rehorn et al., 1996), are found in the identical D-V position as SGPs, visualized by high levels of Zfh-1 protein, in PS10-12 (Fig. 1). We refer to this region of the fat body and gonadal mesoderm collectively as dorsolateral mesoderm. In all parasegments, additional fat body precursors arise in an area ventral to where the SGPs form in PS10-12 (Fig. 1C,D). These cells we have collectively termed ventrolateral mesoderm. As a consequence, while ventral fat body is specified in all parasegments, there is a dorsal gap within the developing fat body in PS10-12 in which SGPs are specified (Fig. 1C,D). Therefore, in PS4-9 and PS13, only fat body develops from lateral mesoderm, whereas in PS10-12, both gonadal mesoderm and fat body are specified. We confirmed these results by sectioning embryos stained with markers recognizing both gonadal mesoderm and fat body (data not shown).

Control of gonadal mesoderm versus fat body cell fate along the A-P axis

The observations that gonadal mesoderm is only specified from dorsolateral mesoderm in PS10-12 and that fat body develops in the same D-V position in PS4-9 and PS13 led us to investigate what controls the cell fate decision between gonadal mesoderm and fat body along the A-P axis. It has been shown that the homeotic genes *abdA* and *AbdB* are required for the specification of gonadal mesoderm, with *abdA* required for gonadal mesoderm in PS10-12 and *AbdB* required only in PS12 (Boyle and DiNardo, 1995). We find that, in *abdA* mutants, Srp-expressing cells are found in the region normally occupied by gonadal mesoderm (Fig. 2B; compare with 2A). Moreover, in embryos lacking *AbdB* function, Srp-expressing cells are now observed in PS12 (Fig. 2C; compare with 2A). This suggests that, in wild-type embryos, *abdA* and *AbdB* function to repress *srp* expression in PS10-12. The *srp* gene has been shown to be required for the proliferation and morphogenesis of fat body precursors (Rehorn et al., 1996). Therefore, *abdA* and *AbdB* result in the inhibition of fat body development within these parasegments. Previous work has shown that ectopic expression of *abdA* in parasegments anterior to PS10-12 results in an expansion of gonadal mesoderm into these parasegments (Boyle and DiNardo, 1995). We find that ectopic *abdA* also represses Srp expression in these same anterior parasegments, suggesting that *abdA* promotes gonadal mesoderm at the expense of fat body (Fig. 2E; compare with 2D). We also find that ectopic *abdA* activity represses Srp expression even in ventrolateral regions of PS10-12 (Fig. 2E, arrowhead; see Discussion). Taken together, these results demonstrate that *abdA* and *AbdB* play key roles in directing the developmental decision between gonadal mesoderm and fat body cell fates along the A-P axis.

We next wanted to determine if the reciprocal cell fate transformation of fat body into gonadal mesoderm could occur by removing a gene activity required for the development of fat body. One gene known to be required for fat body development is the *srp* gene (Rehorn et al., 1996). We therefore analyzed the expression of two gonadal mesoderm cell markers, Zfh-1 and Cli proteins, in *srp* mutant embryos. By stage 11 in wild-type embryos, high levels of Zfh-1 protein are found in gonadal mesoderm (PS10-12), whereas low levels are expressed in other parasegments (Broihier et al., 1998 and Fig. 1C). However, we find that, in *srp* mutant embryos, high levels of Zfh-1 are expressed in every parasegment (Fig. 3A), suggesting that aspects of gonadal mesoderm development are occurring in place of fat body development. Like Zfh-1, Cli protein expression in lateral mesoderm is only found in the gonadal mesoderm within PS10-12 in wild-type embryos (Fig. 1A). In embryos lacking *srp*

