The EMBO Journal Vol. 21 No. 20 pp. 5477–5486, 2002

Two isoforms of Serpent containing either one or two GATA zinc fingers have different roles in *Drosophila* haematopoiesis

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serpent **(*srp*) encodes a GATA transcription factor essential for haematopoiesis in *Drosophila*. Previously, Srp was shown to contain a single GATA zinc finger of C-terminal type. Here we show that *srp* encodes different isoforms, generated by alternative splicing, that contain either only a C-finger (SrpC) or both a C- and an N-finger (SrpNC). The presence of the N-finger stabilizes the interaction of Srp with palindromic GATA sites and allows interaction with the Friend of GATA factor U-shaped (Ush). We have examined the respective functions of SrpC and SrpNC during embryonic haematopoiesis. Both isoforms individually rescue blood cell formation that is lacking in an *srp* null mutation. Interestingly, while SrpC and SrpNC activate some genes in a similar manner, they regulate others differently. Interaction between SrpNC and Ush is responsible for some but not all aspects of the distinct activities of SrpC and SrpNC. Our results suggest that the inclusion or exclusion of the N-finger in the naturally occurring isoforms of Srp can provide an effective means of extending the versatility of *srp* function during development.**

Keywords: *Drosophila*/FOG/GATA/haematopoiesis/*serpent*

Introduction

Members of the GATA family are zinc finger transcription factors, conserved from yeast to vertebrates, that regulate a variety of developmental processes (Patient and McGhee, 2002). These factors bind to the consensus WGATAR DNA site (Martin and Orkin, 1990; Merika and Orkin, 1993) and contain one or two conserved zinc fingers with the characteristic Cys-X2-Cys-X17-Cys-X2-Cys spacing. In vertebrates, all GATA factors contain two distinctive zinc fingers separated by 29 amino acids and referred to as the N-finger (for N-terminal zinc finger) and C-finger (for C-terminal zinc finger), respectively. However, a number of invertebrate GATA factors, including the *Drosophila* GATA factor Serpent/dGATAb (Srp) (Abel *et al*., 1993; Rehorn *et al*., 1996), contain only a C-finger (Lowry and Atchley, 2000). The C-finger is responsible for DNA binding (Martin and Orkin, 1990) and for interaction with other transcription factors (Merika and Orkin, 1995; Rekhtman *et al*., 1999). In vertebrates, the GATA N-finger can modulate the binding of the C-finger to specific GATA sites (Trainor *et al*., 1996, 2000). Moreover, studies in mice have revealed a specific requirement for the GATA-1 N-finger for erythroid differentiation (Weiss *et al*., 1997) and for full rescue of *GATA-1* loss of function (Shimizu *et al*., 2001). Similarly, specific mutations in the N-finger of the *Drosophila* GATA factor Pannier/dGATAa (Pnr) produce dominant effects on the formation of sensory bristles (Ramain *et al*., 1993). Among others, these data suggested that the N-finger could act as a binding domain for a cofactor regulating GATA activity. Most notably, it was shown in vertebrates and in flies that GATA N-finger mediates the interaction with transcriptional coregulators of the Friend of GATA (FOG)/U-shaped (Ush) family (Haenlin *et al*., 1997; Tsang *et al*., 1997).

FOG genes, described in mammals, *Xenopus* and *Drosophila*, code for structurally related proteins with multiple C2H2 and C2HC zinc fingers (Cubadda *et al*., 1997; Tsang *et al*., 1997; Tevosian *et al*., 1999; Deconinck *et al*., 2000). They specifically recognize the GATA N-finger through some of their C2HC fingers (Fox *et al*., 1998, 1999). Initially, FOG-1 was identified in mammals by a two-hybrid screen for factors that interact with the GATA-1 N-finger domain (Tsang *et al*., 1997). The interaction between GATA-1 and FOG-1 is necessary for erythroid and megakaryocytic differentiation (Tsang *et al*., 1998). Recently, human familial dyserythropoietic anaemia and thrombocytopenia has been associated with missense mutations in the GATA-1 N-finger that diminish or abrogate GATA-1–FOG-1 interaction, thereby highlighting the importance of this interaction *in vivo* (Nichols *et al*., 2000; Mehaffey *et al*., 2001). Concomitantly with the cloning of FOG-1, we identified the *Drosophila* FOG homologue Ush by virtue of its antagonism of Pnr during adult neurogenesis (Cubadda *et al*., 1997; Haenlin *et al*., 1997). In addition, *Drosophila* Ush and vertebrate FOG-2 were shown to participate in cardiogenesis in flies and in vertebrates, inhibiting Pnr and GATA-4 functions, respectively (Fossett *et al*., 2000; Tevosian *et al*., 2000). However, the precise mode of action of the GATA–FOG complex remains unclear. In fact, FOG can either repress or enhance GATA-mediated transactivation, depending on the cell and promoter context (Tsang *et al*., 1997; Fox *et al*., 1999; Holmes *et al*., 1999).

Recent evidence suggests that blood cell differentiation in vertebrates and in *Drosophila* shares a common molecular basis (Fossett and Schulz, 2001). During *Drosophila* embryogenesis, blood cells (haemocytes) originate from the procephalic mesoderm and differentiate into two known lineages: plasmatocytes and crystal cells (Tepass *et al*., 1994; Lebestky *et al*., 2000). The plasmatocytes migrate throughout the embryo along several invariant paths and act as macrophages (Cho *et al*., 2002). They contribute to host defence by phagocyting microbes, and they play a crucial role in normal development by eliminating apoptotic bodies. This

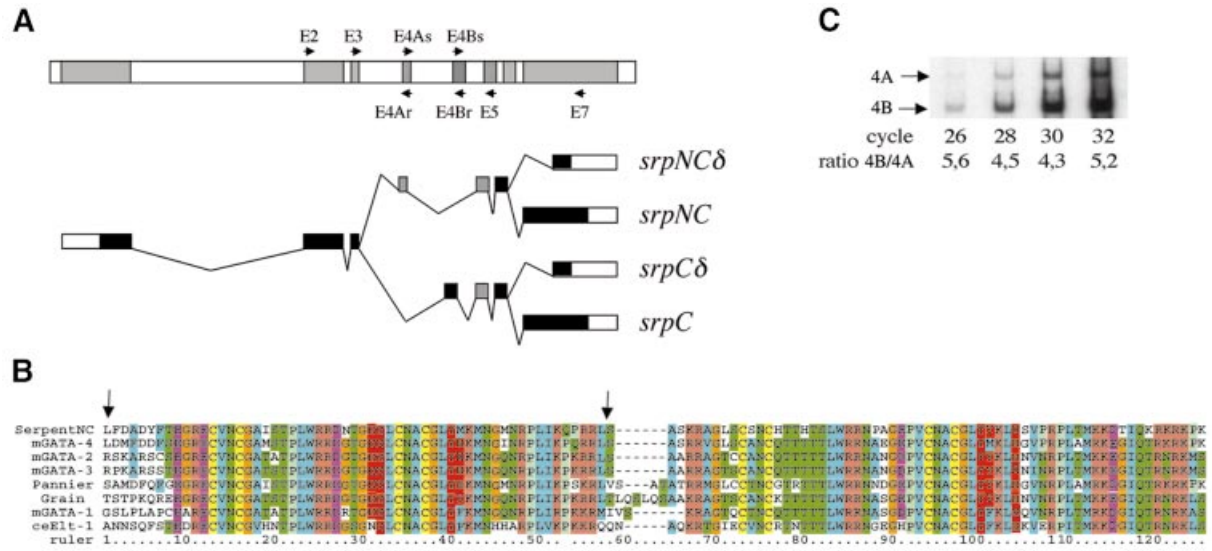

Fig. 1. (**A**) Schematic representation of the *srp* locus and alternatively spliced transcripts. The location and orientation of the primers used for RT–PCR analysis are indicated. The non-coding regions in *srp* transcripts are indicated as open boxes. Exons 4A and 5, coding for the N- and the C-finger, respectively, are indicated as grey boxes. Exon 4A starts at position 121 031 and ends at 121 202 with reference to *Drosophila* scaffold region AE003711 (Flybase). Similarly, the internal splice acceptor site in exon 7 is located at position 123 288. (**B**) Alignment of the GATA zinc finger domains of SerpentNC with Pannier, Grain, mouse GATA-1, -2, -3 and -4 and *Caenorhabditis elegans* Elt-1. Arrows above the SrpNC sequence delineate the region coded by exon 4A. Conserved residues in each column are coloured according to the consensus character assigned to that column; brown, R and K; green, N, T, Q and S; purple, E and D; blue, I, F, L, A, V and M; red, H and Y; orange, G; light green, P; and yellow, C. (**C**) Semi-quantitative RT–PCR analysis of *srp* transcripts containing either exon 4A or exon 4B. RT–PCR was performed with E3 and E5 primers on RNA extracted from stage 5–14 embryos. Aliquots of the PCR were taken after different numbers of cycles. Products containing exon 4A or 4B were resolved on acrylamide gel, and their relative amount was quantified.

activity is largely dependent on the expression of *croquemort*, a member of the CD36 receptor family (Franc *et al*., 1999). They also participate in the synthesis of extracellular matrix components such as peroxidasin (Nelson *et al*., 1994). Crystal cells remain located around the proventriculus during embryogenesis and play a role in melanization, a defence-related process, during larval stages (Rizki *et al*., 1980).

