serpent, a GATA-like transcription factor gene, induces fat-cell development in Drosophila melanogaster

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SUMMARY


Misexpression of serpent also affects the differentiation of muscle cells. Few body-wall muscle precursors are specified and there is a loss of most body-wall muscle fibers. The precursors of the visceral mesoderm are also absent and concomitantly the visceral muscle is absent. We suggest that the ectopic fat cells might originate from cells that have the potential, but do not normally, differentiate into fat cells or from cells that have acquired a fat-cell fate. In light of our results, we discuss the role of serpent in fat-cell specification and in cell fate choices.

Key words: Mesoderm, Cell fate, Fat cells, GATA factors, Drosophila melanogaster

INTRODUCTION

During the course of embryogenesis the potential of cells becomes increasingly restricted as they are programmed to adopt a specific cell fate or lineage. The subdivision of the Drosophila embryo by anteroposterior and dorsoventral patterning genes plays a critical role in providing cells with a progressively restricted identity (Azpiazu et al., 1996; Frasch, 1995; Riechmann et al., 1997; Staehling-Hampton et al., 1994). Although it is well established that positional information helps to guide cell-fate choices, little is known about the molecular mechanisms that control the final steps of cell specification and the irreversible commitment (or determination) of cells to differentiate as a specialized cell type.

Fat body, visceral and somatic muscle, heart (dorsal) muscle, hemocytes, gonad-sheath cells (gonadal mesoderm), and peritracheal cells arise from the mesoderm (Hartenstein and Jan, 1992; Hoshizaki et al., 1994; Technau, 1987). In Drosophila, the subdivision of the mesoderm provides positional cues for individual cell fates. For example, in the dorsoventral axis, decapentaplegic (dpp)-dependent activation of tinman (tin) is necessary for the specification of the dorsal mesoderm and consequently the precursors of visceral muscle (Frasch, 1995; Staehling-Hampton et al., 1994). In the anteroposterior axis, pair-rule genes such as even-skipped (eve) and sloppy paired (slp) subdivide the mesoderm into metamerically repeating units and aid in defining mesodermal sub-populations such as the precursors of fat cells and gonadal mesoderm (Azpiazu et al., 1996; Riechmann et al., 1997).

Although numerous genes responsible for anteroposterior and dorsoventral patterning of the mesoderm have been identified, only a few genes (e.g. tin, dpp) have been shown to respond to this patterning information and to regulate the commitment of cells to a single developmental pathway. The gene serpent (srp) responds to patterning information (Azpiazu et al., 1996) and is a candidate target gene for specifying fat cells and triggering fat-cell differentiation (Sam et al., 1996; Rehorn et al., 1996). Fat cells are derived from several spatially distinct clusters of cells that coalesce into a single-cell thick fat body. The fat body is morphologically made up of three domains: the lateral fat body, the dorsal fat-cell projection and the ventral collar (Hoshizaki et al., 1994; Moore et al., 1998; Riechmann et al., 1998). The bulk of the fat body is organized into a lateral fat body that forms a bilateral ribbon of cells interrupted by the protrusion of internal organs. Extending...
from the posterior of the lateral fat body and lying on the dorsal side of the embryo are the dorsal fat-cell projections that form oblong protrusions of fat cells flanking the dorsal vessel (Hoshizaki et al., 1994; Riechmann et al., 1998). The third morphological domain is the ventral collar that extends from the anterior region of the lateral fat body and spans the ventral midline (Campos-Ortega and Hartenstein, 1997; J. M. M. and D. K. H., unpublished). Each morphological domain arises from spatially distinct clusters of cells. The lateral fat body arises from primary fat-cell clusters located in the eve domain of parasegment (PS) 4-9 and from two secondary sets of fat-cell clusters. One set arises immediately posterior to the primary fat-cell clusters and the other set arises in a position ventral to the primary clusters in the slp domain in PS 3-12. The dorsal fat-cell projections arise from a large cluster of cells in PS 13 (Riechmann et al., 1998).

