A molecular aspect of hematopoiesis and endoderm development common to vertebrates and Drosophila

Klaus-Peter Rehomer¹, Heidi Thelen¹, Alan M. Michelson² and Rolf Reuter¹,*

¹Institut für Genetik, Universität zu Köln, Weyertal 121, D-50931 Köln, Germany
²Howard Hughes Medical Institute, Brigham and Women’s Hospital, 20 Shattuck Street, Boston, MA 02115, USA

*Author for correspondence (e-mail: reuter@gen1.genetik.uni-koeln.de)

SUMMARY

In vertebrates, transcriptional regulators of the GATA family appear to have a conserved function in differentiation and organ development. GATA-1, -2 and -3 are required for different aspects of hematopoiesis, while GATA-4, -5 and -6 are expressed in various organs of endodermal origin, such as intestine and liver, and are implicated in endodermal differentiation. Here we report that the Drosophila gene serpent (srp) encodes the previously described GATA factor ABF. The multiple functions of srp in Drosophila suggest that it is an ortholog of the entire vertebrate GATA family. srp is required for the differentiation and morphogenesis of the endodermal gut. Here we show that it is also essential for Drosophila hematopoiesis and for the formation of the fat body, the insect organ analogous to the liver. These findings imply that some aspects of the molecular mechanisms underlying blood cell development as well as endodermal differentiation are early acquisitions of metazoan evolution and may be common to most higher animals.

Key words: Drosophila, gut, endoderm, hemocyte, fat body, GATA factor

INTRODUCTION

The alimentary canal of Drosophila is an excellent model system for studying molecular mechanisms which underlie organogenesis. It is composed of four major components: the foregut, the midgut, the Malpighian tubules and the hindgut. The midgut is the endodermal part of the gut. It is formed from two primordia which are spatially separated by the mesoderm primordium ventrally and by the primordium of the ectoderm in the dorsolateral region of the blastoderm embryo. The anterior aspect of the endoderm (anterior midgut) is derived from the ventral side of the anterior pole. In addition, cells that originate from the anterior tip of the ventral furrow, also called anterior midgut invagination, may contribute to the anterior midgut. The posterior part of the endoderm (the posterior midgut) derives from the posterior pole of the embryo and becomes internalized during the amnioserosal evagination. Both parts of the midgut later lose their epithelial character and become mesenchymal. The foregut develops from the stomodeum which invaginates in the region anterior to the ventral furrow about two hours after the proctodeum and the posterior midgut have invaginated.

The terminal gap gene huckebein (hkb) establishes the primordia of both anterior and posterior midgut (Weigel et al., 1990; Brömmner et al., 1994; Reuter and Leptin, 1994). It sets the border between endoderm and mesoderm, and it is essential for the invagination of the posterior midgut primordium from the posterior pole. However, its action is not entirely specific to the endoderm, since in hkb embryos the stomodeum also fails to invaginate. Hence, most of the foregut does not form (Reuter and Leptin, 1994). hkb encodes a transcription factor with a zinc finger binding domain of the Sp1/egr type (Brömmner et al., 1994). We have proposed previously that the gene serpent (srp) is one of the genes which are regulated by hkb, and acts as a selector gene in midgut development (Reuter, 1994). srp is essential for organ-specific morphogenesis and differentiation, and it is required to prevent the midgut primordia from adopting the fate of ectodermal foregut or hindgut, respectively. srp does not participate in other functions of hkb, since it is not implicated in setting the borders of the midgut primordia and is not required for the gastrulation movements of these cells or in the invagination of the stomodeum. However, it should be noted that in addition to its function in midgut development, srp is also essential for differentiation of the yolk cell and of the extraembryonic amnioserosa (Reuter, 1994). We were interested to elucidate
the molecular nature of the srp gene product because of its fundamental role in endodermal development. In this paper we present evidence that srp encodes a protein which belongs to the GATA family of transcription factors, previously described as ABF or dGATAb (Abel et al., 1993). In vertebrates transcriptional regulators of the GATA family apparently have a conserved function in differentiation and organ development. GATA-1 is required for primitive and definitive erythropoiesis (Pevny et al., 1991), GATA-2 for early hematopoiesis (Tsai et al., 1994), while GATA-3 is implicated in the differentiation of T-lymphocytes (Ko et al., 1991). Other members of the family, GATA-4, -5 and -6, are expressed during development in various organs of endodermal origin (Arcacci et al., 1993; Laverriere et al., 1994) and are implicated in endodermal differentiation (Tamura et al., 1993; Soudais et al., 1995). Since srp is essential for endodermal development and, as we also show, for hematopoiesis in Drosophila, we consider srp as a gene which is functionally homologous to several, if not all members of the vertebrate Gata family.

