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Transcription factor interplay during *Drosophila* haematopoiesis

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ABSTRACT Transcription factors play a key role in regulating blood cell fate choice and differentiation. In this review, we examine current knowledge of the function and mode of action of the transcription factors implicated in haematopoiesis in *Drosophila*. We particularly emphasize regulation by transcription factors and cofactors, such as GATA, FOG and RUNX, whose homologues in mammals also control blood cell formation and we discuss the cross talks between these transcriptional regulators at the different stages of haematopoietic cell fate decision.

KEY WORDS: *Drosophila, haematopoiesis, transcription*

Introduction

Over the last few years, *Drosophila* has emerged as a valuable model system to gain insights into several aspects of blood cell formation. In particular, despite the evolutionary distance between *drosophila* and vertebrates, many key players controlling blood cell development have been conserved. For instance, transcription factors of the RUNX, GATA or EBF family, as well as signalling from the JAK/STAT, Toll/NF-κB or Notch/CSL pathways, which play key roles in haematopoiesis in vertebrates, also control blood cell development in *Drosophila* (Hartenstein, 2006). Transcription factors play a pivotal role during development as they establish the gene expression programmes intrinsic to cell diversification. The aim of this review is to draw the emerging picture of the transcription factors network implicated in *Drosophila* blood cell development.

Briefly (for details, see the accompanying reviews by Crozatier *et al*. and Banerjee *et al*.), haematopoiesis in *Drosophila* occurs in two waves: blood cell progenitors arise from the head mesoderm in the early embryo and from a specialised organ, the lymph gland, in the larva. These progenitors (prohemocytes) give rise to three differentiated cell types (collectively called hemocytes), which most closely resemble vertebrate myeloid lineages: plasmatocytes, crystal cells and lamellocytes. The plasmatocytes constitute around 95% of the blood cell population and function as macrophages. The crystal cells participate in melanisation, an insect-specific defence response involved in wound healing and encapsulation of foreign invaders. Finally, the lamellocytes are only produced under particular conditions to encapsulate bodies too large to be phagocytosed.

So far, we have a better understanding of the transcriptional network controlling blood cell development in the embryo than in the larva. Accordingly, we shall present in details the molecular and genetic characteristics of the different transcription factors controlling embryonic haematopoiesis. Then, we shall give an overview of the other transcription factors and cofactors that have been shown to participate more specifically in larval haematopoiesis. Alongside, we shall discuss their function and mode of action in *Drosophila* haematopoiesis as compared to vertebrates.

The GATA transcription factor Serpent and its friend U-shaped

The *Drosophila* gene *serpent* (*srp*), which encodes a transcription factor of the GATA family, was the first gene described as implicated in blood cell formation in this organism (Rehorn *et al*. 1996; Sam *et al*. 1996). GATA transcription factors are so called because they bind to the consensus DNA sequence WGATAR (Yamamoto *et al.* 1990; Ko and Engel, 1993; Merika and Orkin, 1993; Whyatt *et al.* 1993;). They form a small, evolutionarily conserved, family of transcription factors and one of their most conspicuous and best-studied functions is during blood cell formation (Haenlin and Waltzer, 2004). In mammals, three of the six GATA genes (GATA-1, -2 and -3) control different aspects

Abbreviations used in this paper: Gcm, glial cells missing; lz, lozenge; ush, u-shaped; srp, serpent.

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of'haematopoiesis from stem cell emergence to differentiation into various lineages (Shimizu and Yamamoto, 2005). In *Drosophila*, among the 5 GATA genes, only *srp* appears to directly participate in haematopoiesis but it is reiteratively used at several stages, from blood cell fate specification to terminal differentiation.