srp is an essential gene for fat-cell development. srp is one of three D. melanogaster genes belonging to the GATA transcription factor family and it was initially identified as a transcriptional activator of the alcohol dehydrogenase (Adh) gene in fat cells (Lin et al., 1995; Ramain et al., 1993; Winick et al., 1993; Abel et al., 1993). Analysis of srp expression during embryogenesis reveals the presence of SRP protein at stage 10-11 in all the fat-cell precursors (Sam et al., 1996). srp is the earliest expressed fat-cell gene and is likely to play a role in fat-cell specification or differentiation (Abel et al., 1993; Rehorn et al., 1996; Sam et al., 1996). Mutational analysis identified srp as being necessary for maintaining a fat-cell identity in the lateral fat body (Sam et al., 1996). In transcript- and protein-expressing srp null mutants, srp cells are readily detected in the position of the fat-cell precursors but later undergo apoptosis (Sam et al., 1996). We find that loss of srp function also does not prevent early wild-type expression of an enhancer-trap line that marks the precursor fat cells that give rise to the lateral fat body (Sam et al., 1996). Thus, srp appears not to be necessary for specification of the lateral fat body, but it is necessary for the maintenance of fat-cell differentiation.

Based on several lines of evidence, however, Riechmann et al. (Riechmann et al., 1998) and Moore et al. (Moore et al., 1998) have suggested that srp also controls the fate of a common precursor in PS 4-12. The presence of srp in the common precursor in PS 4-9 specifies the primary fat-cell clusters while the absence of srp activity in the common precursor in PS 10-12 allows specification of the somatic gonadal precursors (SGPs). A prediction of the common precursor model is that srp is capable of inducing fat-cell formation and suppressing SGP specification. In this report, we have directly tested srp’s role by forcing expression of srp in the mesoderm. We show that expression of srp throughout the mesoderm results in the formation of ectopic fat cells. These excess fat cells do not arise from hyperproliferation of pre-existing precursors and thus srp is able to induce fat cells from cells that normally would not give rise to fat cells. While on surface these results are in favor of the common precursor model, we find that forced expression of srp does not affect the specification of the SGPs; rather, the SGPs fail to migrate and coalesce to form the gonads. We present an alternative model for the choice between precursor fat cells and SGPs and discuss the role of srp in fat-cell and SGP specification.

**MATERIALS AND METHODS**

**Fly stocks**

The twist-GAL4, 24B-GAL4, and 69B-GAL4 stocks were provided by the Drosophila Stock Center, Bloomington, Indiana. The twist-GAL4 driver contains the entire regulatory region of the twist (twi) promoter and drives expression in a pattern similar to that of the endogenous twi gene. twi-driven expression is first detected in the mesoderm and in the mesectoderm at gastrulation. Uniform expression persists in the mesodenderm and in the mesoderm until early stage 10 and late stage 11, respectively. twi-driven expression also persists in a subset of muscle progenitor cells until at least stage 12 (Baylies and Bate, 1996). The 24B-GAL4 line drives expression in the mesoderm at stage 11 and later in embryonic muscle (Brand and Perrimon, 1993). The 69B-GAL4 line directs expression in the ectoderm starting at stage 9 (Brand and Perrimon, 1993; Castelli-Gair et al., 1994; Staeling-Hampton et al., 1994). UAS-srp flies were generated by P-element mediated germline transformation of the pUAST-srp plasmid into w^{1118} embryos.

**Plasmids**

pUAST-srp was assembled by subcloning the entire srp coding sequence into pUAST (Brand and Perrimon, 1993). The srp cDNA clone pABF (Abel et al., 1993) does not contain the complete 5′-end. To construct a full-length clone, the 5′ end of srp was amplified from genomic DNA using the polymerase chain reaction (PCR). Primers were designed based on the full-length cDNA sequence from GenBank (accession no. Y07662) and included an engineered EcoRI site at the 5′ end. The PCR product was isolated after restriction enzyme digestion by EcoRI and NolI; the incomplete srp cDNA was cut at the endogenous NolI site and SpeI site of the polylinker and isolated. These two fragments were then ligated into pUAST that had been previously digested with EcoRI and XbaI. The clifl plasmid, pBlue-eyII (Bonini et al., 1993), was a generous gift from S. DiNardo. Robert Storti generously provided Tropomyosin 1 (Tml) (Baucht et al., 1982) and S9A (Dohrmann et al., 1990) contained in pBluescript KS+.

**In situ hybridization to whole-mount embryos**

In situ hybridization was carried out as previously described (Hoshizaki et al., 1994). Antisense RNA probes for Adh, DCG1, nau and the DNA probe for 412 have been described previously (Hoshizaki et al., 1994; Tan et al., 1996). Digoxigenin substituted antisense RNA probes were synthesized in vitro. Following hybridization to fixed embryos, transcripts were detected using alkaline phosphatase-conjugated anti-digoxigenin antibody (Boehringer Mannheim), followed by the addition of chromogenic substrates, X-phosphate and NBT (Boehringer Mannheim).