MATERIALS AND METHODS

Fly stocks

St srp<sup>6G</sup> e and ru h th st cu srp<sup>HO1</sup> sr e ca: amorphic alleles and probably strong hypomorphic allele (Jürgens et al., 1984). Both alleles form RNA in homozygous srp<sup>6G</sup> embryos so srp protein is detectable (data not shown). St e and ru h th st cu sr e ca: parental chromosomes of srp<sup>6G</sup> and srp<sup>HO1</sup>, respectively (obtained from the Tübingen stock collection). srp<sup>AS</sup> (known as Ins(3R)neo<sup>45</sup>) and srp<sup>PZ</sup> (known as Ins(3R)PZI<sup>349</sup>); non-complementing P element insertions in 89B (Cooley et al., 1988). The new srp alleles srp<sup>H01</sup> to srp<sup>H03</sup> were generated by mobilization of the P element of srp<sup>AS</sup> (which is not marked by an eye color gene) with the transposase source Δ2-3 on a TM3 balancer (Laski et al., 1986; Reuter et al., 1993b). They were isolated by screening about 700 candidate lines for the visible embryonic srp phenotype. The other srp alleles were isolated as eye color revertants of srp<sup>AS</sup> after mobilization of the P element with the stable transposase source and later classified as either full revertants, partial revertants (srp<sup>PZHO1-20</sup>) or new amorphic alleles (srp<sup>PZHO1-20</sup>). hkb: hypomorphic hkb allele; Df(3R)hkb<sup>5</sup>; commonly used as hkb deficiency (Weigel et al., 1990).

In situ detection of RNA and protein

mRNA was detected in situ as described by Tautz and Pfeifle (1989) and protein as described previously (Reuter et al., 1993a). The anti-Peroxidase antibody has been described by Nelson and coworkers (1994).

Microscopy

Pictures were taken on a Zeiss Axiohot microscope using Kodak Ektachrome 64T or Agfachrome 100RS slide film and were digitized by transfer to Kodak PhotoCDs. The figures were assembled in Adobe Photoshop on a Macintosh PowerPC and were printed on a Kodak dye sublimation printer.

Isolation of the srp locus

Genomic DNA flanking the P element insertion of srp<sup>AS</sup> was isolated by plasmid rescue (Cooley et al., 1988). Using this DNA as probe a cosm id library was screened (Tamkun et al., 1992). One of the identified genomic clones (89B) contained the srp transcription unit. The P element insertion of srp<sup>AS</sup> and the deletions associated with srp<sup>H01</sup> to srp<sup>H03</sup> were mapped by Southern blotting. The genomic organization of srp, the sequence of the srp<sup>PZ</sup> and srp<sup>HO1</sup> alleles, the site of the P element insertion of srp<sup>PZ</sup> and the molecular nature of some of the new srp alleles derived from srp<sup>PZ</sup> were determined by sequencing of genomic DNA using appropriate primers. DNA from Oregon R flies, from the cosmid #9 and the cDNAs served as wild-type DNA. Mutant DNA was prepared from homozygous srp embryos which were identified on the apple juice agar plates by their visible embryonic phenotype. In the genomic regions where srp<sup>AS</sup> and srp<sup>6G</sup> deviated from wild-type also the corresponding parental chromosomes st e and ru h th st cu sr e ca were sequenced. The 5’ end of the abf cDNA is not present in the srp locus and is probably an artifact. The bases 3-84 of the abf cDNA (Abel et al., 1993) are a direct repeat of the bases 398-479. The accession number of the srp cDNA at the EMBL Nucleotide Sequence Database is Y07662.