Molecularly, GATA factors are characterized by the presence of a highly conserved zinc finger with the characteristic Cys-X2-Cys-X17-Cys-X2-Cys spacing followed by a short basic region. In vertebrates, all GATA factors contain two distinctive GATA zinc fingers separated by 29 amino acids and referred to as the N-finger and the C-finger, respectively. The C-finger, which is present in all GATA factors, mediates high affinity binding to WGATAR DNA sequences (Evans and Felsenfeld, 1989; Martin and Orkin, 1990; Omichinski *et al.* 1993). The N-finger is dispensable for DNA binding but it stabilises the interaction to tandem and palindromic GATA sites (Trainor *et al.* 1996; Trainor *et al.* 2000;). The N-finger also mediates the interaction with members of the Friend of GATA (FOG) family such as U-shaped (Ush) (Haenlin *et al.* 1997; Tsang *et al.* 1997). So far, all the functions of the FOG family members seem to depend on a GATA factor. In mouse, FOG-1 behaves as a transcriptional coactivator or corepressor in a context-dependant manner (Fox *et al.* 1999; Pang *et al.* 2006; Tsang *et al.* 1997; Wang *et al.* 2002) and it is required with GATA-1 for erythro/megakaryocytic differentiation (Tsang *et al.* 1998). Importantly, several missense mutations in the N-finger of GATA-1 affecting its binding to FOG-1 and/or to DNA are associated to red blood cell disorders in human (Cantor and Orkin, 2005). In *Drosophila*, *ush*, which is mainly expressed in the plasmatocytes, also participates in haematopoiesis (see below). Actually, *srp* codes for different isoforms generated by alternative splicing, which contain only a C-finger (SrpC) or both a C- and an N-finger (SrpNC) (Waltzer *et al.* 2002). These isoforms are coexpressed in the embryo, but their relative abundance at the protein level is not known. Nevertheless, they exhibit both specific and common properties during haematopoiesis (Waltzer *et al.* 2002). For instance, SrpNC activates more efficiently than SrpC the expression of *gcm* (see below), which contains palindromic GATA sites in its promoter. On the opposite, SrpC activates the expression of the apoptotic body receptor *croquemort* (*crq*), while SrpNC represses its expression by recruiting Ush as a corepressor. However, beside Ush-induced phenotypes that are attributable to its interaction with SrpNC (Fossett *et al.* 2001; Waltzer *et al.* 2002; Fossett *et al.* 2003; Sorrentino *et al.* 2007), the exact contribution of each isoform to the global *srp* function in blood cell development remains to be clarified.

Indeed, *srp* is first required for blood cell specification. In the early embryo, *srp* expression defines the anlage of the embryonic