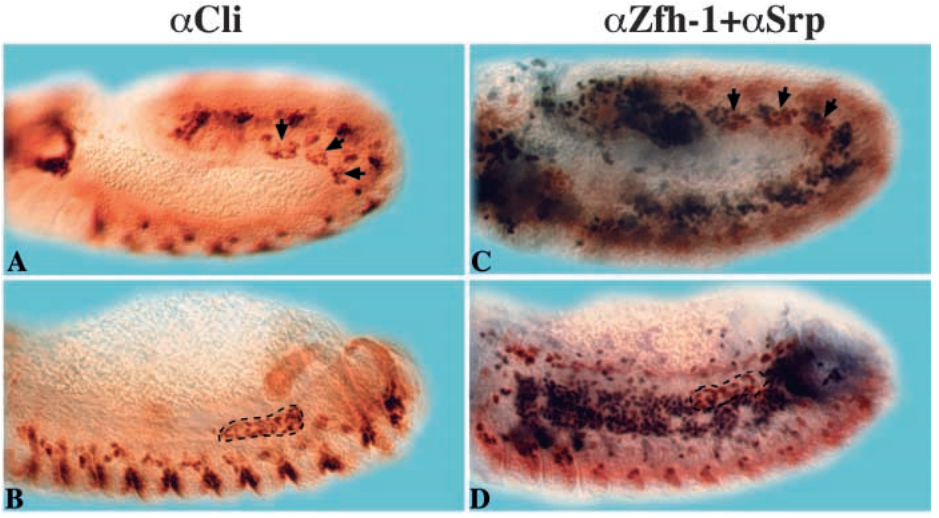

Fig. 1. Gonadal mesoderm and fat body precursors occupy identical positions within different parasegments. Anterior left in all panels; lateral views. Embryos in A and C are at stage 11; embryos in B and D are at stage 13 (stages according to Campos-Ortega and Hartenstein, 1997). (A,B) Somatic gonadal precursors (SGPs) highlighted using an anti-Cli antibody (A, arrows; B, dashed outline). (C,D) SGPs highlighted in brown using an anti-Zfh-1 antibody (C, arrows; D, dashed outline); fat body precursors visualized in blue using an anti-Srp antibody. In PS10-12, SGPs occupy the most dorsal region of staining, whereas fat body precursors are found in more ventral and, in C, posterior areas. The ventral and posterior fat body precursors in C most likely give rise to the ventral fat body cells in D. In PS4-9 and PS13, fat body precursors span the entire region highlighted by staining.

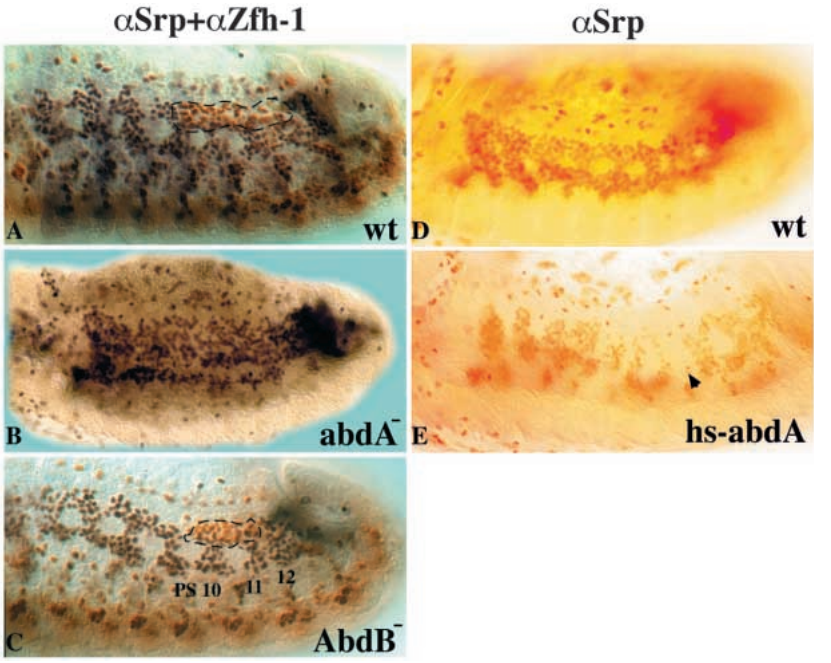

Fig. 2. *abdA* and *AbdB* promote gonadal mesoderm development at the expense of fat body. Anterior left in all panels; lateral views. (A-C) SGPs highlighted using an anti-Zfh-1 antibody (brown, dashed outlines); fat body precursors visualized using an anti-Srp antibody (blue). (D,E) Fat body precursors identified using an anti-Srp antibody (brown). (A,D) Wild type. (B) *abdA*⁻. SGPs are absent and have been replaced by fat body precursors. (C) *AbdB*⁻. SGPs have been replaced by fat body precursors in PS12 (see designations under stained cells), where *AbdB* is known to function (Boyle and DiNardo, 1995). (E) *hs-abdA*. Fewer fat body precursors are found in PS8-9 than in wild type. This is precisely where ectopic gonadal mesoderm has been found to develop in *hs-abdA* embryos (Boyle and DiNardo, 1995). Inhibition of *srp* expression extends into ventral regions of the fat body tissue in PS10 (arrow). This area has not been shown to be occupied by SGPs in *hs-abdA* embryos (Boyle and DiNardo, 1995; see Discussion).

function, Cli expression is expanded anteriorly and posteriorly, indicating that gonadal mesoderm cell types develop in these parasegments (Fig. 3C, compare with Fig. 1A). Taken together, these results demonstrate that *srp* activity results in the repression of gonadal mesoderm development outside of PS10-12 and therefore, like *abdA*, plays a role in the decision between gonadal mesoderm and fat body cell fates. Thus, the combined results of the effect of *abdA*, *AbdB* and *srp* on the development of fat body and gonadal mesoderm suggest that a switch mechanism is involved in specifying gonadal mesoderm versus fat body cell fates along the A-P axis. *abdA* and *AbdB* switch 'off' fat body cell fate, thereby allowing gonadal mesoderm development, whereas *srp* is involved in a mechanism switching 'off' gonadal mesoderm identity and 'on' the developmental program toward fat body differentiation.