**Immunohistochemistry**

Immunohistochemistry was performed as described previously (Hoshizaki et al., 1994). Mouse anti-β-galactosidase and anti-mouse alkaline phosphatase were obtained from Promega. Anti-serpent antiserum was raised against the DNA-binding domain of srp fused to GST protein (Pharmacia) and affinity purified (Sam et al., 1996). Alkaline phosphatase was detected using X-phosphate and NBT (Boehringer Mannheim).

**Microscopy**

For microscopic analysis, embryos were equilibrated in mounting solution (50% glycerol, 150 mM NaCl, 10 mM Tris-HCl pH 8.0). Whole-mounted embryos were imaged on the Zeiss Axiosplan2 microscope using Nomarski optics and documented using either a Kodak MDS120 digital camera attached to a Zeiss Axiosplan2 microscope or photographed. Slides and prints were scanned at 1600 dpi and 600 dpi, respectively with a U-Max S-12 scanner. CorelDraw 8 software was used to prepare figures from digital images.
RESULTS

srp can promote widespread fat-cell development

To test whether srp is sufficient to induce fat-cell development we employed the GAL4 targeted expression system described by Brand and Perrimon (Brand and Perrimon, 1993), twist-GAL4 (Baylies and Bate, 1996) and UAS-srp constructs were used to express srp throughout the mesoderm beginning at gastrulation. In stage-10 to -11 embryos, srp expression is readily detected in the primary fat-cell clusters in PS 4-9 and in the secondary fat-cell clusters in PS 10-12 (Fig. 1A). In twist-GAL4/UAS-srp embryos, srp expression is expanded into a continuous band of cells throughout the mesoderm (Fig. 1B). Several molecular cell markers were used to monitor the effects of ectopic srp expression on the segregation of mesodermal derivatives.

Two genes, alcohol dehydrogenase (Adh) and Drosophila collagen gene 1 (DCg1) were used to detect the fat body. Both Adh and DCg1 are embryonic terminal fat-cell differentiation markers. Adh expression is first detected during embryogenesis at stage 14 in the anterior wall of the midgut and the atrium of the posterior spiracle, and in the late precursor fat cells at stage 15 (Hoshizaki et al., 1994). By stage 16, high levels of Adh transcripts are detected in the lateral fat body and lower levels are found in the dorsal fat-cell projections (Fig. 2A) (Hoshizaki et al., 1994). Within the developing fat cells, the temporal expression pattern of DCg1 is similar to Adh although DCg1 transcripts are easily detected at equivalent levels in both the dorsal fat-cell projections and the lateral fat body by stage 16 (Fig. 2C,E). DCg1 is also expressed in the hemocytes (Hoshizaki, 1994; references therein). In twist-GAL4;UAS-srp embryos, the premature expression of srp throughout the mesoderm does not induce premature expression of Adh. As in wild-type embryos, Adh transcripts were first detected in the fat cells of experimental embryos at stage 15. The Adh-positive fat cells however, occupied most of the lateral mesoderm and formed an expanded lateral fat body (Fig. 2B).

Because srp is a direct transcriptional activator of the Adh promoter in transient transfection assays (Abel et al., 1993), the increase in Adh-expressing cells might be the result of ectopic transactivation of Adh by srp rather than a reflection of the activation of the entire fat-cell genetic program. To test this possibility, we examined the expression pattern of a second fat-cell marker, DCg1. In embryos carrying twist-GAL4;UAS-srp, the temporal pattern of DCg1 expression was also normal. DCg1 transcripts were first detected in hemocytes and then in fat cells at stage 15. The number of DCg1-expressing hemocytes was comparable to that of wild type. However, similar to the results obtained with Adh, there was an increase in the number of DCg1-expressing fat cells (Fig. 2D). The DCg1-expressing fat cells formed an expanded lateral fat body and dorsal fat-cell projections were expanded and fused with the lateral fat body in the posterior region of the embryo (Fig. 2D,F). An increase in lateral fat body cells was also detected in experimental embryos using a third fat-cell marker, the imaginal disc growth factor 3 gene, IDGF3 (Kawamura et al., 1999) (data not shown). Similar results, albeit less extreme, were obtained using a second mesodermal GAL4 driver, 24B, that is first active in the presumptive mesoderm (data not shown) (Brand and Perrimon, 1993). These data demonstrate that ectopic expression of srp in the mesoderm leads to an increase in cells composing the lateral fat body and dorsal fat-cell projections.