Several genes that control blood cell formation and differentiation in *Drosophila* have been identified. Expression of the GATA transcription factor Srp in the procephalic mesoderm is required for the formation and differentiation of both classes of haemocytes (Rehorn *et al*., 1996). The transcription factor encoded by *glial cell missing* (*gcm*) is involved in plasmatocyte formation (Bernardoni *et al*., 1997), whereas the Runt factor Lozenge (Lz) is absolutely required for crystal cell formation (Lebestky *et al*., 2000). Finally, the *Drosophila* FOG protein, Ush, appears to repress crystal cell production (Fossett *et al*., 2001). All these three genes require the activity of *srp* since in its absence none of them is expressed in the haematopoietic anlage. Yet it is still not understood how these genes control blood cell formation at the molecular level. Of particular interest is the case of Srp and Ush. So far, all known functions of FOG proteins seem to be mediated by GATA factors (see for example Chang *et al*., 2002). Since Srp contains only a C-finger, it should be unable to interact with Ush. This suggests either that Ush has a GATA-independent function in haematopoiesis or that Ush acts via an uncharacterized GATA protein containing an N-finger.

In order to gain insight into the molecular mechanisms controlling blood cell formation, we sought new potential GATA protein-encoding genes in the *Drosophila* genome. We found that the *srp* locus contains an N-finger-coding exon that is alternatively spliced to give rise to proteins that contain either a C-finger (SrpC) only or both an N-finger and a C-finger (SrpNC). We have characterized these two isoforms *in vitro* and *in vivo* during haematopoieisis. Interestingly, we show that SrpC and SrpNC have both common and distinct activities. Finally, we provide evidence that Ush interacts with SrpNC and regulates its activity with respect to specific target genes. We propose that the co-expression of Srp proteins containing either one or two zinc fingers provides an extension of the regulatory properties of *srp*, consistent with its broad range of functions during development.

Results

serpent encodes isoforms including N and C zinc fingers

In a systematic search for GATA zinc finger-coding sequences in the *Drosophila* genome, we found five genes (see Materials and methods for details): *dGATA-E* (*CG10278*), *dGATA-D* (*CG5034*), *pnr*, *grain* and *srp*. dGATA-E and dGATA-D appear to include only a C-finger, while Pnr and Grain have already been shown to contain both an N- and a C-finger (Ramain *et al*., 1993; Lin *et al*., 1995). Interestingly, while Srp was reported previously to contain a single C-finger, our search revealed the presence of a putative exon (E4A) coding for an

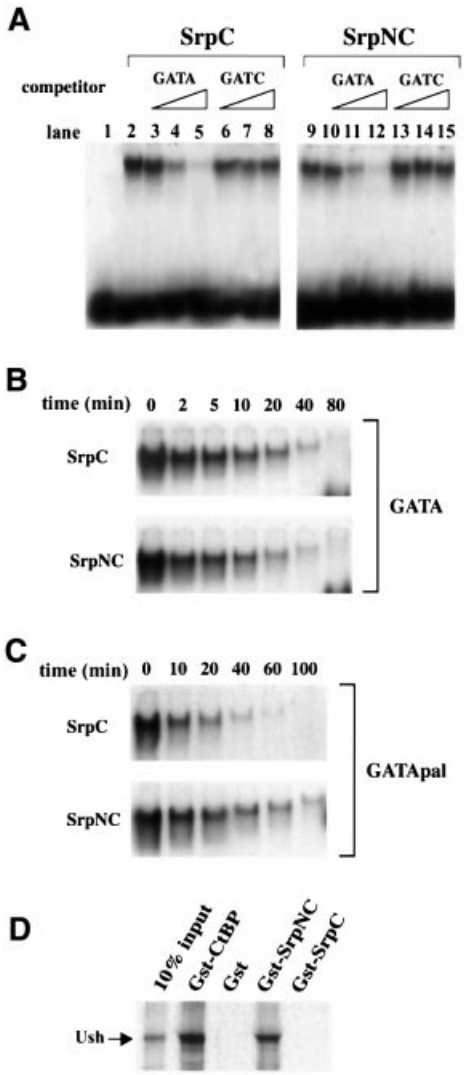

Fig. 2. (A–C) EMSAs using *in vitro* translated SrpC or SrpNC proteins. (**A**) SrpC and SrpNC both specifically bind a consensus GATA probe. Increasing concentrations (5- to 500-fold excess) of unlabelled wild-type (GATA) or mutant (GATC) competitors were added to the reaction as indicated in the upper part of the panel. No GATA-binding activity was observed with unprogrammed reticulocyte lysate (lane 1). (**B** and **C**) SrpC and SrpNC have distinct site-dependent binding properties as revealed by dissociation rate assays. After formation of the complexes between SrpC (upper panel) or SrpNC (lower panel) with either a single GATA site (B) or a palindromic double GATA site (C), an excess of the corresponding unlabelled oligonucleotides was added to the reaction mix and samples were loaded on the gel at various times, as indicated. (**D**) Only SrpNC interacts with Ush *in vitro*. Equivalent molar amounts of the GST fusion proteins were tested for their interaction with *in vitro* translated ^{35}S-labelled Ush as indicated in the upper part of the panel.

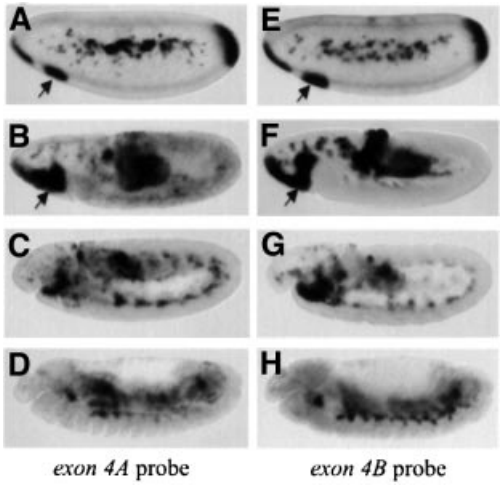

Fig. 3. The transcripts containing either exon 4A (srpNC and srpNCδ) or exon 4B (srpC and srpCδ) have similar expression patterns during embryogenesis. Side views of stage 5 (**A** and **E**), stage 8 (**B** and **F**), stage 11 (**C** and **G**) or stage 14 (**D** and **H**) embryos hybridized with an RNA probe directed against either exon 4A (A–D) or exon 4B (E–H). Arrows indicate the expression of *srp* in the haemocyte primordium.

N-finger motif in *srp*. Using RT–PCR assays with various combinations of oligonucleotides (see Materials and methods and Figure 1A), we showed that E4A is expressed and that E4A and E4B are alternatively spliced to exon 5 (data not shown). In the course of these experiments, we also identified an additional splice acceptor site within E7 (Figure 1A). This downstream acceptor site in E7 is out-of-frame and leads to the deletion of the Srp glutamine-rich C-terminal region. Our data indicate that four alternatively spliced mRNAs are transcribed from *srp*, two encoding products with a single C-finger (SrpC and SrpCδ) and two encoding products with both N- and C-fingers (SrpNC and SrpNCδ) (Figure 1A). Interestingly, in SrpNC and SrpNCδ, the two fingers present the same conserved organization as in other GATA factors (Figure 1B). Notably, they are separated by 29 amino acids, as in all vertebrate GATA. In the following experiments, we used the two isoforms that contain the full-length exon 7, i.e. *srpC* and *srpNC*, to address the functional consequences of the alternative splicing of E4A and E4B.