Our results demonstrating that *srp* is expressed in dorsolateral mesoderm within PS10-12 in *abdA* mutants suggests that *abdA* normally acts upstream of *srp* to negatively affect its expression within this region. In order to directly test the epistatic relationship between *abdA* and *srp*, we investigated the effect of removing the activities of both genes on the development of dorsolateral mesoderm. We found that like embryos lacking *srp* function alone, embryos mutant for both *abdA* and *srp* express gonadal mesoderm-specific markers in PS4-13 (Fig. 3B,D). Thus, *abdA* acts upstream of *srp* to negatively regulate its function, thereby allowing the development of gonadal mesoderm. This result further demonstrates that in the absence of fat body development, *abdA* is no longer necessary for the specification of SGPs.

Gonadal mesoderm and fat body share common genes for their development

The observation that, at stage 13, the dorsal fat body and

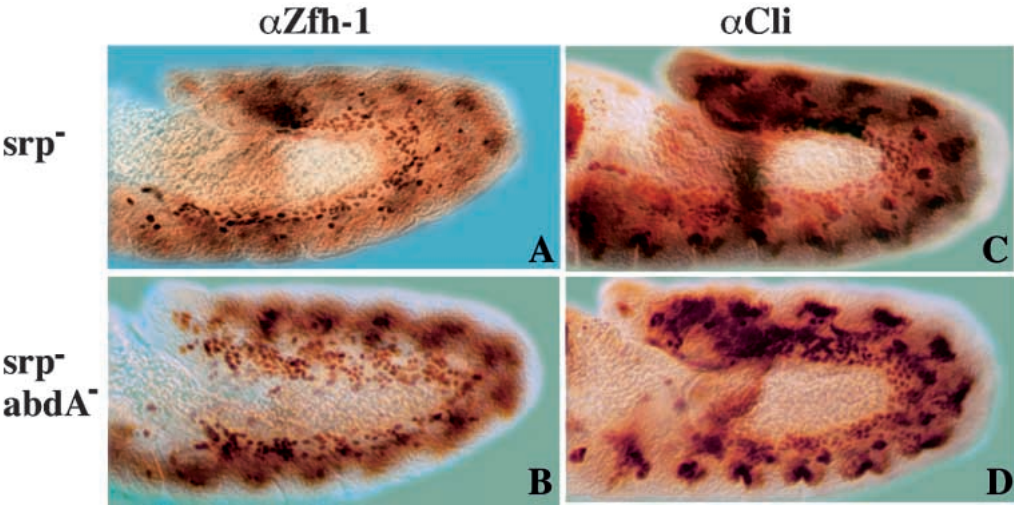

Fig. 3. Expression domains of gonadal mesoderm markers are expanded in *srp* and *abdA srp* mutants. Anterior left in all panels; lateral views. All embryos are at approximately stage 11. (A,C) *srp*⁻. (B,D) *abdA*⁻ *srp*⁻. (A,B) Expression of *zfh-1* (brown) using an anti-Zfh-1 antibody. In wild type, high levels of Zfh-1 mesodermal protein are only found in PS10-12 (see Fig. 1C, arrows). In *srp* and *abdA srp* mutants, high levels are detected in all parasegments. (C,D) Cli expression (brown) using an anti-Cli antibody. Cli mesodermal protein expression is only detected in PS10-12 in wild-type embryos (see Fig. 1A, arrows). In *srp* and *abdA srp* mutants, Cli expression expands anteriorly and posteriorly.

gonadal mesoderm occupy the same D-V position within different parasegments suggests that they may be developmentally closely related tissues. In principle, these two tissues could develop by two different mechanisms. On the one hand, at the time that *zfh-1* and *tin* define lateral mesoderm, gonadal mesoderm and fat body could be already specified as distinct cell types. This might be manifested in the requirement of different genes for early steps in the development of each tissue. On the other hand, precursors of the gonadal mesoderm and fat body could initially follow the same developmental pathway, therefore requiring the same genes, and only later would follow alternate paths toward gonadal mesoderm or fat body development.

