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

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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 ARF. 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 amnioproctodeal invagination. Both parts of the midgut later lose their epithelial properties, become mesenchymal and migrate towards each other. When the two parts have met on both sides of the yolk, they have adopted an epithelial organization again. Subsequently, the cells migrate ventrally and dorsally to surround the yolk and form the tube of the midgut (for a review see Skaer, 1993).

The terminal gap gene *huckebein (hkb)* establishes the primordia of both anterior and posterior midgut (Weigel et al., 1990; Brönnner 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 (Brönnner 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; Lavriere 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 srp6G* and *ru h th st cu srpHL sr e ca*: amorphic alleles and probably strong hypomorphic allele (Jürgens et al., 1984). Both alleles form RNA, in homozygous srp6G embryos no SRP protein is detectable (data not shown), *st e* and *ru h th st cu sr* e *ca*: parental chromosomes of srpHL and srp6G, respectively (obtained from the Tübingen stock collection). *srp6L* (known as *Ins(3R)neo45*) and *srpHL* (known as *Ins(3R)P13349*); non-complementing P element insertions in 89B (Cooley et al., 1988). The new srp alleles *srp101* to *srp103* were generated by mobilization of the P element of *srpAs* (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 *srp102* after mobilization of the P element with the stable transposase source and later classified as either full revertants, partial revertants (*srpF280-24*) or new amorphic alleles (*srpF2801-20*). *hkb*: hypomorphic hkb allele; *Df(3R)hkbA*: 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-PEOXIDASE antibody has been described by Nelson and coworkers (1994).

**Microscopy**

Pictures were taken on a Zeiss Axiopt microscope using Kodak Ektachrome 64T or Agfachrome 100RS slide film and were digitized by a dye sublimation printer.

**Isolation of the srp locus**

Genomic DNA flanking the P element insertion of *srpAs* 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 (#9) contained the srp transcription unit. The P element insertion of *srpAs* and the deletions associated with *srp101* to *srp103* were mapped by Southern blotting. The genomic organization of srp, the sequence of the *srpHL* and *srp6G* alleles, the site of the P element insertion of *srp102* and the molecular nature of some of the new srp alleles derived from *srp102* 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 *srpHL* and *srp6G* 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 Phylic 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 srp45S (Fig. 1). This allele fails to complement the lethality of the two independently isolated, EMS-induced alleles srp102 and srp6G, 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 srp45S 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 srp101, srp102 and srp103 were isolated after mobilization of the P element of srpAS. srp101 and srp103 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). srp102, 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 srp6G, which also results in the lack of srp transcription. The P element of srp6G is inserted about 30 bp 5' of the putative srp transcription start site. The insertion itself affects the srp function in srp6G, 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 srp6G with respect to the midgut or germ band retraction phenotype, but which do not complement the other srp alleles (with the exception of srpAS) 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
enhancer trap and is inserted about 30 bp 5′ completely abolishes (Fig. 2A). This finding is consistent with the amorphic translation of a truncated protein lacking the zinc finger domain associated with a nonsense mutation that would lead to the function phenotype of homozygous srp embryos. In the srp 6G 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 srp 6G 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 srp 6G 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) and 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 part also 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 down-regulated (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 sps 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 mesoderm, phagocytose apoptotic cells in the embryo, and express a number of specific gene products, such as PERX and CED-9. The ventral furrow. Expression of mutant sps RNA is maintained during germ band extension (Fig. 5B,D), however, the cells fail to proliferate or to migrate (Fig. 5B,D) and subsequently die (data not shown). As a consequence, sps embryos are devoid of any mature hemocytes (Fig. 5G) which by stage 12 would be required for the development of hemocytes. (data not shown). As a consequence, sps embryos are devoid of any mature hemocytes (Fig. 5G) which by stage 12 would be required for the development of hemocytes. Our data suggests that the sps 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 sps allele, triangles indicate the position of the introns. (B) Comparison of the zinc finger domain of sps with the (if applicable C-terminal) zinc finger of other GATA factors. In the sps allele the conserved asparagine N29 is replaced by a lysine. The amino acids diagnostic for GATA-1/2/3 (Evans and Felsenfeld, 1989; Zen et al., 1990, 1991; Yamamoto et al., 1990) and GATA-4/5/6 (Tamura et al., 1993; Kelley et al., 1993; Laverriere et al., 1994) and shared by sps are boxed. (C) Phylogenetic tree of the 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) and the fungal GATA factors regulating nitrogen or siderophore metabolism (Araea: Kudla et al., 1990; Haas et al., 1995; Nit-2: Fu and Marchluf, 1990; GlN3: Minehart and Magasanik, 1991; UtrI1: 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.
does not affect the function of srp in gut, yolk or amnioserosa, specifically disturbs hemocyte differentiation. In homozygous srp<sup>AS</sup> embryos the number of hemocytes is severely reduced (data not shown), and no hemocytes are formed in embryos trans-heterozygous for srp<sup>AS</sup> and either of the strong EMS-induced srp alleles: srp<sup>6G</sup> and srp<sup>9L</sup> (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<sup>AS</sup> embryos (Fig. 5J). Other aspects of srp expression are not altered. Thus, the P element insertion of srp<sup>AS</sup> 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<sup>9L</sup> or the srp<sup>6G</sup> 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 ABR (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; Losicky and Wensink, 1995). Here we have shown that it is required for the early steps in fat body morphogenesis.

**Fig. 4.** Expression of srp in the primordia of anterior and posterior midgut depends on hkb. srp mRNA was detected in (A) wild-type and (B) hkb embryos at early gastrulation. In the hkb embryo srp expression at the poles, i.e. in the anterior midgut primordium and the posterior midgut primordium, is not initiated, while other aspects are not affected. The weak staining in the yolk nuclei of the embryos in both panels reflects the variability in the detection of this aspect of srp expression.

**Fig. 5.** srp is essential for Drosophila hematopoiesis. (A-D) The hemocyte precursors do not proliferate in srp embryos. The hemocyte precursor cells are visualized by their srp expression in (A,C) wild-type embryos and in (B,D) embryos homozygous for the srp6G allele at stage 11. (A,B) Optical sagittal section; (C,D) tangential horizontal section, srp9L gives the same result (E,F) Hemocytes, visualized by an anti-PEROXIDASIN antibody (Nelson et al., 1994), populate the interstitial space in wild-type embryos. (E) Stage 12, lateral view; (F) stage 15, horizontal view. (G) They are not detectable in srp embryos carrying strong or amorphic alleles or (H) in embryos trans-heterozygous for the P-element-induced allele srpAS and the strong srp allele srp9L. (I,J) The srp expression in the putative hemocyte primordium within the mesoderm (arrowhead) is specifically decreased in (J) hemizygous srpAS embryos as compared to (I) wild type.
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 srp68 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, Panier (pnr) or dGATAa, 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
orthologous GATA factor, srp, functions in this capacity in the fly. The two other GATA factors described in Drosophila, pnr (Ramain et al., 1993; Winick et al., 1993) and dGATAc (Lin 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 (Lin 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 pnr, 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.

We thank Drs M. Brennan, N. Brown, L. Fessler, J. Fessler M. Mldzik and J. Tamkun and the Bloomington and Tübingen stock centers for reagents and Drs E. Knust, M. Leptin and particularly R. Wilson for comments on the manuscript. We are indebted to Dr José Casal for advice and help during the initial phase of the project. This work was supported by the Deutsche Forschungsgemeinschaft as part of the SFB 243.

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(Accepted 30 August 1996)