SrpNC shows specific features of a two-fingered GATA factor

We first determined whether SrpC and SrpNC displayed different properties *in vitro*. While the C-finger is necessary and sufficient for specific DNA binding, it has also been shown in vertebrates that the N-finger can stabilize the binding to particular double GATA sites (Trainor *et al*., 1996). We tested, by electophoretic mobility shift assays (EMSAs), whether SrpNC and SrpC had similar DNA-binding properties. As shown in Figure 2A (lanes 2 and 9), both *in vitro* translated SrpC and SrpNC proteins bound to an oligonucleotide containing a consensus GATA site. The binding was specific, since it could be competed out efficiently by an excess of cold GATA oligonucleotide (Figure 2A, lanes 3–5 and 10–12), but not by an excess of the GATC oligonucleotide (Figure 2A, lanes 6–8 and 13–15). The stability of the SrpN and SrpNC complex on a single or on a palindromic GATA site was assessed by dissociation experiments. While the rate of dissociation was similar for SrpC and SrpNC on a single GATA probe (Figure 2B), SrpNC bound more stably than SrpC to the palindromic GATA sites (Figure 2C).

The GATA N-finger allows interaction with cofactors of the FOG family (Fox *et al*., 1998). Key residues that are required for the interaction between GATA and FOG are conserved in the Srp N-finger. In order to test the binding between Ush and *srp* products, we performed pull-down

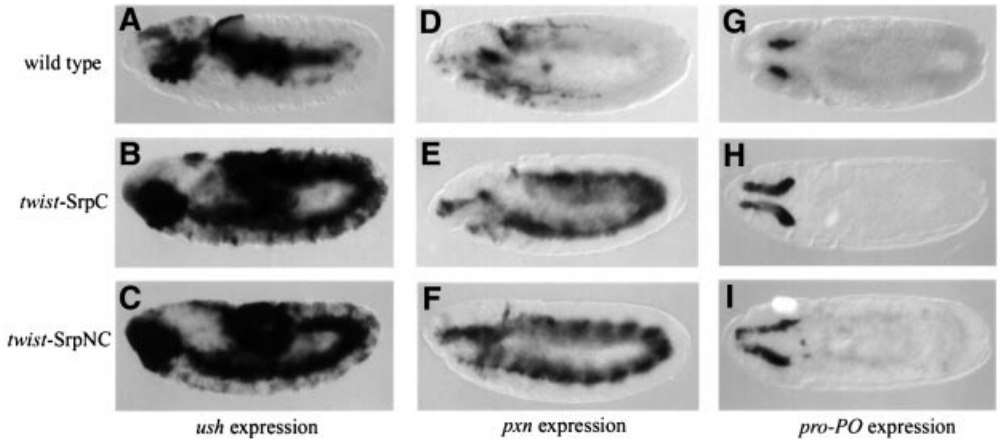

Fig. 4. SrpC and SrpNC have a similar capacity to activate ectopically the expression of *ush*, *pxn* and *pro-PO*. (**A–C**) Side views of *ush* mRNA expression in stage 10 embryos. (**D–F**) Side views of *pxn* mRNA expression in stage 11 embryos. (**G–I**) Dorsal views of *pro-PO* mRNA expression in stage 11 embryos. (A, D and G) Wild type, (B, E and H) *twist-Gal4*; UAS-SrpC and (C, F and I) *twist-Gal4*; UAS-SrpNC.

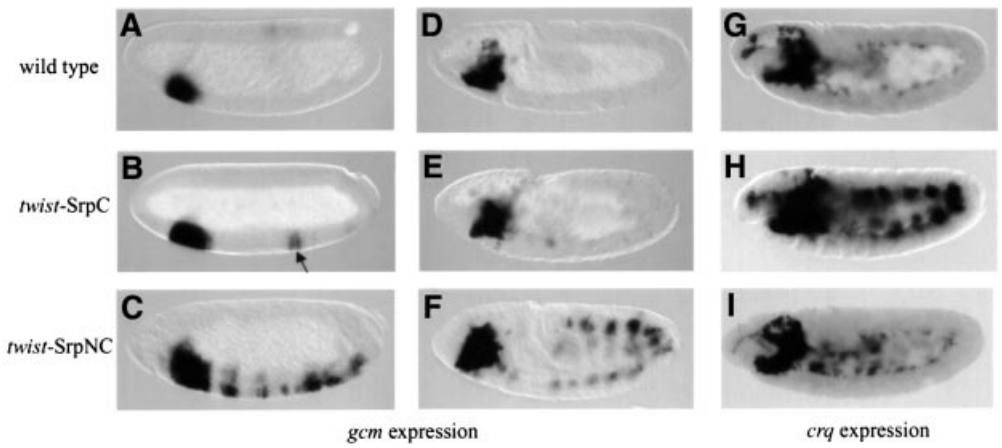

Fig. 5. SrpC and SrpNC differentially activate *gcm* and *crq* expression. (A–F) Side views of *gcm* mRNA expression in stage 5 (**A–C**) or stage 9 (**D–F**) embryos. (**G–I**) Side views of *crq* mRNA expression in stage 10 embryos. (A, D and G) Wild type, (B, E and H) *twist-Gal4*; UAS-SrpC and (C, F and I) *twist-Gal4*; UAS-SrpNC. The arrow in (B) points to the few cells that express *gcm* ectopically in response to SrpC.

assays *in vitro*. We found that *in vitro* translated [^{35}S]methionine-labelled Ush bound to GST–SrpNC, but not to GST alone nor to GST–SrpC (Figure 2D). Thus, Ush specifically interacts with Srp isoforms that contain the N-finger. In addition, like its vertebrate homologues (Fox *et al.*, 1999), Ush interacted with the transcriptional corepressor dCtBP in this assay (Figure 2D).

Taken together, our results indicate that SrpNC displays features characteristic of two-fingered GATA factors. The two types of naturally occurring isoforms encoded by *srp* (with or without the N-finger) have different DNA-binding properties, and only the isoforms including an N-finger can interact with Ush.

srpC and srpNC transcripts have identical expression patterns

In order to determine whether a spatial regulation of the alternative splicing leading to SrpC and SrpNC occurs during embryonic development, we assessed the distribution of the corresponding *srp* transcripts by *in situ* hybridization using specific probes for exon 4A or 4B. At the blastoderm stage and during gastrulation, *srp*C and *srp*NC show the same expression pattern (Figure 3A, B, E and F). They are expressed in the procephalic mesoderm, the haemocyte primordium (arrows in Figure 3), at the anterior and posterior pole, in the primordium of the anterior and posterior midgut as well as in the amnioserosa and in the yolk cells. Later, during germ band extension, and after germ band retraction, *srp*C and *srp*NC are expressed identically in the developing fat body (Figure 3C, D, G and H) (for a full description of *srp* expression see Rehorn *et al.*, 1996). Thus, *srp*C and *srp*NC transcripts are not differentially regulated spatially during embryonic development. However, the level of the transcripts is not identical. Indeed, by means of semi-quantitative RT–PCR, we determined that exon 4B-containing mRNA is five times more abundant than exon 4A-containing mRNA (Figure 1C), suggesting that two-fingered isoforms of Srp are less abundant than single-fingered isoforms.

SrpC and SrpNC differ in their capacity to activate certain target genes in vivo

In order to analyse SrpC and SrpNC activities, we tested their capacities to activate gene expression *in vivo* during *Drosophila* embryonic haematopoiesis. Using the