In an effort to distinguish between these two possibilities, we analyzed fat body development in embryos lacking the functions of genes required for gonadal mesoderm development that were identified in a screen for mutations affecting germ cell migration in *Drosophila* (Moore et al., 1998). We find that the *zfh-1*, *tin* and *cli* genes are all required for fat body development (Fig. 4). In *zfh-1* mutants, fewer fat body precursors develop, often resulting in gaps within the developing tissue (Fig. 4A). *tin* is also required for the proper number of fat body cells to develop correctly. In *tin* mutant embryos, the bridges of fat body cells that normally span the parasegments at stage 13 fail to form (Fig. 4B, arrow). At this stage, fat body tissue instead remains in a state that morphologically resembles a structure normally seen at stage 12 (data not shown). Previous work has shown that *tin* does not depend on *dpp* for its function in gonadal mesoderm development, suggesting that it is the early, *dpp*-independent expression of *tin* throughout the mesoderm that is necessary for gonadogenesis (Broihier et al., 1998). We have found that, in *dpp* mutants, fat body cells do develop, although the morphology of the fat body structure is difficult to assess given the severe developmental defects associated with this genetic background (Fig. 4C). However, a larger number of Srp-expressing cells are present in *dpp* mutants than in embryos lacking *tin* function (compare Fig. 4C with Fig. 4B), implicating the early function of *tin* in the pathway leading toward the specification of fat body as well as gonadal mesoderm. Whereas *tin* and *zfh-1* act at an early stage in gonadal mesoderm development, the *cli* gene is later required for the differentiation of this tissue (Boyle et al., 1997; Broihier et al., 1998; our observations). We find that *cli* also affects differentiation of the fat body. In *cli* mutants, fat body precursors form, but do not differentiate into the characteristic 'ladder' structure found at this stage in wild-type embryos (Fig. 4D).

It has been shown that the above genes can be placed into a genetic hierarchy based on epistasis experiments. We have shown that gonadal mesoderm is completely absent, and that the number of fat body precursors is virtually abolished, in embryos lacking both *zfh-1* and *tin* function (Broihier et al., 1998 and Fig. 4F). Because the phenotypes seen in the double mutant are more severe than those observed in either single mutant, it can be concluded that *tin* and *zfh-1* share overlapping functions in the development of both tissues. It has been recently shown that *cli* expression in gonadal mesoderm is markedly reduced in embryos lacking *zfh-1* activity. In *cli;zfh-1* double mutants, the gonadal mesoderm defect is identical to that seen in *zfh-1* single mutants. Taken together, these results indicate that *zfh-1* acts upstream of *cli* in gonadogenesis (Broihier at al., 1998). We have also examined the effect of removing both *cli* and *zfh-1* function on the development of the fat body. In *cli;zfh-1* double