Phylogenetic analysis

The evolutionary tree displayed in Fig. 2C was generated by the program 'protpars' written by J. Felsenstein as part of the Phylip package (Felsenstein, 1988). The tree is not the only possible tree, but it is the most frequent one in the output of 50 independent runs of the program. 98% of the trees showed the same principal arrangement of the vertebrate, the invertebrate and the fungal GATA factors.

RESULTS

Molecular genetics of the srp locus

We cloned and characterized the srp locus using the P-element-induced allele srp<sup>AS</sup> (Fig. 1). This allele fails to complement the lethality of the two independently isolated, EMS-induced alleles srp<sup>PZ</sup> and srp<sup>6G</sup>, but it does not affect gut development or the other known srp functions (data not shown and Fig. 5H). About 8 kb to the 5’ side of the P-element of srp<sup>AS</sup> we identified a transcription unit which we considered as a candidate gene for srp, since it is expressed in the expected embryonic regions, i.e. in the midgut primordia, the amnioserosa and the yolk nuclei (see Fig. 3). Several srp alleles specifically affect this transcription unit. The alleles srp<sup>H01</sup>, srp<sup>H02</sup> and srp<sup>H03</sup> were isolated after mobilization of the P element of srp<sup>AS</sup>. srp<sup>H01</sup> and srp<sup>H03</sup> are associated with small deletions (Fig. 1) and abolish all known srp functions. The srp candidate gene is not transcribed from these alleles, but no other transcription units to the 3’ side of the P element are affected (data not shown). srp<sup>H02</sup>, associated with a smaller deletion (Fig. 1), is an allele of intermediate strength and partially reduces srp function in the midgut and amnioserosa. It is associated with a significant reduction of expression in the respective embryonic regions (data not shown). Another P-element-induced srp allele is srp<sup>PZ</sup> which also results in the lack of srp transcription. The P element of srp<sup>PZ</sup> is inserted about 30 bp 5’ of the putative srp transcription start site. The insertion itself affects the srp function in srp<sup>PZ</sup>, since mobilization of the P element led in 28 out of 72 cases to a complete reversion of the phenotype, and restored viability. In 20 cases, amorphic srp alleles were isolated that had been generated by imprecise excision of the P element. The remaining 24 cases were novel hypomorphic srp alleles that are partial revertants of srp<sup>PZ</sup> with respect to the midgut or germ band retraction phenotype, but which do not complement the other srp alleles (with the exception of srp<sup>AS</sup>) to adult viability.

Sequence analysis of the transcription unit showed that the srp candidate gene encodes the previously identified Drosophila GATA factor ABF, also known as dGATAb (Abel et al., 1993).
Enhancer trap and is inserted about 30 bp 5' sequenced both EMS-induced phenotype of homozygous (Fig. 2A). This finding is consistent with the amorphic associated with a nonsense mutation that would lead to the blood primordium. The alleles srp603 and srp603 abolish all known srp functions; the srp gene is not transcribed in these alleles. The P element insertion ins(3R)PZ1549 (srpPZ), which also results in the lack of srp transcription, acts as an enhancer trap and is inserted about 30 bp 5' of the putative transcription start site. Within the srp transcription unit the zinc finger motif is indicated in black, the open reading frame is shaded grey and the untranslated regions are open.

To confirm that the srp gene corresponds to abf we have sequenced both EMS-induced srp alleles. The srp6G allele is associated with a nonsense mutation that would lead to the translation of a truncated protein lacking the zinc finger domain (Fig. 2A). This finding is consistent with the amorphic phenotype of homozygous srp6G embryos. In the srp9L allele an asparagine (N29 of the consensus sequence in Fig. 2B) is replaced by a lysine within the second cysteine pair of the zinc finger motif. This mutation probably causes the strong loss-of-function phenotype of homozygous srp9L embryos, since asparagine N29 is apparently essential for the DNA-binding capacity of the GATA factors; this asparagine is present in all members of the family described to date and has been proposed to contact two central DNA residues of the GATA consensus binding site (Omichinski et al., 1993). No polymorphisms were observed which would alter the protein sequence apart from a poly-glutamine stretch 3 amino acids shorter between amino acids 118 and 120 on the st e and the st srp6G e chromosomes.