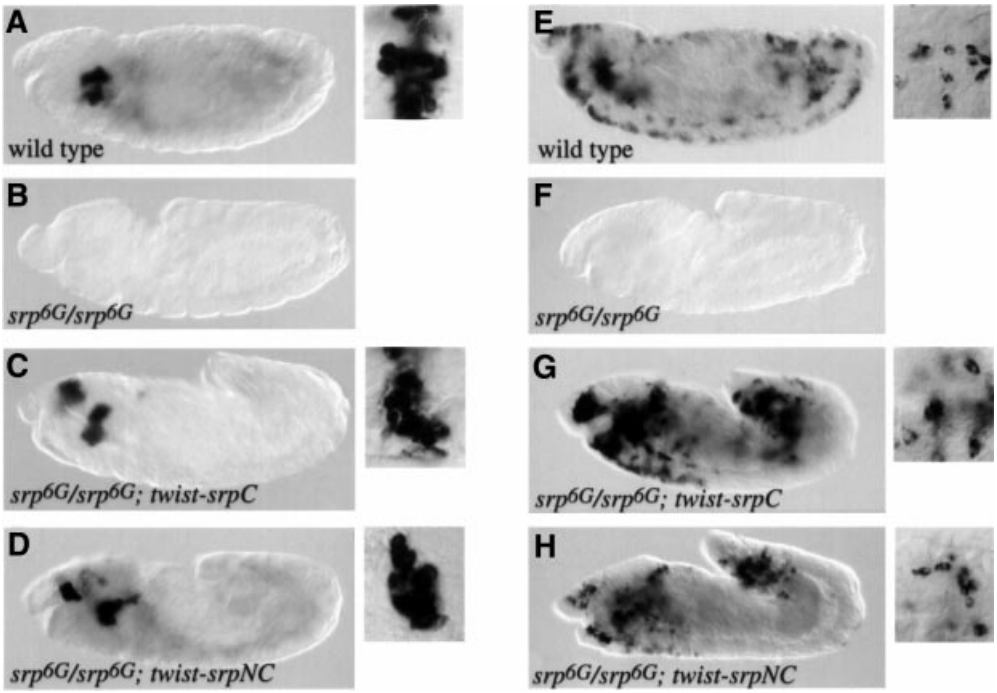

Fig. 6. SrpC and SrpNC individually can rescue the lack of crystal cells and plasmatocytes due to an *srp* null mutation. *In situ* hybridization revealing *pro-PO* (A–D) or *pxn* (E–H) expression in stage 13–14 embryos. (**A** and **E**) Wild type, (**B** and **F**) srp^{6G}/srp^{6G}, (**C** and **G**) *twist-Gal4*/+; UAS-SrpC; srp^{6G}/srp^{6G} and (**D** and **H**) *twist-Gal4*/UAS-SrpNC; srp^{6G}/srp^{6G}. Ten-fold higher magnifications of labelled cells are shown to the right of the wild-type and rescued embryo panels.

UAS-GAL4 system, we ectopically expressed them in the mesoderm and we then assessed the expression pattern of various haematopoietic markers.

The two genes *ush* and *gcm* play critical roles in embryonic haematopoiesis. Their expression in the haematopoietic primordium occurs early and appears to depend on *srp* activity (Bernardoni *et al*., 1997; Fossett *et al*., 2001). Therefore, we decided to determine whether they are transcriptional targets of SrpC and/or SrpNC. We found that, whereas in a wild-type early embryo, *ush* expression is restricted to the anterior mesoderm, *twist*-driven expression of SrpC (*twist*-SrpC) or SrpNC (*twist*-SrpNC) induced strong expression of *ush* throughout the mesoderm (Figure 4, compare A with B and C). In contrast, *twist*-SrpC induced *gcm* expression poorly and in a limited number of mesodermal cells of stage 5 embryos (arrow in Figure 5B), whereas *twist*-SrpNC strongly activated *gcm* expression segmentally from stage 5 to 9 (Figure 5C and F).

Next we looked at the expression of haematopoietic lineage-specific markers. As plasmatocyte markers, we used *peroxidasin* (*pxn*) and *croquemort* (*crq*). Since, Rizki *et al*. (1980) suggested that crystal cells are the only source of prophenoloxidase (pro-PO) in *Drosophila*, we used expression of this gene to monitor crystal cell formation. *pro-PO* transcripts were indeed detected in these cells from early stage 11 to the end of embryogenesis. We confirmed that *pro-PO* expression is specific to the crystal cells since it was not detected in *lz* mutant embryos (data not shown). Analysing these markers, we again observed two situations. On the one hand, *twist*-SrpC and *twist*-SrpNC had similar abilities to induce expression of the plasmatocyte marker *pxn* and of the crystal cell marker *pro-PO* (Figure 4E and F, and H and I, respectively). On the other hand, expression of *crq* was induced by *twist*-SrpC but not by *twist*-SrpNC (Figure 5, compare H with I). Note that *pxn* and *crq* were induced through most of the mesoderm, while *pro-PO* activation was restricted to the head region.

Taken together, our data show that SrpC and SrpNC have both common and different activities during haematopoiesis. Indeed, both isoforms activated the expression of *ush*, *pxn* and *pro-PO* in a similar manner. However, SrpC and SrpNC differentially stimulate the expression of *crq* and *gcm*, respectively, in the mesoderm.

SrpC and SrpNC both induce the crystal cell and plasmatocyte lineages in an srp null embryo

srp is absolutely required for determination of all haematopoietic lineages (Rehorn *et al*., 1996; Lebestky *et al*., 2000). However, the relative contributions of SrpC and SrpNC to this process are unknown, since there is no known mutation in *srp* that affects only one of the two classes of isoforms. To address this question, we asked whether SrpC and SrpNC individually could rescue the plasmatocyte and/or crystal cell lineages in an *srp* mutant background. During haematopoiesis, *srp* expression is first detected at the blastoderm stage in a patch of cells within the mesoderm. Therefore, we used the *twist-Gal4* driver to express UAS-SrpC or UAS-SrpNC in the mesoderm of *srp* mutant embryos. To monitor crystal cell formation and plasmatocyte formation, we assessed the expression of *pro-PO* and *pxn*, respectively. Whereas no *pro-PO* expression was detected in *srp* mutant embryos, *pro-PO* expression was restored around the proventriculus and in an additional patch of cells located above the pharynx

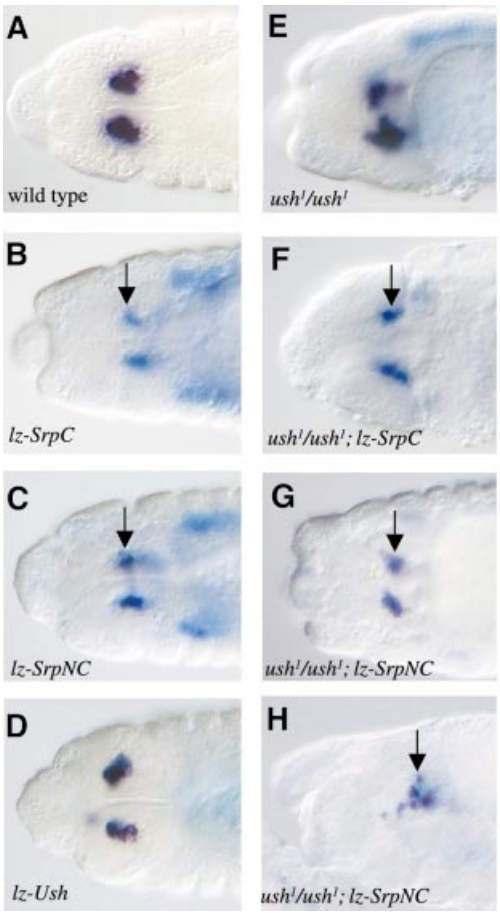

Fig. 7. Forced expression of SrpC or SrpNC in the crystal cells represses their formation independently of *ush*. Side views (A–G) or dorsal view (H) of stage 13 embryos processed to reveal *pro-PO* mRNA expression (black staining) and either *srp* mRNA expression (blue staining in B, C, F, G and H) or *ush* mRNA expression (blue staining in D). (**A**) Wild type, (**B**) *lz-Gal4*/+; UAS-SrpC, (**C**) *lz-Gal4*/+; UAS-SrpNC, (**D**) *lz-Gal4*/+; UAS-Ush, (**E**) ush^1/ush^1, (**F**) *lz-Gal4*/+; ush^1/ush^1; UAS-SrpNC and (**G** and **H**) *lz-Gal4*/+; ush^1/ush^1; UAS-SrpNC. Arrows indicate crystal cells expressing *srp* but not *pro-PO*.

upon expression of *twist*-SrpC or *twist*-SrpNC (Figure 6, compare B with C and D). This phenotype is equivalent to that which we observed previously in wild-type embryos expressing *twist*-SrpC or *twist*-SrpNC. Higher magnification views showed that the *pro-PO*-expressing cells had the typical morphology of crystal cells. Similarly, no expression of *pxn* was detected in *srp* mutant embryos, while scattered *pxn*-positive cells were observed in srp^- embryos expressing *twist*-SrpC or *twist*-SrpNC (Figure 6, compare F with G and H). Morphological analysis confirmed that these cells were genuine plasmatocytes (Tepass *et al*., 1994). Thus our results suggest that SrpC and SrpNC are each able to induce the formation of both lineages.