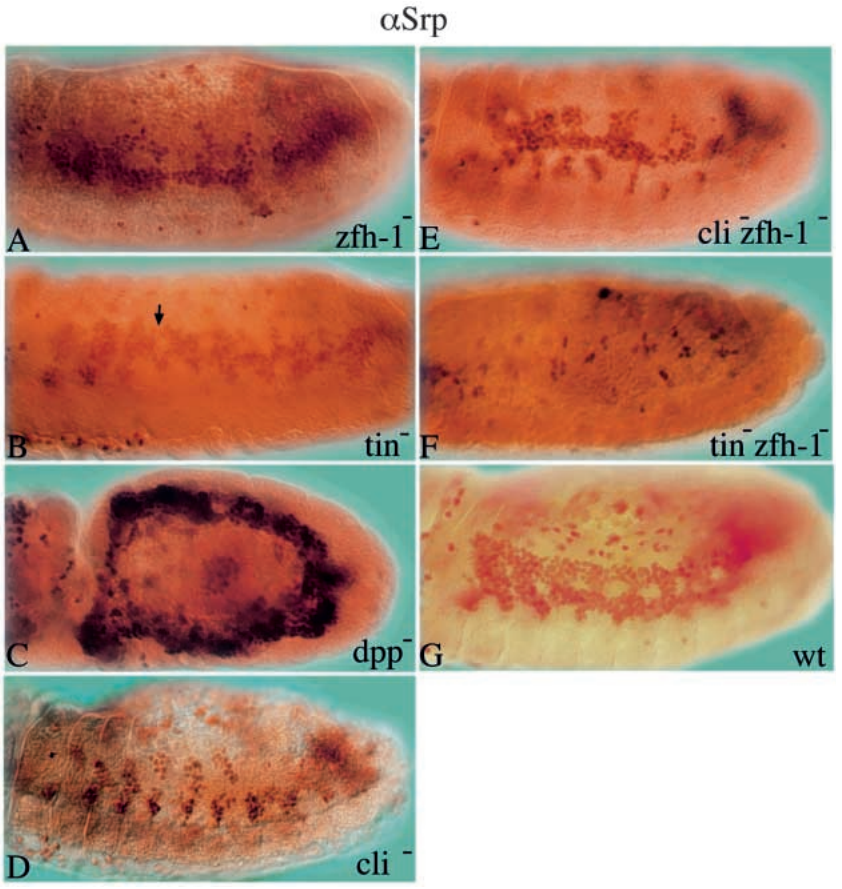

Fig. 4. Genes required for gonadal mesoderm development have a similar requirement in the development of the fat body. Anterior left in all panels; lateral views. All embryos are approximately at stage 13. (A-G) Fat body development visualized using an anti-Srp antibody. (A-F) Mutants; (G) wild type. (A) *zfh-1*$^{-}$. The number of fat body cells is reduced when compared to G, often resulting in gaps within the tissue. (B) *tin*$^{-}$. The characteristic bridges of fat body cells between parasegments fail to form (arrow). This structure resembles that seen in wild-type stage 12 embryos (data not shown). (C) *dpp*$^{-}$. Fat body precursors develop, although the tissue morphology cannot be assayed due to the severe developmental defects associated with these embryos. However, the number of fat body cells observed is greater than in *tin* mutants (compare with B). (D) *cli*$^{-}$. Fat body precursors form, but fail to differentiate into the proper structure. (E) *cli*$^{-}$; *zfh-1*$^{-}$. Fewer fat body cells develop, resulting in a phenotype indistinguishable from that seen in *zfh-1* mutants (compare with A). Interestingly, this phenotype is less severe than that observed in *cli* mutants (compare with D, see Discussion). (F) *tin*$^{-}$, *zfh-1*$^{-}$. Fat body cells are virtually abolished.

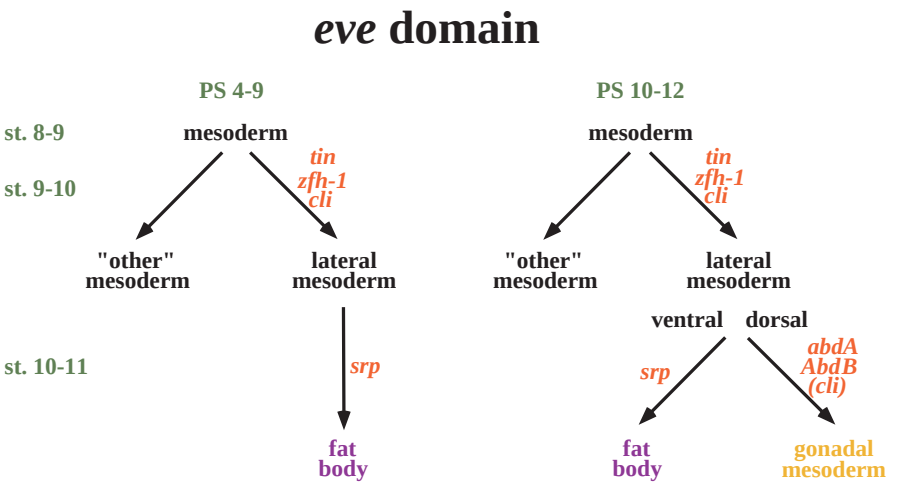

Fig. 5. Model for lateral mesoderm development within the *eve* domain of the mesoderm. In PS4-9, *tin*, *zfh-1*, and *cli* are required before stage 10 to determine lateral mesoderm. During stages 10-11, *srp* is involved in specifying fat body cell fates. In PS10-12, the same genes are required before stage 10 for lateral mesoderm determination. During stages 10-11, the combined functions of *abdA* and *AbdB* specify gonadal mesoderm identity at the expense of fat body development in dorsolateral mesoderm. It is possible that *cli* has a continued requirement in gonadal mesoderm development at this stage. *srp* is required for specifying fat body identity in ventrolateral mesoderm. Although *abdA*, *AbdB*, and *srp* are all required for specifying gonadal mesoderm and fat body cell fates, respectively, along the A-P axis, it is not known what controls the cell fate decision between these two tissues along the D-V axis.

mutants, the defect observed in the fat body is identical to that seen in *zfh-1* mutants alone (compare Fig. 4E with Fig. 4A). Interestingly, a larger number of fat body cells are present in the double mutant than in *cli* single mutants (compare Fig. 4E with 4D; see Discussion). These results suggest that *zfh-1* and *cli* interact similarly in both gonadal mesoderm and fat body development.

Thus, we have demonstrated that many genes required for development of gonadal mesoderm act in a similar manner to direct fat body development. These combined results favor the hypothesis that steps leading to the specification of gonadal mesoderm and fat body initially occur via the same genetic pathway.

DISCUSSION

Previous work has shown that both gonadal mesoderm and the fat body develop from lateral mesoderm derived from the *eve* domain of the mesoderm (Broihier et al., 1998; Moore et al., 1998; Riechmann et al., 1997). Our analysis suggests that prior to their specification as distinct cell types, gonadal mesoderm and fat body precursors exist as a common pool of cells that require a unique set of genes for their determination and development.

Positional relationship between gonadal mesoderm and fat body

We have shown that gonadal mesoderm and dorsal fat body precursors are found in identical positions in different parasegments from stages 11-13 of embryogenesis. Specifically, we found that in PS4-9, the dorsal component of fat body develops in the same D-V location as that in which gonadal mesoderm forms in PS10-12. Moreover, in PS10-12, fat body precursors are found immediately ventral to where gonadal mesoderm develops. These observations provide the first evidence suggesting that gonadal mesoderm and fat body are developmentally closely related tissues.

Genetic relationship between gonadal mesoderm and fat body

Our results demonstrate that mutations in genes disrupting gonadal mesoderm development have similar phenotypic consequences on the development of the fat body. Moreover, we found that the genetic hierarchy controlling gonadal mesoderm development is the same as that functioning in the development of the fat body. These results provide further evidence that both tissues follow a common developmental pathway.