Expression of the srp gene
During the blastoderm stage and gastrulation srp is expressed in five regions of the Drosophila embryo (Abel et al., 1993). At the anterior pole srp is found in a region (Fig. 3A,B) which later invaginates through the stomodeum (Fig. 3C) before expression of srp is down-regulated (Fig. 3D). This region is the primordium of the anterior midgut (see Discussion). At the posterior pole, the srp-expressing cells invaginate as the posterior midgut primordium (Fig. 3A,B) and likewise cease expression some time after the invagination (Fig. 3C). The expression of srp in the anterior and the posterior midgut primordium is not observed in hkb embryos (Fig. 4B), while expression in the other domains is initiated normally. Thus, as predicted, srp is downstream of hkb within the genetic hierarchy that directs midgut development.

On the dorsal side of the embryo srp gene product is seen in the primordium of the amnioserosa (Fig. 3A,B) and later in the amnioserosa itself while it connects the dorsal edges of the germ band (Fig. 3C-F). In the center of the embryo srp is expressed by the yolk nuclei (Fig. 3A), most of which subsequently migrate to the periphery of the yolk (Fig. 3B). Finally, srp is expressed in a patch of cells within the mesoderm primordium, which was not expected from the previously described phenotype of srp. These cells invaginate with the ventral furrow anterior to the cephalic furrow (Fig. 3A,B) and become located laterally to the stomodeum which in turn expresses srp (Fig. 3C). Slightly later they differentiate into prohemocytes which migrate into the head (Fig. 3D-F). Subsequently, these cells become distributed throughout the body and differentiate as mature hemocytes, at which point srp expression is downregulated (Fig. 3G). We propose that the mesodermal patch of srp expression constitutes the hemocyte primordium at blastoderm stage.

**srp function in hematopoiesis**
The expression of srp in the putative hemocyte primordium...
raised the question of whether srp has a function in hematopoiesis. The *Drosophila* blood cells (hemocytes) are part of the insect immune system and mainly develop into macrophages. They are known to derive from the anterior part of the insect immune system and mainly develop into hematopoiesis. The **OXIDASIN** (Tepass et al., 1994; Nelson et al., 1994). In **B**

**Fig. 2.** (A) Predicted sequence of the srp protein. Our data suggests that the srp protein is 949 amino acids in size. The C-terminal 680 amino acids (including the zinc finger domain; underlined) are identical with the reported sequence of **ABF** (Abel et al., 1993). An asterisk indicates the position of the stop codon found in the srp**Δ** allele, triangles indicate the position of the introns.

(B) Comparison of the zinc finger domain of srp with the (if applicable C-terminal) zinc finger of other GATA factors. In the srp**Δ** allele the conserved asparaginase N29 is replaced by a lysine. The amino acids diagnostic for GATA-1/2/3 (Evans and Felsenfeld, 1989; Zon et al., 1990, 1991; Yamamoto et al., 1990) and GATA-4/5/6 (Tamura et al., 1993; Felsenfeld, 1989; Zon et al., 1990, 1991; Yamamoto et al., 1990) and the fungal GATA factors regulating nitrogen or siderophore metabolism (AreA: Kudla et al., 1990; Haas et al., 1995; Nrt-2: Fu and Marzluf, 1990; GlN3: Minehart and Magasanik, 1991; UrtB1: Voisard et al., 1993) each form a branch. There is no significance to the length or to the angle of the lines. Every node represents an inferred intermediate in the evolution of the sequences.

**GATA factors based on the domain of 66 amino acids shown in B. The unrooted tree displays the most parsimonious way in which the protein sequences could have evolved.**

The sub-families GATA-1/2/3, GATA-4/5/6 (Tamura et al., 1993; Felsenfeld, 1989; Zon et al., 1990, 1991; Yamamoto et al., 1990) and the fungal GATA factors regulating nitrogen or siderophore metabolism (AreA: Kudla et al., 1990; Haas et al., 1995; Nrt-2: Fu and Marzluf, 1990; GlN3: Minehart and Magasanik, 1991; UrtB1: Voisard et al., 1993) each form a branch. There is no significance to the length or to the angle of the lines. Every node represents an inferred intermediate in the evolution of the sequences.

