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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 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 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 major components of the digestive tract apart from the midgut are ectodermal in origin. The hindgut and the Malpighian tubules develop from the proctodeum which originates from the region anterior to the primordium of the posterior midgut in the blastoderm. Both of these primordia are internalized during the amnioproctodeal invagination. They become morphologically distinct when the pouches of the developing Malpighian tubules begin to evaginate from the proctodeum and when the posterior midgut cells slightly 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; Brönner 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 (Brönner 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 (Arceci 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^{6G} *e* and *ru h th st cu* srp^{9L} *sr e ca*: amorphic alleles and probably strong hypomorphic allele (Jürgens et al., 1984). Both alleles form RNA, in homozygous srp^{6G} embryos no SRP protein is detectable (data not shown). *st e* and *ru h th st cu sr e ca*: parental chromosomes of srp^{6G} and srp^{9L}, respectively (obtained from the Tübingen stock collection). srp^{AS} (known as *Ins(3R)neo45*) and srp^{PZ} (known as *Ins(3R)PZ1549*): non-complementing P element insertions in 89B (Cooley et al., 1988). The new *srp* alleles srp^{HO1} to srp^{HO3} were generated by mobilization of the P element of srp^{AS} (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^{PZ} after mobilization of the P element with the stable transposase source and later classified as either full revertants, partial revertants ($srp^{PZRV1\text{-}24}$) or new amorphic alleles ($srp^{PZHO1\text{-}20}$). hkb^{2}: hypomorphic *hkb* allele; $Df(3R)hkb^{A}$: 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-PEROXIDASIN antibody has been described by Nelson and coworkers (1994).

Microscopy

Pictures were taken on a Zeiss Axiophot 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^{AS} was isolated by plasmid rescue (Cooley et al., 1988). Using this DNA as probe a cosmid library was screened (Tamkun et al., 1992). One of the identified genomic clones (#9) contained the *srp* transcription unit. The P element insertion of srp^{AS} and the deletions associated with srp^{HO1} to srp^{HO3} were mapped by Southern blotting. The genomic organization of *srp*, the sequence of the srp^{6G} and srp^{9L} alleles, the site of the P element insertion of srp^{PZ} and the molecular nature of some of the new *srp* alleles derived from srp^{PZ} 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^{9L} and srp^{6G} 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^{AS} (Fig. 1). This allele fails to complement the lethality of the two independently isolated, EMS-induced alleles srp^{9L} and srp^{6G}, 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^{AS} 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^{HO1}, srp^{HO2} and srp^{HO3} were isolated after mobilization of the P element of srp^{AS}. srp^{HO1} and srp^{HO3} 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^{HO2}, 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^{PZ} which also results in the lack of *srp* transcription. The P element of srp^{PZ} is inserted about 30 bp 5′ of the putative *srp* transcription start site. The insertion itself affects the *srp* function in srp^{PZ}, 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^{PZ} with respect to the midgut or germ band retraction phenotype, but which do not complement the other *srp* alleles (with the exception of srp^{AS}) 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

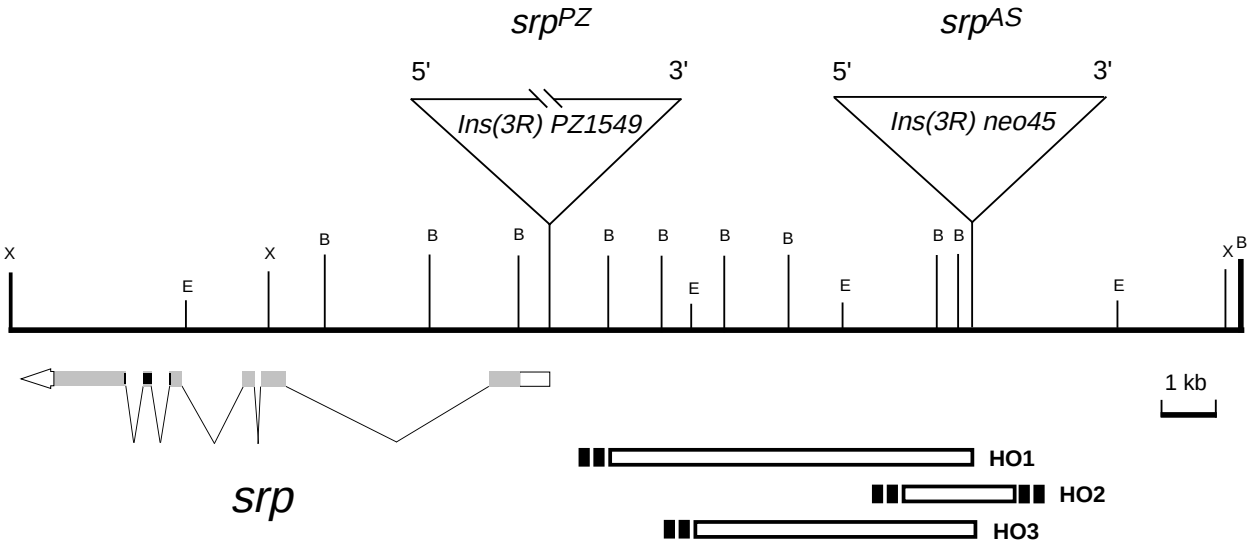

Fig. 1. Structure of the *srp* locus. The P element insertion *Ins(3R)neo45* (*srp*^{AS}) (Cooley et al., 1988) was used to isolate the *srp* locus and specifically affects the function of *srp* in hemocyte development (see Fig. 5). The *srp* alleles *srp*^{HO1} to *srp*^{HO3} were isolated after mobilization of the P element of *srp*^{AS}. *srp*^{HO2} is an allele of intermediate strength which partially reduces *srp* function in the midgut and amnioserosa, and completely abolishes *srp* function in the blood primordium. The alleles *srp*^{HO1} and *srp*^{HO3} abolish all known *srp* functions; the *srp* gene is not transcribed in these alleles. The P element insertion *Ins(3R)PZ1549* (*srp*^{PZ}), 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.