We have shown that *tin* and *zfh-1* are required for the development of both gonadal mesoderm and fat body. Moreover, we have demonstrated that both tissues require the *dpp*-independent expression of *tin* throughout the mesoderm that occurs before stage 10 of embryogenesis. This is consistent with the observation that *tin* expression cannot be detected in lateral mesoderm from stage 10 onward (Azpiazu and Frasch, 1993). However, the effect of loss of *tin* function on both gonadal mesoderm and fat body cannot be detected until later embryonic stages. It is only when both *tin* and *zfh-1* are simultaneously removed that the formation of gonadal mesoderm and fat body is virtually abolished, revealing the early and overlapping functions of both genes in the developmental pathways of both tissues. These partially redundant functions for *tin* and *zfh-1* suggest that *zfh-1* acts at the same time as *tin* in the determination of lateral mesoderm. This is consistent with the fact that, like *tin*, *zfh-1* is expressed throughout the mesoderm prior to stage 10 (Lai et al., 1991). Therefore, we propose that, prior to stage 10, gonadal mesoderm and fat body have not yet been specified, but exist as a population of precursor cells requiring the functions of both *tin* and *zfh-1*. This is further supported by the observation that, at stage 10, Zfh-1 is expressed at uniform levels in lateral mesoderm within PS4-12, whereas high levels of Zfh-1 expression specifically in PS10-12 are not detected until stage 11 (Broihier et al., 1998). Furthermore, the expression of all known gonadal mesoderm and fat body cell-specific markers is only observed during or after stage 10 (Boyle et al., 1997; Broihier et al., 1998; Riechmann et al., 1997).

The *cli* gene is also required for both gonadal mesoderm and fat body development. However, in contrast to *tin* and *zfh-1*, *cli* does not affect the determination of lateral mesoderm. In *cli* mutants, precursors of both gonadal mesoderm and fat body form but do not differentiate (Boyle et al., 1997; this work). This demonstrates that, like *zfh-1* and *tin*, *cli* affects both fat body and gonadal mesoderm development in a similar manner. It is not known at what point the *cli* gene is required in the development of either tissue. Cli protein is found throughout the mesoderm prior to stage 11, but becomes specifically expressed in SGPs at later timepoints. In contrast, Cli protein cannot be detected in fat body cells once they have been specified. Therefore, there are two ways by which *cli* could be involved in the development of gonadal mesoderm and fat body. One possibility is that *cli* is required before stage 11 for both tissues, but does not belie its function until later stages in development. A precedence for this type of gene behavior has been shown through our studies of *tin* (see above). In this model, *cli* could also play a role in gonadal mesoderm development at later embryonic stages, consistent with its expression pattern in SGPs throughout embryogenesis. Conversely, *cli* could function early in fat body development, but not play a role in gonadal mesoderm development until later in the differentiation of this tissue. Although both explanations are formally possible, our results favor the first model. The fact that *cli* mutants have similar effects on both fat body and gonadal mesoderm development suggests that *cli* functions at a stage before gonadal mesoderm and fat body have been specified as unique cell types. Moreover, the *cli* expression pattern indicates that it is unlikely to function in fat body development after stage 10. We cannot at this point discern whether or not *cli* continues to play a role in gonadal mesoderm development after this stage.

Our analysis of fat body development in *cli;zfh-1* double mutants demonstrates that these two genes interact in a similar manner for both gonadal mesoderm and fat body development, further indicating that the two tissues initially follow a common genetic pathway. However, it is surprising that more fat body cells develop in the *cli;zfh-1* double mutant than in *cli* single mutant embryos. Interestingly, a similar result has been observed using the 412 retrotransposon as a marker for gonadal mesoderm development. In *cli* mutants, fewer 412-expressing cells are detected than in *zfh-1* mutants, whereas the double mutant is indistinguishable from embryos lacking *zfh-1* function alone (Broihier and Lehmann, unpublished observations). These results indicate that lack of *zfh-1* activity bypasses *cli*'s requirement in both gonadal mesoderm and fat body development. One possible explanation for this result is that without *zfh-1* function, both cell types are developmentally stalled at a stage before *cli* activity is required. Therefore, loss of *cli* function does not affect these precursor cells and they behave as in *zfh-1* single mutants. However, the fact that residual gonadal mesoderm and fat body cells still express tissue-specific markers in *zfh-1;cli* double mutants suggests that some aspects of differentiation proceed in these cells. Further investigation into the functional relationship between *zfh-1* and *cli* will be necessary to address these observations.