Interestingly, srp**Δ** the P-element-induced allele which
does not affect the function of *srp* in gut, yolk or amnioserosa, specifically disturbs hemocyte differentiation. In homozygous *srp* embryos the number of hemocytes is severely reduced (data not shown), and no hemocytes are formed in embryos *trans*-heterozygous for *srp* and either of the strong EMS-induced *srp* alleles: *srp*G and *srp*6G (Fig. 5H). Consistent with the failure of hemocyte development a drastic decrease of *srp* expression is observed in the putative hemocyte primordium of hemizygous *srp* embryos (Fig. 5J). Other aspects of *srp* expression are not altered. Thus, the P element insertion of *srp* specifically affects the *srp* function required for development of the hemocytes, and it is likely that the P-element is inserted into a regulatory region of the gene which is essential for the anterior mesodermal *srp* expression.

**srp** function in fat body development

During germ band extension expression of *srp* is initiated in the developing fat body, one of the subdivisions of the mesoderm (Abel et al., 1993; see also Fig. 3C). It is expressed in a series of nine clusters of cells located bilaterally within the mesoderm of thoracic segment t2 to the abdominal segment a7. These clusters begin to form a sheet of cells on each side of the embryo during late stage 11 (Fig. 3F) from which the fat body develops (Fig. 3G,H). *srp* continues to be expressed in the fat body until the end of embryogenesis. The GATA transcription factor encoded by *srp* has been described to be sufficient to activate the fat body-specific expression of *Alcohol dehydrogenase (Adh)* (Abel et al., 1993). In fact, *srp* is required for that expression, since *Adh* transcription in the mesoderm is not detected in *srp* mutant embryos (data not shown). However, the lack of *Adh* expression in these embryos does not necessarily support the notion of a direct activation of *Adh* transcription by *srp* in vivo, since fat body development is impaired at a very early stage in the mutants.

The fat body precursors, which can be visualized by their *srp* RNA expression, are present in embryos homozygous for the *srp*6G or the *srp*G allele (Fig. 6B). However, the cells do not proliferate and do not rearrange to form the continuous sheet of cells observed in wild-type embryos at late stage 11 (Fig. 6A). Furthermore, the early events of fat body differentiation do not take place in *srp* embryos. *seven-up (svp)* has been described as a gene with an important role in fat body development (Hoshizaki et al., 1994). Expression of *svp* commences in the fat body precursors at about stage 11 and is maintained in the maturing fat body until stage 15 (Hoshizaki et al., 1994). In *srp* embryos *svp* expression is not initiated in the mesoderm at a position which would correspond to the fat body or its precursors (Fig. 6D,F), however the expression of *svp* within the ventral nervous system is not affected. Thus, *srp* is essential for the earliest known steps in the morphogenesis and the differentiation of the fat body.

**DISCUSSION**

We have identified the previously described GATA factor *ABF* (Abel et al., 1993) as the product of the gene *srp*. *srp* is expressed in five principal regions of the embryo: the midgut primordium, the yolk, the putative hemocyte primordium, the amnioserosa and the fat body. In all of these regions *srp* is required for proper development (Reuter, 1994 and Figs 5, 6). Three *srp* alleles are associated with small deletions which specifically affect the *srp* transcription unit (Fig. 1). One P element insertion is associated with a decrease in *srp* transcription in the putative hemocyte primordium and with a specific failure in hemocyte development (Fig. 5). This insertion probably affects a regulatory region within the *srp*
gene that is essential for this aspect of the expression pattern. One other P element insertion abolishes all srp functions: a reversion of the insertion alleviates the phenotype. Finally, the two EMS-induced srp alleles are associated with mutations in the coding sequence which in the case of srp6G eliminate, and in the case of srp9L most likely eliminate the ability of srp to act as a transcriptional regulator. The nonsense mutation in srp6G results in a truncated protein lacking the zinc finger domain. The missense mutation of srp9L replaces an asparagine (N29) within the DNA binding domain which is conserved in all known members of the GATA family (Fig. 2B). Exchanges of amino acids conserved to the same degree in the zinc finger domain of the fungal GATA factor AREA are also associated with a loss of function (Kudla et al., 1990). Furthermore, the asparagine N29 is one of the residues of the GATA zinc finger domain which contacts the core of the consensus DNA binding motif (Omichinski et al., 1993), a function which cannot be fulfilled by the lysine found at this position in srp9L. Taken together, our findings establish that the transcription unit encoding the GATA factor ABF is identical to srp.