et al., 1993). However, in genomic and cDNA sequences we noted a few deviations from the published sequence. Our results indicate that the transcribed mRNA is about 4 kb in size and encodes a protein of 949 amino acids, rather than 779 as published previously (Abel et al., 1993). The C-terminal 680 amino acids (including the zinc finger domain) are identical with the reported sequence of ABF (Abel et al., 1993). The putative translation start site of the *srp* mRNA is in good agreement with the consensus translation start site for that of *Drosophila* (Cavener, 1987). This mRNA, which we consider as full-length, is efficiently translated in the embryo, in contrast to the truncated mRNA directed by the *abf* cDNA (data not shown).

To confirm that the *srp* gene corresponds to *abf* we have sequenced both EMS-induced *srp* alleles. The *srp*^{6G} 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 *srp*^{6G} embryos. In the *srp*^{9L} 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*^{9L} 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). 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 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 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

A

```
1                                                  51
MRNNFAFYSVYTGGGGGVGVGMTSHEHLAAATAAAAAVAQGTTPNIDEVIQDTLKDECFEDGHSTDYHVLTSVSDMHTLKDSSPYALTHEQLHQQQHHHQ
101                                                151
QQLHHHQQQQQQLYHQQQQQQQQQQHHHHHNNSTSSAGGDSPSSSHALSTLQSFTQLTSATQRDSLSPENDAYFAAAQLGSSLQNSSVYAGSLLTQTANG
201                                                251
IQYGMQSPNQTQAHLQQQHHQQQQQQHQQHQQQQLQQQQQQHHHNQHQHHNSSSSSPGPAGLHHSSSSAATAAAVAAATAAVNGHNSSLEDGYGSPRSSH
301                                                351
SGGGGGGTLPAFQRIAYPNSGSVERYAPITNYRGQNDTWFDPLSYATSSSGQAQLGVGVGAGVVSNVIRNGRAISAANAAAAAAAADGTTGRVDPGTFLSA
401                                                451
SASLSAMAAESGGDFYKPNSFNVGGGGRSKANTSGAASSYSCPGSNATSAATSAVASGTAATAATTLDEHVSRANSRRLSASKRAGLSCSNCHTTHTSLW
501                                                551
RRNPAGEPVCNACGLYYKLHSVPRPLTMKKDTIQKRKRKPKGTKSEKSKSKSKNALNAIMESGSLVTNCHNVGVVLDSSQMDVNDDMKPQLDLKPYNSYS
601                                                651
SQPQQQLPQYQQQQQLVMADQHSSAASSPHSMGSTSLSPSAMSHQHQTHPHQQQQQQLCSGWTCRPTQTTKCRRSTCSSISSSNRAACSTHPAHPLHLQH
701                                                751
PSPTHQLHNNNNNNNSSLFNNNNNNNNSSSNENNNKLIQKYLQAQQLSSSSNSDSTSDHQLLAQLLPNSITAAAAAAAAAAAAAIKTEALSLTSQANCST
801                                                851
ASAGLMVTSTPTTASSTLSSLSHSNIISLQNPYHQAGMTLCKPTRPSPPYYLTPEEDEQPALIKMEEMDQSQQQQQQQQHQQQQHGEIMLSRSASLDEHY
901                                                949
ELAAFQRHQQQQQQPEQQTASLLGQHEQHVTNYAMHKFGVDRETVVKME
```

B

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Dm SRP          KRAGLSCSNCHTTHTSLWRRNPAGEPVCNACGLYYKLHSVPRPLTMKKDTIQKRKRKPKGTKSEKS