Misexpression of SrpC or SrpNC controls crystal cell formation independently of Ush

Previous studies have shown that *ush* acts to repress crystal cell formation. Notably, Fossett *et al*. (2001) showed that *lz-Gal4*-driven expression of UAS-Ush led to a variable decrease in the number of crystal cells, as monitored by the expression of a *Uas-lacZ* reporter gene driven by *lz-Gal4*. However, the molecular mechanism of action of *ush* in this process remained largely unknown. The expression of SrpNC during haematopoiesis suggested that it might act with Ush. Thus we decided to analyse the consequences of misexpressing either SrpC or SrpNC in the crystal cells. Because only SrpNC can interact with Ush, we surmised that SrpNC, but not SrpC, would repress crystal cell formation. We used the *lz-Gal4* driver to express UAS-SrpC, UAS-SrpNC or UAS-Ush in the crystal cells. Misexpression of each transgene was visualized by *in situ* hybridization with a probe against *srp* or *ush*, respectively, and differentiation of the crystal cells was monitored by analysing *pro-PO* expression. As expected, UAS-Ush induced a reduction in the number of crystal cells in stage 13–16 embryos (Figure 7, compare A with D). Surprisingly, both UAS-SrpC and UAS-SrpNC also reduced the number of crystal cells (Figure 7B and C). Note that the reduction induced by SrpC or SrpNC was greater than that observed with Ush, although, as for Ush, we observed considerable variations of phenotype within the population of embryos. Most interestingly, we observed strong expression of UAS-SrpC or UAS-SrpNC driven by *lz-Gal4* and no expression of *pro-PO* in the same cells in stage 13–16 embryos (arrows in Figure 7B and C). In contrast, *lz-Gal4*-driven expression of UAS-Ush did not prevent *pro-PO* expression in these cells (Figure 7D). This suggests that SrpC and SrpNC can control both the number and the differentiation of the crystal cells, while Ush only affects their number.

However, it still remained possible that SrpNC was acting in a complex with Ush to prevent crystal cell formation and differentiation. Additionally, the effect of SrpC and SrpNC on crystal cell number could be related to Ush activity, since SrpC and SrpNC can induce its expression (at least in the mesoderm, see above). In order to test this hypothesis, we misexpressed UAS-SrpC or UAS-SrpNC under the control of *lz-Gal4* in an *ush* null mutant background. As shown in Figure 7F, G and H, even under these conditions, SrpC and SrpNC reduced the number of crystal cells and repressed *pro-PO* expression. Therefore, SrpC and SrpNC can both inhibit crystal cell formation and differentiation independently of *ush* activity.

The SrpNC–Ush complex represses crq expression

We have shown that the misexpression of SrpC, but not SrpNC, activates ectopic expression of *crq*. Conversely, SrpNC is a much stronger activator of *gcm* expression than SrpC. These differences in activity on particular target genes could be due to the capacity of SrpNC, unlike SrpC, to form a complex with Ush. Given that *twist*-SrpNC induces *ush* expression in the mesoderm, it is possible that Ush exerts a feedback action on SrpNC, preventing it from activating *crq* expression and/or enhancing its capacity to activate *gcm*. We decided to check this possibility by assaying the expression of *crq* and *gcm* in an *ush* mutant embryo expressing *twist*-SrpNC. The absence of *ush* had no detectable effect on the activation of *gcm* by *twist*-SrpNC, showing that *ush* is not involved in this specific activity of SrpNC (data not shown). On the contrary, we observed ectopic expression of *crq* by *twist*-SrpNC in the absence of *ush* function (Figure 8, compare A and B with C). Note that *twist*-SrpC-mediated

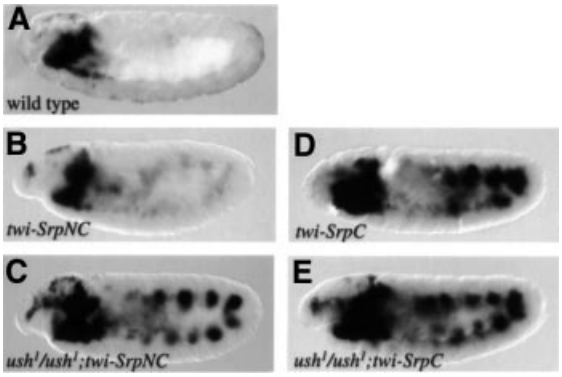

Fig. 8. *ush* inhibits SrpNC-mediated activation of *crq*. Side views of *crq* mRNA expression in stage 10 embryos. (**A**) Wild type, (**B**) *twist-Gal4/+*; UAS-SrpNC, (**C**) *twist-Gal4/+*; ush^1/ush^1; UAS-SrpNC, (**D**) *twist-Gal4/+*; UAS-SrpC and (**E**) *twist-Gal4/+*; ush^1/ush^1; UAS-SrpC.

activation of *crq* was similar in a wild-type and *ush* mutant embryo (Figure 8, compare D with E). These data strongly suggest that Ush can form a complex with SrpNC, thereby modulating the transactivation of *crq*.

This also led us to consider that *ush* might repress the expression of *crq* in the wild-type embryo. We thus assayed *crq* expression in an *ush* mutant. In wild-type stage 11 embryos, the expression of *crq* was barely detectable in migrating plasmatocytes, especially those localized beyond the head (Figure 9A). However, the level of expression of *crq* was increased in *ush* mutants, and *crq*-positive plasmatocytes were clearly visible in the trunk region (Figure 9B). Moreover, in *ush* embryos, the expression of *crq* persisted longer than in wild type (Figure 9, compare C with D). Note that this effect was specific to *crq*, as the expression of *pxn* in the plasmatocytes appeared normal in *ush* mutant embryos (data not shown). These observations are consistent with a role for Ush in the control of *crq* expression in association with SrpNC. In conclusion, these results support the idea that some, but not all, of the differential activities of SrpC and SrpNC *in vivo* depend on a physical interaction between SrpNC and Ush.

Discussion

We have identified new isoforms, encoded by the previously characterized gene *srp*, which are produced by alternative splicing. The alternative use of exon 4A and 4B allows the production of GATA proteins containing either a single C-finger or both N- and C-fingers. Analysis of the expression pattern of these isoforms indicated that the splicing mechanism is not spatially regulated and thus that isoforms with one or two fingers most probably co-exist in the same cells. We also identified a new splice acceptor site within exon 7. Use of this internal site leads to the synthesis of Srp proteins with a shorter C-terminal region. The Srp C-terminal domain has no significant homology to other proteins suggestive of a possible function. Here, we have focused our study on the functional differences of GATA factors harbouring one or two zinc fingers and

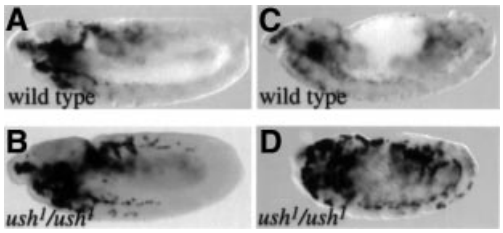

Fig. 9. *ush* downregulates *crq* expression. Side views of *crq* mRNA expression in stage 11 (A and B) or 14 (C and D) embryos. (**A** and **C**) Wild type, (**B** and **D**) ush^1/ush^1.

on the characterization of the two isoforms SrpC and SrpNC.

SrpC and SrpNC have common and different features

It is remarkable that *srp* encodes both single and dual zinc finger-containing products. Our results provide strong evidence that this alternative splicing allows production of transcription factors with specific activities. On the one hand, the two isoforms activated the expression of *ush* and *pxn* with similar efficiency, suggesting that SrpC and SrpNC have similar transactivating properties *in vivo*. On the other hand, SrpC, but not SrpNC, activated *crq* expression, while SrpNC was a much stronger activator of *gcm* expression than SrpC. The domain coded by exon 4B that is present only in SrpC has no known motif and we do not know if and how it participates in SrpC-specific function. However, the presence of the N-terminal zinc finger encoded by exon 4A may explain some of the distinct features of SrpNC as discussed below.

We show that, as in the case of vertebrate GATA-1, the presence of the N-finger in Srp stabilizes binding to double palindromic GATA sites. Although the N-finger of GATA-1 modulates the binding and the transactivating properties of GATA-1 on synthetic promoters (Trainor *et al*., 2000), the functional importance of these effects has remained elusive, particularly as no GATA-1 isoform contains only the C-finger. In the case of *srp*, these distinct binding properties may have direct functional consequences. For instance, the fact that SrpC and SrpNC activate a common target, *ush*, whereas only SrpNC strongly activates a specific target, *gcm*, could be related to the DNA-binding specificity of the two isoforms. A scan of the *ush* upstream regulatory region shows that it contains several GATA consensus sequences, nine of which are clustered in <1 kb and are organized as three repetitions of three sites. In contrast, GATA sites are far less frequent in *gcm* regulatory regions and are often organized in palindromes. Considering that *ush* and *gcm* are likely to be direct target genes for *srp*, the different organization of their regulatory regions may explain the differential effect we observed.