To determine whether mesoderm-specific components are necessary for srp to promote fat-cell development, we ectopically expressed srp in the ectoderm starting at stage 9 using the 68B-GAL4 driver (Castelli-Gair et al., 1994). We found that ectopic expression of srp in the ectoderm is not sufficient to induce either Adh or DCg1 in the ectoderm, nor does it alter the normal development or morphology of the lateral fat body and dorsal fat-cell projections (data not shown). These data suggest that srp requires mesodermal factors for induction of fat cells.

Fig. 1. Ectopic srp expression in a twist-GAL4;UAS-srp embryo. (A,B) Lateral view of stage-11 embryos stained for srp mRNA. (A) Wild-type embryo. srp is expressed in the primary fat-cell clusters in PS 4-9 and in the secondary fat-cell clusters in PS 10-12. (B) twist-GAL4;UAS-srp embryo. srp is expressed throughout the mesoderm.

Fig. 2. Ectopic expression of srp leads to expansion of the fat body. (A,B) Stage-16 embryos stained for Adh mRNA. (A) Lateral view of a wild-type embryo. The lateral fat body consists of a ribbon-like band of cells with multiple disruptions or holes. (B) Lateral view of a twist-GAL4;UAS-srp embryo. The lateral fat body is expanded in both the dorsal/ventral and anterior/posterior axis. (C-F) Stage-16 embryos stained for DCg1 mRNA. (C) Lateral view of a wild-type embryo. DCg1 is expressed in the lateral fat body (arrowhead bracket), and also in the hemocytes (open arrowhead). (D) Lateral view of a twist-GAL4;UAS-srp embryo. The lateral fat body is expanded (arrowhead bracket). (E,F) Higher magnification and dorsal view of (C,D) respectively. (E) The two dorsal fat-cell projections are clearly visible. (F) The dorsal fat-cell projections are fused with the lateral fat body (arrows), and cover the posterior end of the dorsal side of the embryo. Scale bars: in C, 0.1 mm for A-D; in E, 0.2 mm; in F, 0.2 mm.
The increase in fat cells is not due to hyperproliferation of the endogenous fat-cell lineage

At least two distinct mechanisms could account for the expansion of the fat body in the twist-GAL4;UAS-srp embryos. Firstly, the misexpression of srp could alter cell fates within the mesoderm by recruiting cells into the fat-cell developmental pathway. Alternatively, premature and ectopic expression of srp in the mesoderm could cause hyperproliferation of endogenous precursor fat cells. To distinguish between these possibilities, we employed the P-element enhancer-trap line, P[29D], as a lacZ reporter gene for the primary fat-cell clusters in PS 4-9 and in the secondary fat-cell clusters in PS 10-12 (Fig. 3A,C,E; Hoshizaki et al., 1994). Early expression of P[29D] is independent of srp (Sam et al., 1996). By marking precursor fat cell with the P[29D] lacZ reporter we could test whether expression of srp throughout the mesoderm causes hyperproliferation of endogenous fat cells. We found lacZ expression was not significantly altered in twist-GAL4;UAS-srp embryos. Specifically, the number and organization of the fat cells marked by P[29D] appeared normal (Fig. 3B). Thus, the increase in fat cells is likely not to be due to hyperproliferation of endogenous fat-cell lineage although we have not eliminated the possibility that the ectopic fat cells originate from the secondary fat-cell clusters not marked by P[29D].

Misexpression of srp disrupts gonad formation

The forced expression of srp in the mesoderm results in the production of ectopic fat cells. Because it is not likely that the endogenous fat-cell lineage undergoes hyperproliferation, we suggest that srp might be capable of inducing fat-cell development in cells that normally would not contribute to the fat body. Such a capability has been proposed for srp in the developmental choice model between somatic gonadal precursors (SGPs) and precursor fat cells (Moore et al., 1998; Riechmann et al., 1998). If srp activity can direct a fat-cell fate upon a common precursor that otherwise would be specified as
SGPs, then it follows that ectopic expression of \textit{srp} should repress the specification of SGPs in PS 10-12 and these cells should be replaced by fat cells.