Gg GATA-1       ----TVC--CQ-ST-T----S-M-D--C--C--------Q-N-----R--G--T-N--VSSKGKKRR
Hs GATA-1       ----TQCT-CG--T-T-----AS-D--C--C--------Q-N-----R--G--T-N--AS-KGKKRR
Xl GATA-1A      ----TQC--C--ST-T-----AS-D--C--C--------N-N-------EG--T-N--VSSRSKKKK
Gg GATA-2       R---TCCA-CQ--T-T-----AN-D--C--C--------N-N-------EG--T-N--MSNKSKKSK
Xl GATA-2       R---TCCA-CQ-ST-T-----AN-D--C--C--------N-N-------EG--T-N--MSNKSKKNK
Gg GATA-3       R---T-CA-CQ--T-T-----AN-D--C--C--------NIN-------EG--T-N--MSSKSKKCK
Xl GATA-3       R---T-CA-CQ--T-T-----AN-D--C--C--------NIN-------EG--T-N--MSSKSKKSK
Gg GATA-4       R-V---CA-C---T-T-----AE-----C--C---M---G-----A-R-EG--T-------NLNKTKT
Rn GATA-4       R-V---CA-CQ--T-T-----AE-----C--C---M---G-----A-R-EG--T-------NLNKSKT
Gg GATA-5       R----CCT-C---N-T-----AE-----C--C---M---G-----A---ES--T-------NITKGKT
Xl GATA-5A      R----CCT-C--ST-T-----SE-----C--C---M---G-----A---ES--T-------NIGKGKT
Gg GATA-6       R-L---CA-C---T-T-----AE-----C--C---M---G-----A---EG--T-------NINKSKA
Rn GATA-6       R-L---CA-C---T-T-----AE-----C--C---M---G-----A---EG--T-------NINKSKA
Dm PNR          R-M--CCT-CG-RT-T-----ND----C--C-------G-N---A-R--G--T------K-GSGSA
Dm dGATAc       ----T-CA-CK--T-T-----AS----C--C-------N-N-------EG--T-N--LSSKSKKKK
Ce ELT-1        --T-IECV-CR-NT-T-----GE-H--C--C---F---K-E--I-----G--T-N--LSAKGSRRM
Ce ELT-2        R-Q--VC--CNG-N-T-----AE-D--C--C---F---HI---TS---EGL-T----S-SGDSSTP
An AREA         QNGPTTCT-CF-QT-P------E-Q-LC--C--FL---G-V---SL-T-V-K--N-NSANSLAVGS
Pc AREA         AGPEPACT-CF-QT-P------E-Q-LC--C--FL---G-V---SL-T-V-K--N-SSAN-LTVGT
Nc NIT-2        TDTPTTCT-CF-QT-P------D-Q-LC--C--FL---G-V---SL-T-V-K--N-GSGASLPVGG
Sc GLN3         -KPLIQCF-CK-FK-P----S-E-NTLC--C--FQ---GTM---SL-S-V-K--IS-KRAKQTDPN
Um URBS1        VVGA-RCT-CQ--T-P----DED-NNIC--C---H---GTH--IG---SV-KR---I-ANATAQPT

consensus       ......C.NC.T..T.LWRR...G...CNACGL..KLH...RP.......I..R.R..........
                                           ▼
SRP 9L          ------C--C-----------------CK-C---------------------------------
```

C

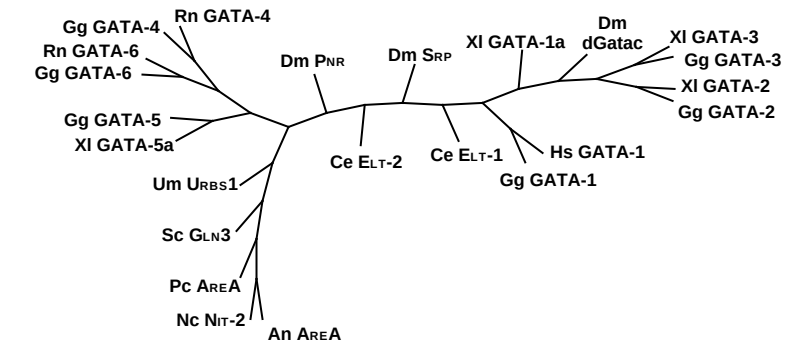

Fig. 2. (A) Predicted sequence of the SRP protein. Our data suggests that the SRP protein is 949 amino acids in size. The C-terminal 680 amino acids (including the zinc finger domain; underlined) are identical with the reported sequence of ABF (Abel et al., 1993). An asterisk indicates the position of the stop codon found in the *srp*^{6G} 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*^{9L} allele the conserved asparagine 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; Kelley et al., 1993; Laverriere et al., 1994) and shared by SRP 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 and the fungal GATA factors regulating nitrogen or siderophore metabolism (AREA: Kudla et al., 1990; Haas et al., 1995; NIT-2: Fu and Marzluf, 1990; GLN3: Minehart and Magasanik, 1991; URBS1: 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.