SrpC and SrpNC can both induce blood cell formation in Drosophila

We were able to rescue the lack of plasmatocyte and crystal cell formation due to an *srp* null mutation by expressing SrpC or SrpNC in the mesoderm. No difference between the two isoforms was seen in this assay, suggesting that the N-finger is not absolutely required for *srp* function in embryonic blood cell formation.

However, in the absence of a functional test, we cannot determine to what extent the formation of embryonic blood cells is fully rescued. Interestingly, rescue experiments with the mouse *GATA-1* mutant indicate that the GATA-1 N-finger is dispensable for primitive erythropoiesis but is required for definitive erythopoiesis (Shimizu *et al*., 2001). In *Drosophila*, a second wave of haematopoiesis, occurring at the larval stage, gives rise to four different lineages: plasmatocytes, crystal cells, secretory cells and lamellocytes (Lanot *et al*., 2001). *srp* is expressed in the dorsal lymph gland (i.e. the main larval haematopoietic organ) and it probably controls larval haematopoiesis (Lebestky *et al*., 2000). By analogy to vertebrate GATA-1, the Srp N-finger may provide an additional function for larval haematopoiesis, perhaps during formation of the new cell types.

In our assay, the expression of the transgene was limited to the mesoderm but it still rescued blood cell formation. This finding suggests that the early expression of *srp* in the haematopoietic primordium is sufficient to initiate the genetic programme that controls haemocyte formation and differentiation. Interestingly, in the wild-type embryo, *srp* transcripts are not expressed detectably in haemocytes after stage 11, but Srp protein is detected in plasmatocytes and crystal cells throughout most of embryogenesis (Sam *et al*., 1996; Lebestky *et al*., 2000). Persistence of *srp* products in haemocytes might be critical for *srp* function, and control of *srp* products at the post-translational level may play a crucial role in the correct regulation of blood cell differentiation. Rescue of crystal cell formation by mesodermal expression of SrpC and SrpNC contrasts with the observation that later expression driven by *lz-Gal4* in crystal cells represses their development. Lebetsky *et al*. (2000) reported that Srp levels were reduced in crystal cells compared with surrounding plasmatocytes. Therefore, our results are consistent with a two-step model in which Srp expression is first necessary to induce *lz* expression and subsequently is downregulated to allow crystal cell differentiation.

Ush regulates SrpNC activity on a specific target gene

One of the best characterized features of GATA N-fingers is their dimerization with cofactors of the FOG family. Consistent with this feature, we found that SrpNC interacts with the *Drosophila* FOG Ush, but SrpC does not. Previous analysis showed that *ush* regulates the number of crystal cells (Fossett *et al*., 2001). It was proposed that this function of *ush* could be mediated by a putative isoform of Srp containing an N-finger. Our findings strongly support this hypothesis. However, it was not possible to address this issue directly, since both SrpC and SrpNC display a strong Ush-independent repressive effect on crystal cell formation and differentiation.

A new function of *ush* revealed here is the regulation of the level of expression of the macrophage receptor *crq*, suggesting that *ush* displays a broader function in haematopoiesis than previously assumed. Notably, we provide evidence that Ush modulates SrpNC transactivation of *crq*. As Ush interacts with the corepressor dCtBP *in vitro*, the Ush–SrpNC complex could repress *crq* expression. However, we do not know whether *crq* is a direct target of *srp*, so we cannot rule out the possibility that the Ush–SrpNC complex activates a transcriptional repressor that regulates *crq*. Vertebrate FOGs can act as either a coactivator or a corepressor of GATA factors (Tsang *et al*., 1997; Crispino *et al*., 1999; Fox *et al*., 1999; Deconinck *et al*., 2000). In *Drosophila*, Ush was clearly shown to be a repressor of Pannier-induced activation in cell culture, and it probably also represses the expression of *achaete* in the dorso-central proneural cluster *in vivo* (Cubadda *et al*., 1997; Haenlin *et al*., 1997; Garcia-Garcia *et al*., 1999). Furthermore, in a heterologous assay in *Drosophila*, the CtBP-binding region of mFOG2 was shown to be required for repressing the formation of crystal cells but not cardiac cells (Fossett *et al*., 2000, 2001). Thus several mechanisms seem to regulate the function of the GATA–FOG complex.

Remarkably, some functions of SrpNC appear to be independent of Ush. Thus, *gcm*-specific activation by SrpNC is not affected in an *ush* mutant embryo. Moreover, SrpNC still represses crystal cell formation in the absence of *ush*. This is reminiscent of mouse erythropoiesis, where both FOG-dependent and FOG-independent regulation of gene expression by GATA-1 have been observed (Crispino *et al*., 1999). The molecular mechanisms underlying the regulation by Ush/FOG-1 of SrpNC/GATA-1 activity on some specific targets remain to be elucidated. It is tempting to speculate that the N-finger of SrpNC is involved in the recognition of promoter sequences, on *gcm* for example, and thus is not available to recruit Ush. Alternatively, other cofactors already localized to the promoter or bound to SrpNC might prevent Ush binding to the N-finger.

srp is a structural and functional homologue of vertebrate GATA genes

We have focused our study on haematopoiesis, but *srp* also participates in other developmental processes, such as germ band retraction (Frank and Rushlow, 1996), midgut differentiation (Reuter, 1994), fat body formation (Hayes *et al*., 2001), induction of the immune response (Petersen *et al*., 1999) and the ecdysone response (Brodu *et al*., 1999). It will be interesting to determine the respective roles of SrpC and SrpNC in these different phenomena. Phylogenetic analysis shows that SrpNC is closely related to vertebrate GATA factors. It has been suggested that *srp* is a functional homologue of the entire vertebrate GATA family, since *srp* is required in *Drosophila* for haematopoiesis, like GATA-1/2/3 in mice, and for endodermal development, like GATA-4/5/6 (Rehorn *et al*., 1996). Nevertheless, this hypothesis was at odds with the fact that Srp seemingly had a single zinc finger while all the vertebrate GATAs have two (Lowry and Atchley, 2000). The present identification of Srp isoforms with two fingers gives new force to this hypothesis. Further, the expression of isoforms of Srp with distinct activities helps to account for the broad range of functions ensured by this gene.

It is worth noting that alternative splicing eliminating the N-finger has also been described in *Bombyx mori GATA*β (Drevet *et al*., 1995) and in chicken *GATA-5* genes (MacNeill *et al*., 1997). Moreover, a BLAST search analysis revealed alternatively spliced human expressed sequence tags coding for two isoforms of a potential GATA factor with either one or two zinc fingers (L.Waltzer, unpublished results). This suggests that

alternative splicing of GATA genes could be more general than previously thought, and as yet unnoticed splice variants of GATA vertebrate genes may generate proteins with only a C-finger.

In conclusion, our results shed further light on the molecular control of haematopoiesis by the GATA factor Srp. The alternative splicing of *srp* gives rise to different Ush-interacting and non-interacting Srp proteins with different target gene specificities, thereby contributing to the exquisite control of *Drosophila* blood cell formation. We speculate that alternative splicing of the GATA N-finger might be an important mechanism regulating the activity of other GATA genes from insects to man.

Materials and methods

Fly stocks

The *twist-Gal4*, *lz-Gal4*, srp^{6G}, ush^{1} stocks were provided by the *Drosophila* Stock Center, Bloomington, IN. Several *Uas-SrpC* and *Uas-SrpNC* transgenic lines were generated by P-element-mediated germline transformation of the pUAST-*srpC* and pUAST-*srpNC* plasmids, respectively, into w^{1118} embryos according to standard protocols. *Uas-Ush* transgenic lines have already been described in Haenlin *et al*. (1997).

Embryos obtained from the mating of *Uas-SrpC*, *Uas-SrpNC* or *Uas-Ush* to *twist-Gal4* or *lz-Gal4* flies were collected at 25°C. To analyse the phenotype of the rescued *srp* mutant by SrpC or SrpNC, *twist-Gal4*; srp^{6G} *e*/TM3, *twist-lacZ* females were crossed to *Uas-SrpC*; srp^{6G} *e*/TM3, *twist-lacZ* or to *Uas-SrpNC*/Y, srp^{6G} *e*/TM3, *twist-lacZ* males. *lacZ* staining was used to genotype the embryos. To analyse the effect of overexpression of SrpC or SrpNC on the production of crystal cells in an *ush* mutant background, ush^{1}/*CyO*; *Uas-SrpC* or ush^{1}/*CyO*; *Uas-SrpNC* females were crossed to *lz-Gal4*/Y, ush^{1}/*CyO* males. In order to observe the effect of mesodermal expression of SrpNC in *ush* mutant embryos, *twist-Gal4*; ush^{1}/*Cyo* females were crossed to ush^{1}/*CyO*; *Uas-SrpNC* males. *ush* embryos were identified by their retraction phenotype. Embryos overexpressing SrpC or SrpNC were identified after *in situ* hybridization against *srp*.