**srp function in the Drosophila embryo**

srp is required for proper differentiation and morphogenesis of the midgut and has been suggested to act as a selector gene in gut development acting downstream of hkb (Reuter, 1994). The pattern of srp expression and its dependence on hkb in the midgut primordia are consistent with such a function (Fig. 4). However, a role in terminal midgut differentiation can probably be excluded. The SRP protein disappears from the tissue before germ band retraction is completed (data not shown). Of particular interest is the domain of srp expression at the anterior pole. This domain is located at the ventral side of the embryo anterior to the ventral furrow. It corresponds to the stomodeal anterior midgut primordium which has been shown by lineage analysis to contribute to the anterior part of the anterior midgut (Technau and Campos-Ortega, 1985). In hkb embryos no anterior midgut is formed, and the only srp expression domain missing in the anterior half of the embryo is the anteroventral domain (Fig. 4). We therefore propose that the anterior midgut is exclusively derived from this single primordium at the ventral side of the anterior pole. That would imply that most cells of the anterior midgut are internalized by the stomodeal invagination (Fig. 3C). Only few cells invaginate as the front edge of the ventral furrow (visible for instance in the Figs 3B or 5I).

SRP (ABF) is capable of activating transcription of fat-body-specific genes (Abel et al., 1993; Loskky and Wensink, 1995). Here we have shown that it is required for the early steps in fat body morphogenesis...
and differentiation (Fig. 6). Based on the early onset of expression, srp might even determine the fat body precursors and integrate the positional information that specifies them in the mesoderm. Since a mature fat body is not formed in srp embryos, it has to date not been possible to rigorously test whether srp is indeed also required for the terminal differentiation in the fat body, e.g. for Adh transcription. srp, however, is the only known GATA factor expressed at this time in the fat body, and therefore it is very likely that it is responsible for tissue-specific gene activation. srp would then have a biphasic function in the physiology of the fat body, for the early morphogenesis and differentiation and for the maintenance of organ-specific gene expression.

A striking finding is the function of srp in the hematopoiesis of Drosophila. No mature hemocytes are formed in srp embryos, and the earlier differentiation of prohemocytes is also impaired in the mutants (Fig. 5). Furthermore, expression pattern and phenotype of srp suggest that a particular region within the anterior mesoderm is the primordium of the hemocytes. The cells of the expression domain located ventrally in front of the cephalic furrow can be traced until they become morphologically distinguishable as hemocytes. Moreover, the srp<sup>ts9</sup> allele, which specifically affects hemocyte development, reduces srp expression in this domain of the mesoderm (Fig. 5H,J). However, lineage analysis is required to confirm that the proposed primordium exclusively gives rise to hemocytes, as the srp expression pattern and srp phenotype suggest. srp expression in the putative hemocyte primordium commences at blastoderm stage (Fig. 3A) and is maintained as protein expression when the mature hemocytes already migrate through the embryo (data not shown). It is therefore conceivable that srp has a biphasic function in hemocyte development as possibly it does in the fat body. srp might be required for the specification of the blood cells within the mesoderm at an early stage and subsequently later for gene expression during their differentiation and maturation.

**srp is a Drosophila ortholog of the vertebrate Gata gene family**

Since srp is required for hematopoiesis in Drosophila, as Gata-1, -2, and probably -3 are in mice, and for endodermal development, as Gata-4, -5 and -6 probably are, srp can be considered as a Drosophila ortholog of the entire vertebrate GATA family. We do not know yet to what degree the processes of hematopoiesis and endodermal development are homologous at the molecular level in insects and in vertebrates. For instance, it is unclear to what extent the sets of genes that are regulated by these GATA factors overlap in different phyla. Nevertheless, we suspect that these processes are far more similar than previously expected, supporting the notion that the principal molecular mechanisms underlying organogenesis are early phylogenetic achievements.