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 mesoderm, phagocytose apoptotic cells in the embryo and express a number of specific gene products, such as PEROXIDASIN (Tepass et al., 1994; Nelson et al., 1994). In *srp* embryos the primordium of the hemocytes invaginates with the ventral furrow. Expression of mutant *srp* 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 *srp* embryos are devoid of any mature hemocytes (Fig. 5G) which by stage 12 would normally be found throughout the embryo (Fig. 5E). Thus, *srp* is required for the development of hemocytes.

Interestingly, *srp*^{AS}, 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^{AS} embryos the number of hemocytes is severely reduced (data not shown), and no hemocytes are formed in embryos *trans*-heterozygous for srp^{AS} and either of the strong EMS-induced *srp* alleles: srp^{6G} and srp^{9L} (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^{AS} embryos (Fig. 5J). Other aspects of *srp* expression are not altered. Thus, the P element insertion of srp^{AS} 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^{9L} or the srp^{6G} 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*

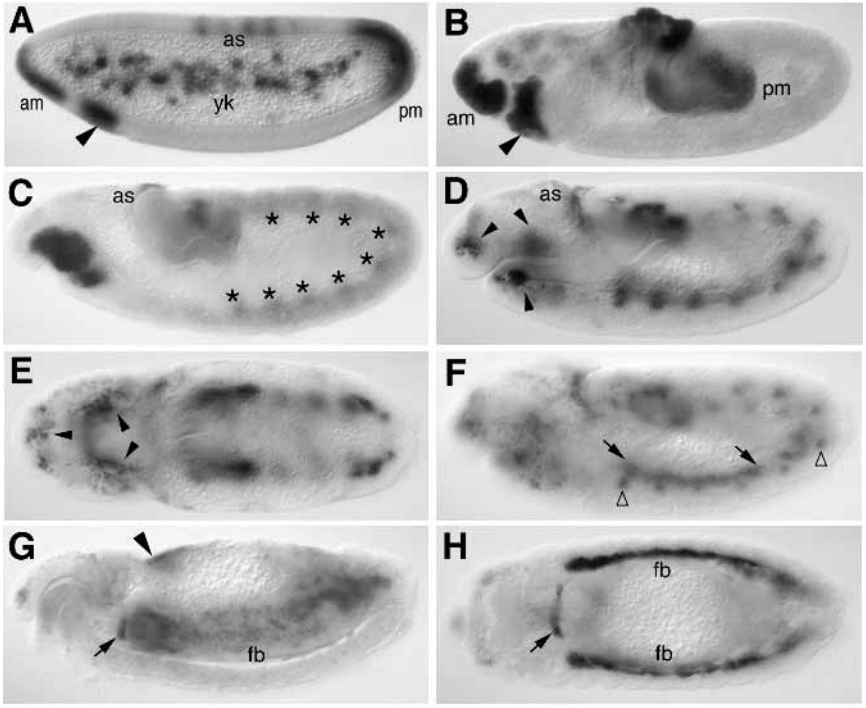

Fig. 3. Expression of *srp* mRNA at (A) blastoderm stage and (B) after gastrulation. The arrowheads indicate *srp* expression in the putative hemocyte primordium within the anterior mesoderm primordium (am, anterior midgut primordium; pm, posterior midgut primordium; as, amnioserosa primordium; yk, yolk cell). (C) At stage 10 *srp* expression disappears from the posterior midgut primordium, but is still strongly present in the anterior midgut primordium which invaginates with the stomodeum. This expression masks part of the mesodermal cells expressing *srp* in the head when viewed from the side. Secondary *srp* expression in the mesoderm has become distinguishable in segmentally repeated clusters of cells (asterisks), the putative fat body precursors (as, amnioserosa). (D) At stage 11 the prohemocytes (arrowheads) migrate into the head. The secondary expression in the putative fat body primordium becomes stronger. (E) Same embryo as in D in a horizontal view. (F) Later during stage 11, the fat body precursor cells rearrange and begin to form a sheet on each side of the germ band (arrows). In addition to the fat body also precursors of the somatic mesoderm transiently express *srp* (open arrowheads). (**G**) After germ band retraction *srp* expression has become very weak in the hemocytes which have already dispersed over the interstitial space. *srp* is strongly expressed in the differentiating fat body (fb) and now also in the developing lymph gland (arrowhead) and the ring gland (arrow). (H) Same embryo as in G seen in a medial horizontal section.