Database search

In order to find all the potential GATA factor-encoding genes in the *Drosophila* genome, we used either the consensus GATA-type zinc finger sequence defined in PRODOM (reference PD000513), or the *Drosophila* GATA Pannier N-finger or C-finger sequences. These sequences were used as queries in three independent iterative PSI-BLAST searches against the database of predicted proteins encoded by the *Drosophila* genome (BLASTP). Pannier C-finger was also used as a query in a TBLASTN search against the whole *Drosophila* genomic sequence. Similar results were obtained in all searches.

RT–PCR

Total RNA was isolated from dechorionated embryos using Trizol™ according to the manufacturer's instructions. A 2 µg aliquot of RNA was used as a template in a 20 µl reverse transcription reaction with 0.5 µM *srp*-specific oligonucleotide reverse primer or 1 µM oligo(dT) primer. Reverse transcription reaction mixture (0.4 µl) was then used in a standard PCR in the presence of 0.5 µM specific primers. The RT–PCR products were checked on agarose gels, subcloned into pGemT easy vector (Promega) and sequenced.

To compare the levels of exon 4A- versus exon 4B-containing transcripts, reverse transcription was performed using the E5 primer. The PCR was performed in the presence of 0.5 µM E3 primer and a 0.5 µM ^{32}P end-labelled E5 primer. From cycle 20 onward, aliquots were preserved for analysis every two cycles to ensure that amplification was in the logarithmic phase. To facilitate the separation of exon 4A- and exon 4B-specific bands, the RT–PCR products were digested by *Pst*I before being run on a 4% acrylamide gel and quantified with a phosphoimager. RT–PCR experiments were repeated with three different preparations of RNA and gave similar results.

Forward primers used were the following: E2, 5′-TTATGCTGGC-TCGTTGCTTACTC-3′; E3, 5′-ATACCTGGTTCGATCCGTTAAGC-3′, E4AS: 5-GTCAATGTGGTGCGATTTCAAC-3′; and E4BS, 5′-TG-AATCAGGCGGGGATTTCTAT-3′. Reverse primers used were the following: E4AR, 5′-GGCTGTTTAATTAGGGGTCGATTC-3′; E4BR, 5-GGCGCGACTAACTGCTCGTCG-3′; E5, 5′-ATGGTGTCCTTTTT-CATGGTCAGT-3′; and E7, 5′-CAGCGTGTCGCGCCTACTCC-3′.

Plasmids

pBS-KS Srp, containing the SrpC open reading frame (ORF) (Brodu *et al*., 1999), was a gift from C.Antoniewski. The full-length SrpNC ORF was cloned into pBS-KS by PCR. The resulting plasmid, pBS-SrpNC, was checked by sequencing. The SrpC or SrpNC ORF was subcloned into pUAST (for transgenesis) or into pGEX2TK (for GST fusion protein expression) by standard cloning techniques.

In situ hybridizations

In situ hybridizations were carried out as described previously using a Dig-UTP- or fluorescein-UTP-labelled antisense RNA probe (Peyrefitte *et al*., 2001). RNA probes for *srp*, *ush*, *gcm* and *lacZ* have been described previously. To generate RNA probes for *srp* exon 4A, *srp* exon 4B, *crq* exon 3 or *pro-PO* exon 3, the corresponding DNA sequences were cloned by PCR in pGemTeasy. The corresponding antisense RNAs were transcribed *in vitro* using T7 or SP6 RNA polymerase.

Pull-down assays

pGEX2TK-SrpC, pGEX2TK-SrpNC, pGEX-dCtBP (a generous gift from M.Levine) and pGEX2TK plasmids were used to produce GST–SrpC, GST–SrpNC, GST–dCtBP and GST proteins, respectively, in *Escherichia coli* BL21. pBS-Ush cDNA (Cubadda *et al*., 1997) was used as a template to produce full-length Ush protein *in vitro* using a coupled transcription/translation system (Promega) in the presence of [^{35}S]methionine. Interaction assays were performed as described in Waltzer and Bienz (1999).

Electrophoretic mobility shift assays

The following double-stranded oligonucleotides were used in EMSAs: GATA (5′-CTCCGGCAACTGATAAGGACTCCC-3′), GATC (5′-CT-CCGGCAACTGATCAGGACTCCC-3′) and GATApal (5′-CTCCGGC-AACTATCAGATAAGGACTCCC-3′). EMSAs were performed by incubating *in vitro* translated SrpC or SrpNC with 5×10^4 c.p.m. of 5′-end ^{32}P-labelled double-stranded GATA probe for 30 min at room temperature in 10 mM HEPES pH 7.9, 50 mM KCl, 1 mM $MgCl_2$, 1 mM EDTA, 5% glycerol and 0.5 µg poly(dI–dC) in a final volume of 20 µl. The stability of the protein–DNA complexes was assessed by dissociation rate experiments as described in Trainor *et al*. (1996), using a 200-fold excess of unlabelled probe. The reactions were loaded on to a 6% polyacrylamide gel with 0.5× TBE and run at room temperature at 15 V/cm. The protein–DNA complexes were visualized by autoradiography.

Acknowledgements

We are grateful to Professor J.Smith and Dr B.G.Monster for critically reading the manuscript, and B.Augé for expert technical assistance. This work was supported by the Centre National de Recherche Scientifique (CNRS) and grants from the Association pour la Recherche sur le Cancer (ARC), from the Fondation pour la Recherche Médicale (FRM) and from the Ministère de l'Education Supérieure et la Recherche (MESR) of France. S.P. was financed by graduate fellowships from the MESR and ARC.

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.

Bernardoni,R., Vivancos,B. and Giangrande,A. (1997) *glide/gcm* is expressed and required in the scavenger cell lineage. *Dev. Biol.*, **191**, 118–130.

Brodu,V., Mugat,B., Roignant,J.Y., Lepesant,J.A. and Antoniewski,C. (1999) Dual requirement for the EcR/USP nuclear receptor and the dGATAb factor in an ecdysone response in *Drosophila melanogaster*. *Mol. Cell. Biol.*, **19**, 5732–5742.

Chang,A.N., Cantor,A.B., Fujiwara,Y., Lodish,M.B., Droho,S., Crispino,J.D. and Orkin,S.H. (2002) GATA-factor dependence of the multitype zinc-finger protein FOG-1 for its essential role in megakaryopoiesis. *Proc. Natl Acad. Sci. USA*, **99**, 9237–9242.

Cho,N.K., Keyes,L., Johnson,E., Heller,J., Ryner,L., Karim,F. and Krasnow,M.A. (2002) Developmental control of blood cell migration by the *Drosophila* VEGF pathway. *Cell*, **108**, 865–876.

Crispino,J.D., Lodish,M.B., MacKay,J.P. and Orkin,S.H. (1999) Use of altered specificity mutants to probe a specific protein–protein

interaction in differentiation: the GATA-1:FOG complex. *Mol. Cell*, **3**, 219–228.

Cubadda,Y., Heitzler,P., Ray,R.P., Bourouis,M., Ramain,P., Gelbart,W., Simpson,P. and Haenlin,M. (1997) *u-shaped* encodes a zinc finger protein that regulates the proneural genes *achaete* and *scute* during the formation of bristles in *Drosophila*. *Genes Dev.*, **11**, 3083–3095.

Deconinck,A.E., Mead,P.E., Tevosian,S.G., Crispino,J.D., Katz,S.G., Zon,L.I. and Orkin,S.H. (2000) FOG acts as a repressor of red blood cell development in *Xenopus*. *Development*, **127**, 2031–2040.

Drevet,J.R., Swevers,L. and Iatrou,K. (1995) Developmental regulation of a silkworm gene encoding multiple GATA-type transcription factors by alternative splicing. *J. Mol. Biol.*, **246**, 43–53.

Fossett,N. and Schulz,R.A. (2001) Functional conservation of hematopoietic factors in *Drosophila* and vertebrates. *Differentiation*, **69**, 83–90.

Fossett,N., Zhang,Q., Gajewski,K., Choi,C.Y., Kim,Y. and Schulz,R.A. (2000) The multitype zinc-finger protein U-shaped functions in heart cell specification in the *Drosophila* embryo. *Proc. Natl Acad. Sci. USA*, **97**, 7348–7353.