Our results further suggest that precursors of gonadal mesoderm and fat body are determined independently of the visceral mesoderm, another *eve*-domain derivative. Although *zfh*-1 and *cli* are required at an early stage for both gonadal mesoderm and fat body development, neither is necessary for visceral mesoderm formation. In addition to its role in visceral mesoderm specification (Azpiazu and Frasch, 1993; Bodmer, 1993), we have shown that *tin* is also required for both gonadal mesoderm and fat body development. However, we have demonstrated that these latter functions for *tin* are dependent on its early, ubiquitous expression throughout the mesoderm. This is in contrast to previous work demonstrating that dorsally restricted *tin* expression is necessary for visceral mesoderm formation (Frasch, 1995; Staehling-Hampton et al., 1994). Therefore, *tin*'s role in visceral mesoderm specification is distinct from its requirement in the development of gonadal mesoderm and fat body. Given that *zfh-1*, *tin* and *cli* all appear to function in gonadal mesoderm and fat body development before stage 10, our results suggest that at this stage, the developmental pathways leading toward gonadal mesoderm and fat body versus visceral mesoderm specification have already diverged.

Control of decision between gonadal mesoderm and fat body cell fates

We have shown that the transcription factors *abdA*, *AbdB* and *srp* are key players in the control of gonadal mesoderm versus fat body development along the A-P axis. In principle, two different mechanisms could account for the initial specification of each cell type within a parasegment. The first possibility is that *abdA* and *srp* could merely act to promote gonadal mesoderm and fat body development, respectively, with no effect on the alternate tissue. Therefore, loss of function of these genes would result in lack of cell differentiation and possibly cell death. The second possibility is that *abdA* and *srp* also function in repressing development of the alternate cell type, thereby creating a switch mechanism that chooses either gonadal mesoderm or fat body cell fates. Our results favor the latter hypothesis. In *abdA* mutants, fat body develops in place of gonadal mesoderm. In *srp* mutants, gonadal mesoderm markers are expressed where fat body normally develops. Moreover, ectopic *abdA* promotes gonadal mesoderm at the expense of fat body development in the dorsal component of lateral mesoderm. Therefore, the progeny of lateral mesoderm cells either give rise to gonadal mesoderm or fat body along the A-P axis, depending on the presence or absence of *abdA* and *srp*.

Our results from the *abdA srp* double mutant demonstrate that the development of gonadal mesoderm from dorsolateral mesoderm in PS10-12 is executed through *abdA*-dependent negative regulation of *srp* function in this region. It is not known at what level this regulation occurs, although a likely possibility is that *abdA* directly affects *srp* transcription, given that *abdA* encodes a homeodomain protein (Karch et al., 1990). The phenotype observed in the *abdA srp* double mutant also shows that aspects of gonadal mesoderm development can occur in the absence of *abdA* activity, as long as fat body development is abolished. This suggests that the developmental 'ground state' of dorsolateral mesoderm is gonadal mesoderm.

It is unclear what mechanism controls the D-V decision between gonadal mesoderm and fat body within PS10-12. Whereas *abdA* is required to promote gonadal mesoderm versus fat body development in dorsolateral mesoderm in PS10-12, fat body develops from ventrolateral mesoderm within the same parasegments. It is possible that *abdA* expression does not

extend into the region where the ventral fat body precursors are found. Alternatively, a ventrally localized factor analogous to *tin* in the specification of dorsal mesoderm derivatives could inhibit *abdA* function in more ventral regions of lateral mesoderm. The results from the *hs-abdA* experiment argue that a combination of these theories could prove correct. We have demonstrated that ectopic *abdA* expression can inhibit *srp* expression and therefore fat body development, in more ventral regions of the embryo. This suggests that, in wild-type embryos, *abdA* is either not present or is not active in the more ventral cells. However, gonadal mesoderm does not develop in place of fat body in this ventral region of ectopic *abdA* activity (Boyle and DiNardo, 1995), arguing for a ventrally localized factor that inhibits some aspects of *abdA* function in these cells. These results suggest that prior to *abdA* function, D-V differences within lateral mesoderm cells have already occurred. Further analysis of the spatial pattern of *abdA* expression in the mesoderm may help to address how these differences along the D-V axis are generated.

A model for gonadal mesoderm and fat body development

Our combined results lead to a developmental and genetic model of the pathway toward specification of two mesodermal tissues, the gonadal mesoderm and embryonic fat body (Fig. 5). Since both tissues are derived from within the *eve* domain of the mesoderm, we will only focus on this mesodermal component. During germ band extension (stage 8-9), the early functions of both *tin* and *zfh-1* determine lateral mesoderm while other mesodermal subtypes, including those that will become visceral mesoderm, are determined as distinct cell populations. The *cli* gene then renders lateral mesoderm cells competent to fully differentiate into either fat body or gonadal mesoderm identity. During late stage 10, the combined functions of *abdA*, *AbdB* and *srp* control the decision between gonadal mesoderm and fat body cell fates along the A-P axis. In PS10-12, *abdA* and *AbdB* function to repress *srp* expression in dorsolateral mesoderm, thereby allowing gonadal mesoderm development in this location. Ventrolateral mesoderm cells, expressing *srp*, develop into fat body. In PS4-9, *abdA* function is absent, resulting in all lateral mesoderm cells adopting a fat body cell fate. It is possible that *cli* has an additional role in gonadal mesoderm development at this stage. Presumably other factors, such as those determining D-V differences within lateral mesoderm cells and their derivatives, remain to be identified. Taken together, these studies provide a model for the events leading to the specification of gonadal mesoderm and fat body cell fates that includes gene functions, developmental steps and regulatory interactions.