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 srp^{6G} eliminate, and in the case of srp^{9L} most likely eliminate the ability of SRP to act as a transcriptional regulator. The nonsense mutation in srp^{6G} results in a truncated protein lacking the zinc finger domain. The missense mutation of srp^{9L} 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 srp^{9L}. Taken together, our findings establish that the transcription unit encoding the GATA factor ABF is identical to *srp*.

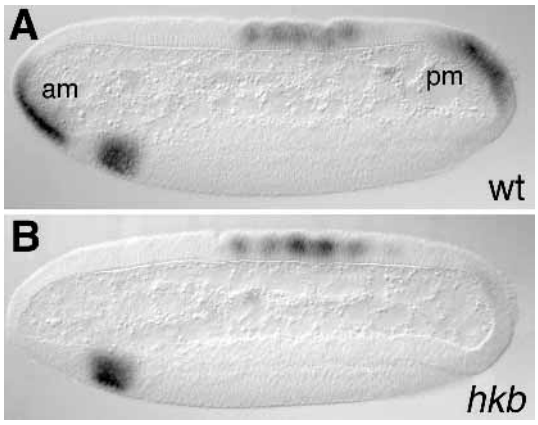

Fig. 4. Expression of *srp* in the primordia of anterior and posterior midgut depends on *hkb*. *srp* mRNA was detected in (A) wild-type and (B) *hkb* embryos at early gastrulation. In the *hkb* embryo *srp* expression at the poles, i.e. in the anterior midgut primordium and the posterior midgut primordium, 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.

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; Lossky and Wensink, 1995). Here we have shown that it is required for the early steps in fat body morphogenesis

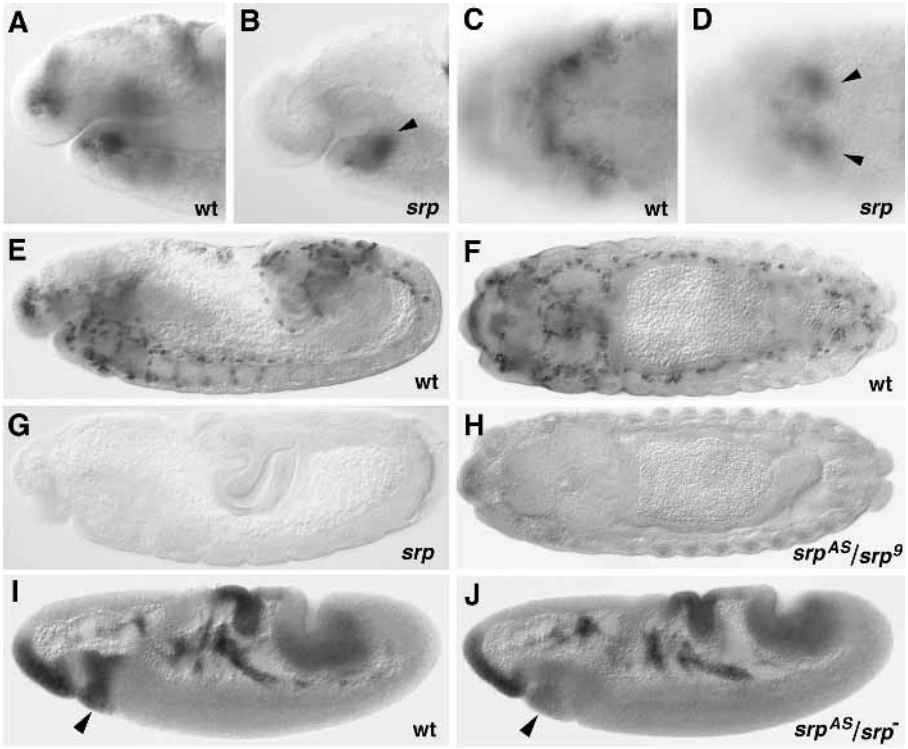

Fig. 5. *srp* is essential for *Drosophila* hematopoiesis. (A-D) The hemocyte precursors do not proliferate in *srp* embryos. The hemocyte precursor cells are visualized by their *srp* expression in (A,C) wild-type embryos and in (B,D) embryos homozygous for the srp^{6G} allele at stage 11. (A,B) Optical sagittal section; (C,D) tangential horizontal section, srp^{9L} 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 srp^{AS} and the strong *srp* allele srp^{9L}. (I,J) The *srp* expression in the putative hemocyte primordium within the mesoderm (arrowhead) is specifically decreased in (J) hemizygous srp^{AS} 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 srp^{AS} 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 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

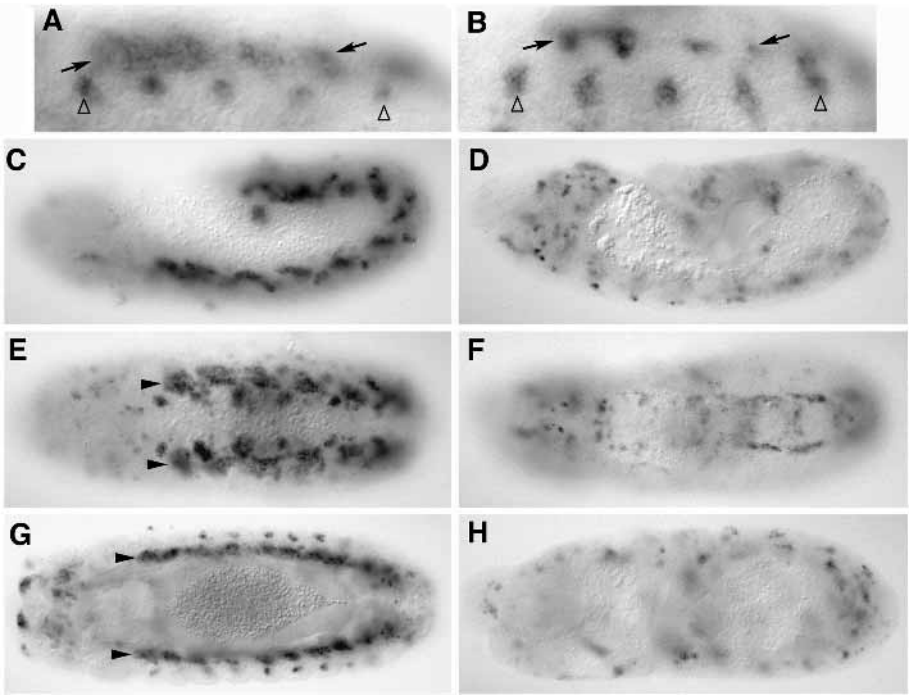