Fossett,N., Tevosian,S.G., Gajewski,K., Zhang,Q., Orkin,S.H. and Schulz,R.A. (2001) The Friend of GATA proteins U-shaped, FOG-1 and FOG-2 function as negative regulators of blood, heart and eye development in *Drosophila*. *Proc. Natl Acad. Sci. USA*, **98**, 7342–7347.

Fox,A.H., Kowalski,K., King,G.F., Mackay,J.P. and Crossley,M. (1998) Key residues characteristic of GATA N-fingers are recognized by FOG. *J. Biol. Chem.*, **273**, 33595–33603.

Fox,A.H., Liew,C., Holmes,M., Kowalski,K., Mackay,J. and Crossley, M. (1999) Transcriptional cofactors of the FOG family interact with GATA proteins by means of multiple zinc fingers. *EMBO J.*, **18**, 2812–2822.

Franc,N.C., Heitzler,P., Ezekowitz,R.A. and White,K. (1999) Requirement for *croquemort* in phagocytosis of apoptotic cells in *Drosophila*. *Science*, **284**, 1991–1994.

Frank,L.H. and Rushlow,C. (1996) A group of genes required for maintenance of the amnioserosa tissue in *Drosophila*. *Development*, **122**, 1343–1352.

Garcia-Garcia,M.J., Ramain,P., Simpson,P. and Modolell,J. (1999) Different contributions of *pannier* and *wingless* to the patterning of the dorsal mesothorax of *Drosophila*. *Development*, **126**, 3523–3532.

Haenlin,M., Cubadda,Y., Blondeau,F., Heitzler,P., Lutz,Y., Simpson,P. and Ramain,P. (1997) Transcriptional activity of Pannier is regulated negatively by heterodimerization of the GATA DNA-binding domain with a cofactor encoded by the *u-shaped* gene of *Drosophila*. *Genes Dev.*, **11**, 3096–3108.

Hayes,S.A., Miller,J.M. and Hoshizaki,D.K. (2001) *serpent*, a GATA-like transcription factor gene, induces fat-cell development in *Drosophila melanogaster*. *Development*, **128**, 1193–1200.

Holmes,M., Turner,J., Fox,A., Chisholm,O., Crossley,M. and Chong,B. (1999) hFOG-2, a novel zinc finger protein, binds the co-repressor mCtBP2 and modulates GATA-mediated activation. *J. Biol. Chem.*, **274**, 23491–23498.

Lanot,R., Zachary,D., Holder,F. and Meister,M. (2001) Postembryonic hematopoiesis in *Drosophila*. *Dev. Biol.*, **230**, 243–257.

Lebestky,T., Chang,T., Hartenstein,V. and Banerjee,U. (2000) Specification of *Drosophila* hematopoietic lineage by conserved transcription factors. *Science*, **288**, 146–149.

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 embryos. Identification of homozygous lethal mutants with P-element insertion at the promoter region. *J. Biol. Chem.*, **270**, 25150–25158.

Lowry,J.A. and Atchley,W.R. (2000) Molecular evolution of the GATA family of transcription factors: conservation within the DNA-binding domain. *J. Mol. Evol.*, **50**, 103–115.

MacNeill,C., Ayres,B., Laverriere,A.C. and Burch,J.B. (1997) Transcripts for functionally distinct isoforms of chicken GATA-5 are differentially expressed from alternative first exons. *J. Biol. Chem.*, **272**, 8396–8401.

Martin,D.I. and Orkin,S.H. (1990) Transcriptional activation and DNA binding by the erythroid factor GF-1/NF-E1/Eryf 1. *Genes Dev.*, **4**, 1886–1898.

Mehaffey,M.G., Newton,A.L., Gandhi,M.J., Crossley,M. and Drachman,J.G. (2001) X-linked thrombocytopenia caused by a novel mutation of *GATA-1*. *Blood*, **98**, 2681–2688.

Merika,M. and Orkin,S.H. (1993) DNA-binding specificity of GATA family transcription factors. *Mol. Cell. Biol.*, **13**, 3999–4010.

Merika,M. and Orkin,S.H. (1995) Functional synergy and physical interactions of the erythroid transcription factor GATA-1 with the Kruppel family proteins Sp1 and EKLF. *Mol. Cell. Biol.*, **15**, 2437–2447.

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.

Nichols,K.E., Crispino,J.D., Poncz,M., White,J.G., Orkin,S.H., Maris, J.M. and Weiss,M.J. (2000) Familial dyserythropoietic anaemia and thrombocytopenia due to an inherited mutation in *GATA-1*. *Nat. Genet.*, **24**, 266–270.

Patient,R.K. and McGhee,J.D. (2002) The GATA family (vertebrates and invertebrates). *Curr. Opin. Genet. Dev.*, **12**, 416–422.

Petersen,U.M., Kadalayil,L., Rehorn,K.P., Hoshizaki,D.K., Reuter,R. and Engstrom,Y. (1999) Serpent regulates *Drosophila* immunity genes in the larval fat body through an essential GATA motif. *EMBO J.*, **18**, 4013–4022.

Peyrefitte,S., Kahn,D. and Haenlin,M. (2001) New members of the *Drosophila* Myc transcription factor subfamily revealed by a genome-wide examination for basic helix–loop–helix genes. *Mech. Dev.*, **104**, 99–104.

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.

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

Rekhtman,N., Radparvar,F., Evans,T. and Skoultchi,A. (1999) Direct interaction of hematopoietic transcription factors PU.1 and GATA-1: functional antagonism in erythroid cells. *Genes Dev.*, **13**, 1398–1411.

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

Rizki,T.M., Rizki,R.M. and Grell,E. (1980) A mutant affecting the crystal cells in *Drosophila melanogaster*. *Wilhelm Roux's Arch. Dev. Biol.*, **188**, 91–99.

Sam,S., Leise,W. and Hoshizaki,D.K. (1996) The *serpent* gene is necessary for progression through the early stages of fat-body development. *Mech. Dev.*, **60**, 197–205.

Shimizu,R., Takahashi,S., Ohneda,K., Engel,J.D. and Yamamoto,M. (2001) *In vivo* requirements for GATA-1 functional domains during primitive and definitive erythropoiesis. *EMBO J.*, **20**, 5250–5260.

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.

Tevosian,S.G., Deconinck,A.E., Cantor,A.B., Rieff,H.I., Fujiwara,Y., Corfas,G. and Orkin,S.H. (1999) FOG-2: a novel GATA-family cofactor related to multitype zinc-finger proteins Friend of GATA-1 and U-shaped. *Proc. Natl Acad. Sci. USA*, **96**, 950–955.

Tevosian,S.G., Deconinck,A.E., Tanaka,M., Schinke,M., Litovsky,S.H., Izumo,S., Fujiwara,Y. and Orkin,S.H. (2000) FOG-2, a cofactor for GATA transcription factors, is essential for heart morphogenesis and development of coronary vessels from epicardium. *Cell*, **101**, 729–739.

Trainor,C.D., Omichinski,J.G., Vandergon,T.L., Gronenborn,A.M., Clore,G.M. and Felsenfeld,G. (1996) A palindromic regulatory site within vertebrate GATA-1 promoters requires both zinc fingers of the GATA-1 DNA-binding domain for high- affinity interaction. *Mol. Cell. Biol.*, **16**, 2238–2247.

Trainor,C.D., Ghirlando,R. and Simpson,M.A. (2000) GATA zinc finger interactions modulate DNA binding and transactivation. *J. Biol. Chem.*, **275**, 28157–28166.

Tsang,A.P., Visvader,J.E., Turner,C.A., Fujiwara,Y., Yu,C., Weiss,M.J., Crossley,M. and Orkin,S.H. (1997) FOG, a multitype zinc finger protein, acts as a cofactor for transcription factor GATA-1 in erythroid and megakaryocytic differentiation. *Cell*, **90**, 109–119.

Tsang,A.P., Fujiwara,Y., Hom,D.B. and Orkin,S.H. (1998) Failure of megakaryopoiesis and arrested erythropoiesis in mice lacking the GATA-1 transcriptional cofactor FOG. *Genes Dev.*, **12**, 1176–1188.

Waltzer,L. and Bienz,M. (1999) A function of CBP as a transcriptional co-activator during Dpp signalling. *EMBO J.*, **18**, 1630–1641.

Weiss,M.J., Yu,C. and Orkin,S.H. (1997) Erythroid-cell-specific properties of transcription factor GATA-1 revealed by phenotypic rescue of a gene-targeted cell line. *Mol. Cell. Biol.*, **17**, 1642–1651.

Received July 9, 2002; revised August 22, 2002;
accepted August 26, 2002