To test for \textit{srp}'s role in the repression of SGP specification, we examined \textit{twist-GAL4;UAS-srp} embryos using 412 as a cell marker for the SGPs and the gonadal mesoderm (Brookman et al., 1992). In wild-type stage-12 embryos, 412 is expressed in PS 10-12 in the SGPs and in cell clusters in PS 2-9 and 14 (Fig. 4A). During germband retraction, expression of 412 declines but persists in the SGPs (PS 10-12; Brookman et al., 1992). It is unclear which cell lineage(s) the PS 2-9 and PS 14 clusters of cells represent (Tan et al., 1996). However, by over-staining for 412 transcripts we can detect late 412 expression in the dorsal-most cells of the lateral fat body (Hoshizaki and Hayes, unpublished). At stage 13, the SGPs migrate and coalesce to form the gonadal mesoderm cells that will eventually ensheathe the germ cells (Fig. 4C,E). At stage 16, 412 transcripts are still detected in the gonadal mesoderm (Fig. 4G,I; Brookman et al., 1992).

In stage-12 \textit{twist-GAL4;UAS-srp} embryos the expression of 412 is similar to that observed in wild-type embryos (compare Fig. 4A and 4B). The SGPs are specified and initial association of pole cells with gonadal precursors appears normal (data not shown). The first alteration in the development of the gonadal mesoderm is detected at stage 13 when the SGPs fail to migrate and coalesce (Fig. 4D,F). The number of 412-expressing cells are dispersed in the posterior region of the embryo (Fig. 4H,J). Misexpression of \textit{srp} in the mesoderm does not affect the formation of the SGPs, however it does disrupt the ability of the SGPs to migrate and coalesce into a gonad.

To confirm the effects of \textit{srp} on SGP and gonad formation, we examined \textit{twist-GAL4;UAS-srp} embryos for expression of a second gonadal mesoderm cell maker, \textit{clift} (\textit{cli}) (Boyle et al., 1997). \textit{cli} is expressed throughout the mesoderm but by early stage 11 is lost in most mesodermal cells. During late stage 11, \textit{cli} expression is detected in SGPs and in lateral muscle precursors as well as in the ectoderm (Boyle et al., 1997). Based on mutational analysis of a transcript-producing \textit{cli} mutant, \textit{cli} is necessary for maintenance of SGPs and their migration and coalescence into a gonad (Boyle et al., 1997).

In \textit{twist-GAL4;UAS-srp} embryos, misexpression of \textit{srp} does not affect early \textit{cli} expression in the mesoderm (Fig. 5B). However, in older embryos, \textit{cli} transcripts are not detected in either the SGPs or in the precursors of the lateral muscles although \textit{cli} expression is still detected in the ectoderm (see Fig. 5D). Because \textit{cli} is necessary for the migration and coalescence of the SGPs, the inability of the SGPs to form a mature gonad is most likely due to the loss of \textit{cli} expression in these cells. We suggest that in the experimental embryos, \textit{srp} does not prevent the specification of the SGPs but can block the differentiation of the SGPs by repressing \textit{cli} expression in these cells.

**Ectopic fat cells are correlated with the loss of muscle cells**

To test whether other lineages are affected by misexpression of \textit{srp}, we examined \textit{twist-GAL4;UAS-srp} embryos for heart and visceral muscle precursors. To mark heart precursors, we employed the homeobox gene \textit{tinman} (\textit{tin}; Bodmer, 1993). \textit{tin} is involved in the specification of the dorsal mesoderm and in the formation of heart muscle precursors (Azpiazu and Frasch, 1993). In embryos carrying \textit{twist-GAL4;UAS-srp}, heart precursors develop normally to form the heart (data not shown). Thus, ectopic expression of \textit{srp} does not affect heart development.

We used the \textit{bagpipe} (\textit{bap}) gene to mark the visceral mesoderm, which gives rise to visceral muscle precursors. The visceral mesoderm is made up of 10 metameric clusters of cells located in the dorsal mesoderm (Fig. 6A). \textit{bap} is expressed in these cells and is necessary for the formation of the visceral muscle (Azpiazu and Frasch, 1993). We found that \textit{twist-GAL4;UAS-srp} embryos lacked the \textit{bap}-expressing cells (Fig. 6B) and exhibited a \textit{bap}-like phenotype, in which the midgut failed to undergo its normal constrictions (Fig. 6D; Azpiazu and Frasch, 1993). The loss of \textit{bap}-expressing cells in the experimental embryos might reflect a cell-fate change that...
allows the replacement of visceral muscle precursors by fat-cell precursors (see Discussion).