Fig. 6. Fat body development is affected in *srp* embryos. (A,B) The fat body precursor cells (arrows) do not proliferate and do not form the typical continuous sheet of cells in *srp* embryos. *srp* expression was detected in (A) wild-type and (B) srp^{9L} embryos at late stage 11 (the same result is seen with srp^{6G}). 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 magnification is about 2× higher than in C-H. (C-H) *svp* expression in the fat body depends on *srp* function. *svp* expression is shown (C,E,G) in wild-type and (D,F, H) in *srp* embryos at about stage 11 (C-F) and at about stage 14 (G,H). (C,E,G) While *svp* is strongly expressed in the developing fat body of wild-type embryos (arrowheads), (D,F,H) no such *svp* expression is detectable in the *srp* embryos. *svp*-expressing cells are visible in the ventral nervous system (D,F,H) and also in the periphery. The embryos are viewed in (C,D) parasagittal, (E,F) tangential horizontal and (G,H) medial horizontal sections. C and E as well as D and F show the same embryo, respectively. The *srp* embryo shown in H is grossly distorted due to the failure of germ band retraction.

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.

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REFERENCES

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

Arceci, R. J., King, A. A. J., Simon, M. C., Orkin, S. H. and Wilson, D. B. (1993). Mouse GATA-4: a retinoic acid-inducible GATA-binding transcription factor expressed in endodermally derived tissues and heart. *Mol. Cell. Biol.* **13**, 2235-2246.

Borkowski, O. M. D., Brown, N. H. and Bate, M. (1995). Anterior-posterior subdivision and the diversification of the mesoderm in *Drosophila*. *Development* **121**, 4183-4193.

Brönner, G., Chu-la Graff, Q., Doe, C. Q., Cohen, B., Weigel, D., Taubert, H. and Jäckle, H. (1994). Sp1/egr-like zinc-finger protein required for endoderm specification and germ-layer formation in *Drosophila*. *Nature* **369**, 664-668.

Cavener, D. R. (1987). Comparison of the consensus flanking translational start sites in *Drosophila* and vertebrates. *Nucl. Acids Res.* **15**, 1353-1361.

Cooley, L., Kelley, R. and Spradling, A. (1988). Insertional mutagenesis of the *Drosophila* genome with single P elements. *Science* **329**, 1121-1128.

Evans, T. and Felsenfeld, G. (1989). The erythroid-specific transcription factor Eryf1: a new finger protein. *Cell* **58**, 877-885.

Felsenstein, J. (1988). Phylogenies from molecular sequences: inference and reliability. *Ann. Rev. Genet.* **22**, 521-565.