The Gata-4/5/6 genes are also implicated in the differentiation of the vertebrate heart. srp does not have a corresponding role in Drosophila, and it is open to discussion whether a GATA factor exists in the fly which functions in this respect. One other GATA factor, PANNIER (PNR) or dGATAaa, has been found in Drosophila which is reportedly expressed in the amnioserosa and the dorsal epidermis adjacent to the cardiac progenitor cells (Ramain et al., 1993; Winick et al., 1993). However, so far there is no indication that pnr fulfills a function homologous to the one of the vertebrate Gata genes. The failure of pnr mutant embryos to form a proper heart (data not shown) is probably due to the general defects in differentiation and morphogenesis on the dorsal side of the body.

The hematopoietic system of Drosophila is far simpler than that of a vertebrate. Thus, it is plausible that in vertebrates three structurally distinct GATA factors are required in overlapping compartments of the hematopoietic system, while only one

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**Fig. 6.** Fat body development is affected in srp embryos. (A,B) The fat body precursor cells (arrows) do not proliferate and do not form the typical continuous sheet of cells in srp embryos. srp expression was detected in (A) wild-type and (B) srp<sup>ts9</sup> embryos at late stage 11 (the same result is seen with srp<sup>9L</sup>). The region from the second thoracic to the third abdominal segment is shown; the embryonic midline corresponds to the bottom of the panel. The srp-expressing cells between the fat body and the midline are probably precursors of the somatic mesoderm (open triangles), as judged by their position (Borkowski et al., 1995). The cells of the expression domain located ventrally in front of the cephalic furrow can be traced until they become morphologically distinguishable as hemocytes. Moreover, the srp<sup>ts9</sup> allele, which specifically affects hemocyte development, reduces srp expression in this domain of the mesoderm (Fig. 5H,J). However, lineage analysis is required to confirm that the proposed primordium exclusively gives rise to hemocytes, as the srp expression pattern and srp phenotype suggest. srp expression in the putative hemocyte primordium commences at blastoderm stage (Fig. 3A) and is maintained as protein expression when the mature hemocytes already migrate through the embryo (data not shown). It is therefore conceivable that srp has a biphasic function in hemocyte development as possibly it does in the fat body. srp might be required for the specification of the blood cells within the mesoderm at an early stage and subsequently later for gene expression during their differentiation and maturation.
orthologous GATA factor, srp, functions in this capacity in the fly. The two other GATA factors described in Drosophila, prn (Ramain et al., 1993; Winick et al., 1993) and dGATAc (Liu et al., 1995), are not expressed in the hemocyte primordium. Similarly, the derivatives of the endoderm are more complex in vertebrates than in Drosophila, and thus only one GATA factor might be required in the fly. However, it is highly probable that the function of srp only corresponds to earlier aspects of the function of GATA-4/5/6, since srp expression disappears from the midgut primordium before terminal differentiation is initiated. This later aspect might be carried out by dGATAc which is expressed in these cells after the decline in the level of srp transcripts (Liu et al., 1995).

It is striking that srp combines functions of both the ‘blood-specific’ (GATA-1/2/3) and the ‘endoderm-specific’ (GATA-4/5/6) vertebrate GATAs. This is particularly astonishing if one considers the presence of only one zinc finger domain in srp as opposed to the two zinc finger domains in the vertebrate GATAs. Future studies will show whether this property is a new acquisition of advanced insects or whether it is an original feature of more primitive organogenesis. The ‘combined’ function of srp may in part derive from the chimaeric nature of its DNA-binding domain (Fig. 2B,C). Like three of the other four invertebrate GATA factors, elt-1 (Spieth et al., 1991), elt-2 (Hawkins and McGhee, 1995) and prn, the zinc finger domain of srp has a structure that lies between the two vertebrate GATA sub-families (Fig. 2C). Therefore, these invertebrate GATA factors might be structurally closer to the ancestors of the vertebrate factors. However, the specific activity of srp in the Drosophila embryo is certainly determined by the expression pattern of the gene. The specificity must depend on the direct or indirect interaction with other gene products which are present in the same primordium or tissue, and it will be interesting to investigate the extent to which these interactions are conserved between Drosophila and vertebrates.

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