Finally, we investigated the effect of srp misexpression on somatic or body-wall muscle. The absence of cli-expressing lateral (body wall) muscle precursors suggests that these muscles might be absent in the experimental embryos (see below). The body-wall muscle is derived from the lateral region of the slp domain that exhibits the highest levels of twist expression (Borkowski et al., 1995). We used nautilus (nau) and S59 as cell markers for a subset of the founder or precursor cells for body-wall muscle (Dohrmann et al., 1990; Michelson et al., 1990; Paterson et al., 1991) and the tropomyosin 1 (Tml) gene as a marker for body-wall muscle fibers (Bautch et al., 1982). nau is first active at stage 10 and is expressed in a dynamic pattern (Michelson et al., 1990; Paterson et al., 1991). nau expression is detected in cells flanking the ventral midline and later in lateral and dorsolateral cell clusters. S59 expression is initially detected in a single, large mesodermal cell in a segmentally repeating pattern. At late stage-11 these cells give rise to two founder muscle cells and in each segment a second cluster of four S59-expressing cells that also contributes to precursor muscles. (B,D) twist-GAL4;UAS-srp embryos. The initial nau expression pattern is disrupted and by late stage 11 variable cell clusters are missing. (D) S59 expression is also disrupted and there is a loss of S59-expressing cells.

\[srp\] gene has several roles in the developing fat cell. srp is a direct transcriptional activator of Adh in the fat body (Abel et al., 1993) and is involved in the larval fat-cell specific expression of Cecropin A1 (Petersen et al., 1999). Based on genetic studies, using mis-sense and non-sense srp mutants, srp is also necessary for maintaining the fat-cell lineage (Sam et al., 1996 and see figure 6 in Rehorn et al., 1996). Recently, Moore et al. (1998) and Riechmann et al. (1998) have suggested that the primary fat-cell clusters in PS 4-9 and the SGPs in PS 10-12 are derived from homologous metameric clusters of cells. Central to determining the fate of these cells is srp, where srp activity specifies a fat-cell fate in the precursor cells that otherwise would adopt an SGP fate.

Here, we have further examined the role of srp in fat-cell development and its possible role in this developmental switch. We find that srp, in addition to maintaining the fat-cell lineage, is sufficient to promote fat-cell development. We favor the idea that production of ectopic fat cells is not due to the hyperproliferation of the endogenous fat cells but rather is the result of changes in either cell fate or proliferation of potential fat cells. The capability of srp to generate fat cells is also dependent upon other factors because premature expression of srp does not result in premature expression of fat-cell genes nor does misexpression of srp in the ectoderm result in ectopic fat cells. It is likely that the preservation of the correct temporal sequence reflects the dependence of srp upon temporally restricted mesoderm factors and/or whether a cell has previously acquired the potential to develop as a fat cell.

**Is srp involved in a developmental choice between fat cells and SGPs?**

It has been proposed that within PS 4-12 of the lateral mesoderm there resides metamERICALLY repeating precursors that have the potential to give rise to either precursor fat cells or SGPs. Based on mutational studies, segmentation and dorsoventral patterning genes control srp activity within the common precursors (Riechmann et al., 1998; Moore et al., 1998). In PS 4-9 srp is active in the common precursor while in PS 10-12 the presence of abdominal-A represses srp in the common precursor. Double mutant analysis of abdA and srp...
confirms that abdA’s role in SGP formation is solely to repress srp (Moore et al., 1998). The choice between fat-cell precursors and SGPs is dependent upon srp, where srp activity induces fat-cell specification while blocking SGP specification. The ground or default state of the common precursor is a somatic gonadal mesoderm cell fate. Thus, in the absence of srp the SGPs are specified (Riechmann et al., 1998; Moore et al., 1998).

We have tested srp for some of its proposed activities in this cell-fate switch model. We find that srp is indeed capable of inducing fat-cell formation. srp can also disrupt gonad formation and appears to do so by preventing cli expression in the SGPs. cli function is necessary for 412 expression and for the migration and coalesce of the SGPs to form the gonads but not for the specification of the SGPs (Boyle et al., 1997). In the srp misexpression embryos, early expression of cli and 412 is normal; both wild-type pan-mesoderm expression of cli and the metamerically repeating expression of 412 are readily detected. The normal decline of 412 expression takes place in PS 2-9 and 412 expression persists in the SGPs (PS 10-12). The two most notable effects of misexpression of srp are the absence of cli expression in the SGPs and the inability of the SGPs to migrate and coalesce to form the gonads. It is likely that the defect in gonad formation is due to loss of cli expression specifically in the SGPs. We also note that loss of cli expression in the SGP does not affect the 412-specific SGP expression. It is likely that the early pan-mesoderm expression of cli is responsible for 412 expression.