Fu, Y.-H. and Marzluf, G. A. (1990). *nit-2*, the major nitrogen regulatory gene of *Neurospora crassa*, encodes a protein with putative zinc finger DNA-binding domain. *Mol. Cell. Biol.* **10**, 1056-1065.

Haas, H., Bauer, B., Redl, B., Stöffler, G. and Marzluf, G. A. (1995). Molecular cloning and analysis of *nre*, the major nitrogen regulatory gene of *Penicillium chrysogenum*. *Curr. Genet.* **27**, 150-158.

Hawkins, M. G. and McGhee, J. D. (1995). *elt-2*, a second GATA factor from the nematode *Caenorhabditis elegans*. *J. Biol. Chem.* **270**, 14666-14671.

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

Jürgens, G., Wieschaus, E., Nüsslein-Volhard, C. and Kluding, H. (1984). Mutations affecting the pattern of the larval cuticle in *Drosophila melanogaster*. II. Zygotic loci on the third chromosome. *Roux's Arch. Dev. Biol.* **193**, 283-295.

Kelley, C., Blumberg, H., Zon, L. I. and Evans, T. (1993). GATA-4 is a novel transcription factor expressed in endocardium of the developing heart. *Development* **118**, 817-827.

Ko, L. J., Yamamoto, M., Leonard, M. W., George, K. M., Ting, P. and Engel, J. D. (1991). Murine and human T-lymphocyte GATA-3 factors mediate transcription through a cis-regulatory element within the human T-cell receptor δ gene enhancer. *Mol. Cell. Biol.* **11**, 2778-2784.

Kudla, B., Caddick, M. X., Langdon, T., Martinez-Rossi, N. M., Bennett, C. F., Sibley, S., Davies, R. W. and Arst, H. N. (1990). The regulatory gene *areA* mediating nitrogen metabolite repression in *Aspergillus nidulans*. Mutations affecting specificity of gene activation alter a loop residue of a putative zinc finger. *EMBO J.* **9**, 1355-1364.

Laski, F. A., Rio, D. C. and Rubin, G. M. (1986). Tissue specificity of *Drosophila* P element transposition is regulated at the level of mRNA splicing. *Cell* **44**, 7-19.

Laverriere, A. C., MacNeill, C., Mueller, C., Poelmann, R. E., Burch, J. B. E. and Evans, T. (1994). GATA-4/5/6, a subfamily of three transcription factors transcribed in developing heart and gut. *J. Biol. Chem.* **269**, 23177-23184.

Lin, W.-H., Huang, L.-H., Yeh, J.-Y., Hoheisel, J., Lehrach, H., Sun, Y. H. and Tsai, S.-F. (1995). Expression of a *Drosophila* GATA transcription factor in multiple tissues in the developing embryo. *J. Biol. Chem.* **270**, 25150-25158.

Lossky, M. and Wensink, P. C. (1995). Regulation of *Drosophila yolk protein* genes by an ovary-specific GATA factor. *Mol. Cell. Biol.* **15**, 6943-6952.

Minehart, P. L. and Magasanik, B. (1991). Sequence and expression of *GLN3*, a positive nitrogen regulatory gene of *Saccharomyces cerevisiae* encoding a protein with a putative zinc finger DNA-binding domain. *Mol. Cell Biol.* **11**, 6216-6228.

Nelson, R. E., Fessler, L. I., Takagi, Y., Blumberg, B., Keene, D. R., Olson, P. F., Parker, C. G. and Fessler, J. H. (1994). Peroxidasin: a novel enzyme-matrix protein of *Drosophila* development. *EMBO J.* **13**, 3438-3447.

Omichinski, J. G., Clore, G. M., Schaad, O., Felsenfeld, G., Trainor, C., Appella, E., Stahl, S. J. and Gronenborn, A. M. (1993). NMR structure of a specific DNA complex of Zn-containing DNA binding domain of GATA-1. *Science* **261**, 438-446.

Pevny, L., Simon, M. C., Robertson, E., Klein, W. H., Tsai, S.-F., D'Agati, V., Orkin, S. H. and Costantini, F. (1991). Erythroid differentiation in chimaeric mice blocked by a targeted mutation in the gene for transcription factor GATA-1. *Nature* **349**, 257-260.

Ramain, P., Heitzler, P., Haenlin, M. and Simpson, P. (1993). *pannier*, a negative regulator of *achaete* and *scute* in *Drosophila*, encodes a zinc finger protein with homology to the vertebrate transcription factor GATA-1. *Development* **119**, 1277-1291.

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

Reuter, R., Grunewald, B. and Leptin, M. (1993a). A role for the mesoderm in endodermal migration and morphogenesis in *Drosophila*. *Development* **119**, 1135-1145.