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REFERENCES

Abel, T., Michelson, A. M. and Maniatis, T. (1993). A *Drosophila* GATA family member that binds to *Adh* regulatory sequences is expressed in the developing fat body. *Development* **119,** 623-633.

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

Azpiazu, N., Lawrence, P. A., Vincent, J.-P. and Frasch, M. (1996). Segmentation and specification of the *Drosophila* mesoderm. *Genes Dev.* **10,** 3183-3194.

Bate, M. (1993). The mesoderm and its derivatives. In *The Development of Drosophila melanogaster* (ed. M. Bate and A. M. Arias), pp. 1013-1090. Plainview: Cold Spring Harbor Laboratory Press.

Bodmer, R. (1993). The gene *tinman* is required for specification of the heart and visceral muscles in *Drosophila*. *Development* **118,** 719-729.

Boyle, M. and DiNardo, S. (1995). Specification, migration, and assembly of the somatic cells of the *Drosophila* gonad. *Development* **121,** 1815-1825.

Boyle, M., Bonini, N. and DiNardo, S. (1997). Expression and function of *clift* in the development of somatic gonadal precursors within the *Drosophila* mesoderm. *Development* **124,** 971-982.

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

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

Eldon, E. D. and Pirrotta, V. (1991). Interactions of the Drosophila gap gene *giant* with maternal and zygotic pattern forming genes. *Development* **111**, 367-378.

Ephrussi, A., Dickenson, L. and Lehmann, R. (1991). *oskar* organizes the germ plasm and directs localization of the posterior determinant *nanos*. *Cell* **66**, 37-50.

Frasch, M. (1995). Induction of visceral and cardiac mesoderm by ectodermal Dpp in the early *Drosophila* embryo. *Nature* **374,** 464-467.

Gavis, E. R. and Lehmann, R. (1992). Localization of *nanos* RNA controls embryonic polarity. *Cell* **71**, 301-313.

Hopmann, R., Duncan, D. and Duncan, I. (1995). Transvection in the *iab-5,6,7* region of the bithorax complex of Drosophila: homology independent interactions in *trans. Genetics* **139**, 815-833.

Hoshizaki, D. K., Blackburn, T., Price, C., Ghosh, M., Miles, K., Ragucci, M. and Sweis, R. (1994). Embryonic fat-cell lineage in *Drosophila melanogaster*. *Development* **120,** 2489-2499.

Karch, F., Bender, W. and Weiffenbach, B. (1990). *abdA* expression in *Drosophila* embryos. *Genes Dev*. **4**, 1573-1587.

Lai, Z., Fortini, M. E. and Rubin, G. (1991). The embryonic expression patterns of *zfh-1* and *zfh-2*, two *Drosophila* genes encoding novel zinc-finger homeodomain proteins. *Mech. Dev.* **34,** 123-134.

Moore, L. A., Broihier, H. T., Van Doren, M., Lunsford, L. and Lehmann, R. (1998) Identification of genes controlling germ cell migration and embryonic gonad formation in Drosophila. *Development* **125**, 667-678.

Rehorn, K.-P., Thelen, H., Michelson, A. M. and Reuter, R. (1996). A molecular aspect of hematopoiesis and endoderm development common to vertebrates and *Drosophila*. *Development* **122,** 4023-4031.

Reuter, R. (1994). The gene *serpent* has homeotic properties and specifies endoderm versus ectoderm within the *Drosophila* gut. *Development* **120**, 1123-1135.

Riechmann, V., Irion, U., Wilson, R., Grosskortenhaus R. and Leptin, M. (1997). Control of cell fates and segmentation in the *Drosophila* mesoderm. *Development* **124,** 2915-2922.

Rizki, T. M. (1978). Fat Body. In *Genetics and biology of Drosophila* (ed. M. Ashburner and T. R. F. Wright), Vol. 2b, pp. 561-601. New York: Academic Press.

Staehling-Hampton, K., Hoffmann, F. M., Baylies, M. K., Rushton, E. and Bate, M. (1994). *dpp* induces mesodermal gene expression in *Drosophila*. *Nature* **372,** 783-786.

St. Johnston, R. D., Hoffmann, F. M., Blackman, R. K., Segal, D., Grimaila, R., Padgett, R. W., Irick, H. A. and Gelbart, W. M. (1990). Molecular organization of the *decapentaplegi*c gene in *Drosophila melanogaster*. *Genes Dev*. **4**, 1114-1127.