We find that srp has only one of its two predicted activities required for the common precursor model. srp is capable of inducing fat-cell formation but is not able to repress SGP specification. To reconcile these results, we suggest that the precursors of the SGPs and the fat cells are derived from independent groups of cells and that positional cues within each parasegment specify which group will differentiate and be maintained. This model, in general terms, is analogous to the interaction between the Wolffian and Müllerian ducts. In early mammalian development, before gonadal differentiation both ducts are present. In females the Müllerian duct develops into the oviduct and the Wolffian duct degenerates, while in males the production of Müllerian inhibition substance in the testes causes the degeneration of Müllerian ducts and the Wolffian duct develops into the vas deferens (Higgins et al., 1989).

In this alternative model, the absence of srp activity in PS 10-12 would allow the SGPs to be specified and differentiate, while the fat-cell precursors would not be maintained. We have previously demonstrated that srp is necessary for the maintenance of the fat-cell lineage (Sam et al., 1996). In PS 4-12 srp activity would allow the proliferation and maintenance of the precursor fat cells and the SGPs would not differentiate. We report here that srp has the capability to block SGP differentiation by repressing cli expression.

**srp can disrupt muscle differentiation**

The misexpression of srp in the mesoderm also results in the loss of body wall and visceral muscle. The body wall muscle consists of a syncytium that is derived from a single founder muscle cell that serves as a focal point for fusion with the surrounding myoblasts (Knirr et al., 1999; Rushton et al., 1995). We used nau and S59 to mark some of the founder muscle cells and find that misexpression of srp results in the loss of both nau-expressing and S59-expressing founder muscle cells and consequently loss of body wall muscles. Based on genetic analysis, nau is a non-essential gene that is not necessary for the formation of muscle precursors. However, a distinct subset of muscle fibers is missing in individuals lacking nau activity (Keller et al., 1998). The misexpression of srp results in a severe muscle defect where most muscle fibers and myoblasts are absent. It is likely that this is due to the absence of the founder muscle cells. Whether the loss of myoblasts is a secondary effect caused by the loss of founder muscle cells or is a direct consequence of srp expression is not known.

The misexpression of srp also results in the loss of the visceral muscle. This loss is due to the absence of the precursor cells as marked by bap expression in stage-10 embryos. Because bap is the earliest known marker for the visceral muscle precursors, we cannot distinguish between loss of bap expression and loss of the precursor cells. We favor the idea, however, that srp prevents the specification of the precursors of the visceral mesoderm prior to bap expression.

It is clear that srp is capable of disrupting the establishment and differentiation of specific mesodermal derivatives and inducing fat-cell formation. The origin of the ectopic fat cells however is unknown. The fat body is derived from cells that originate in distinct positions. In the lateral mesoderm the primary fat-cell clusters and the SGPs represent metamerically repeating clusters. We suggest that the precursors of the primary fat-cell clusters and the SGPs coexist in each parasegment and misexpression of srp results in the formation of ectopic fat cells by allowing the differentiation of precursor fat cell in PS 10-12.

In the dorsal mesoderm in PS 2-13 the precursors of the visceral muscle and the dorsal fat-cell projections might represent homologous metameric repeating cell clusters (Riechmann et al., 1998; Miller and Hoshizaki, unpublished results). Misexpression of srp also leads to the complete absence of visceral muscle precursors. We suggest that a common precursor might exist between the precursors of visceral muscle and of the dorsal fat-cell projection and srp might control a developmental switch between these two cell types. Alternatively, the precursors of visceral muscle and the dorsal fat-cell projection might coexist in each parasegment (PS 2-13) and srp determines which cell type will flourish. In either model, the misexpression of srp would result in ectopic fat cells. In the common precursor model, misexpression of srp would drive the common precursors in PS 2-12 to a fat-cell fate and in the alternative model, misexpression would allow the coexisting precursor fat cells in PS 2-12 to differentiate. Experiments to further test these ideas and to identify factors that cooperate with srp in fat-cell development are in progress.

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