Reuter, G., Hoffmann, G., Dorn, R., Gausz, J. and Saumweber, H. (1993b). Construction and characterization of a TM3 balancer carrying P[(ry[+])Δ2-3] as a stable transposase source. *Dros. Inf. Serv.* **72**, 78.

Reuter, R. and Leptin, M. (1994). Interacting functions of *snail*, *twist* and

huckebein during the early development of germ layers in *Drosophila*. *Development* **120**, 1137-1150.

Skaer, H. (1993). The alimentary canal. In *The Development of Drosophila melanogaster*. (ed. Bate, M. Martinez-Arias, A.), pp 941-1012. Plainview, NY: Cold Spring Harbor Laboratoy Press.

Soudais, C., Bielinska, M., Keikinheimo, M., MacArthur, C. A., Narita, N., Saffitz, J. E., Simon, M. C., Leiden, J. M. and Wilson, D. B. (1995). Targeted mutagenesis of the transcription factor GATA-4 gene in mouse embryonic stem cells disrupts visceral endoderm differentiation in vitro. *Development* **121**, 3877-3888.

Spieth, J., Shim, Y. H., Lea, K., Conrad, R. and Blumenthal, T. (1991). *elt-1*, an embryologically expressed *Caenorhabditis elegans* gene homologous to the GATA transcription factor family. *Mol. Cell. Biol.* **11**, 4651-4659.

Tamkun, J. W., Deuring, R., Scott, M. P., Kissinger, M., Pattatucci, A. M., Kaufman, T. C. and Kennison, J. A. (1992). *brahma*: A regulator of *Drosophila* homeotic genes structurally related to the yeast transcriptional activator *SNF2/SWI2*. *Cell* **68**, 561-572.

Tamura, S., Wang, X.-H., Maeda, M. and Futai, M. (1993). Gastric DNA-binding proteins recognize upstream sequence motifs of parietal cell-specific genes. *Proc. Natl. Acad. Sci. USA* **90**, 10876-10880.

Tautz, D. and Pfeifle, C. (1989). A non-radioactive in situ hybridization method for the localization of specific RNAs in *Drosophila* embryos reveals translational control of the segmentation gene hunchback. *Chromosoma* **98**, 81-85.

Technau, G. M. and Campos-Ortega, J. A. (1985). Fate-mapping in wild-type Drosophila melanogaster. II. Injections of horseradish peroxidase in cells of the early gastrula stage. *Roux's Arch. Dev. Biol.* **194**, 196-212.

Tepass, U., Fessler, L. I., Aziz, A. and Hartenstein, V. (1994). Embryonic origin of hemocytes and their relationship to cell death in *Drosophila*. *Development* **120**, 1829-1837.

Tsai, F. Y., Keller, G., Kuo, F. C., Weiss, M., Chen, J. Z., Rosenblatt, M., Alt, F. W. and Orkin, S. H. (1994). An early haematopoietic defect in mice lacking the transcription factor GATA-2. *Nature* **371**, 221-226.

Voisard, C., Weang, J., McEvoy, J. L., Xu, P. and Leong, S. A. (1993). *urbS1*, a gene regulating siderophore biosynthesis in *Ustilago maydis*, encodes a protein similar to the erythroid transcription factor GATA-1. *Mol. Cell Biol.* **13**, 7091-7100.

Weigel, D., Jürgens, G., Klingler, M. and Jäckle, H. (1990). Two gap genes mediate maternal terminal pattern information in *Drosophila*. *Science* **248**, 495-498.

Winick, J., Abel, T., Leonard, M. W., Michelson, A. M., Chardon-Loriaux, I., Holmgren, R. A., Maniatis, T. and Engel, J. D. (1993). A GATA family transcription factor is expressed along the embryonic dorsoventral axis in *Drosophila melanogaster*. *Development* **119**, 1055-1065.

Yamamoto, M., Ko, L. J., Leonard, M. W., Beug, H., Orkin, S. H. and Engel, J. D. (1990). Activity and tissue-specific expression of the transcription factor NF-E1 multigene family. *Genes Dev.* **4**, 1650-1662.

Zon, L. I., Tsai, S. F., Burgess, S., Matsudaira, P., Bruns, G. A. and Orkin, S. H. (1990). The major human erythroid DNA-binding protein (GF-1): primary sequence and localization of the gene to the X chromosome. *Proc. Natl. Acad. Sci. USA* **87**, 668-672.

Zon, L. I., Mather, C., Burgess, S., Bolce, M. E., Harland, R. M. and Orkin, S. H. (1991). Expression of GATA-binding proteins during embryonic development in *Xenopus laevis*. *Proc. Natl. Acad. Sci. USA* **88**, 10642-10646.

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