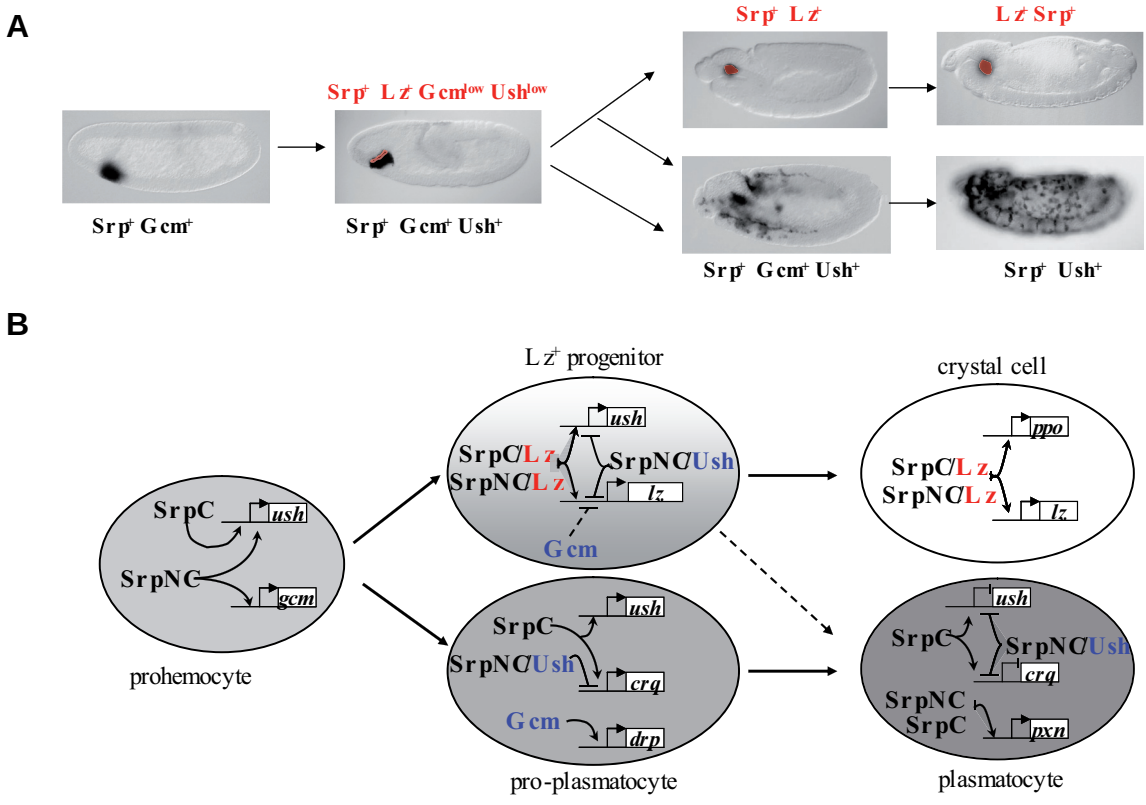

Fig. 1. Transcriptional regulation of embryonic blood cell development. (A) *At stage 5, all the prohemocytes express* srp *and* gcm *By stage 7-8,* ush *expression is activated in all the cells, while* gcm *expression is switched off and* lz *expression induced in the anterior row of prohemocytes (red) Srp⁺ Gcm⁺ Ush⁺ cells (90% of the prohemocytes) differentiate into plasmatocytes that migrate throughout the embryo Among the Lz⁺ progenitors, 60% maintain* lz *expression and differentiate into crystal cells that remain clustered around the proventriculus, whereas the remaining 40% differentiate into plasmatocytes.* **(B)** *Schematic representation of the transcription factors regulating the different stages of embryonic blood cell fate decision Some of the target genes regulated by the different combination of transcription factors are indicated. See text for details.*

hemocytes in the head mesoderm (Rehorn *et al.* 1996; de Velasco *et al.* 2006) (Fig. 1A) and, at the end of embryogenesis, it is expressed in the lymph gland precursors (Mandal *et al.* 2004). Srp is required for the maintenance of embryonic blood cells as well as for the specification of definitive blood cell progenitors in the lymph gland (Rehorn *et al.* 1996; Sam *et al.* 1996; Mandal *et al.* 2004). Interestingly, these functions of *srp* are similar to that of *GATA-2,* which is essential for the development of both primitive and definitive blood cell progenitors in mammals (Fujiwara *et al.* 2004; Ling *et al.* 2004; Rodrigues *et al.* 2005). What controls the onset of *srp* expression in the embryonic hematopoietic anlage is not known although its expression partially depends on the early head-patterning genes *buttonhead* (Yin *et al.* 1997) and *empty spiracle* (Bataille, 2006). In the late embryo, the expression of *srp* in the lymph gland progenitors depends on Notch signalling, which specifies the blood cell fate among the mesodermal precursors (Mandal *et al.* 2004). Accordingly, the ectopic expression of Srp or an activated Notch in the cardiogenic mesoderm promotes blood cell fate choice (Mandal *et al.* 2004). Strikingly, a similar Notch/GATA cascade is employed in mammals during the specification of definitive haematopoietic stem cells in the dorsal aorta region (Robert-Moreno *et al.* 2005; Robert-Moreno *et al.* 2008).

Second*, srp* also controls the differentiation of the three *Drosophila* blood cell lineages. In the embryo, Srp is still detected in mature plasmatocytes and crystal cells (Sam *et al.* 1996; Lebestky *et al.* 2003). The ectopic expression of *srpC or srpNC* throughout the mesoderm is sufficient to activate the expression of several plasmatocyte markers and mutations affecting *ush* affect plasmatocyte differentiation, indicating that *srp* participates in the differentiation of this lineage (Waltzer *et al.* 2002) (Fig. 1B). Furthermore, *srp* has a dual role in the crystal cell lineage. On the one hand, SrpNC represses crystal cell fate choice by associating to Ush (Fossett *et al.* 2001): crystal cell number slightly increases in *ush* mutant embryos and decreases upon enforced expression of Ush. The mechanism of crystal cell repression by FOG proteins appears to be conserved and might require the transcriptional corepressor CtBP. Indeed mouse FOG-1 and FOG-2, but not a FOG-2 protein unable to interact with CtBP, can impair crystal cell formation (Fossett *et al.* 2001). On the other hand, *srp* is required for crystal cell differentiation in conjunction with the RUNX transcription factor Lozenge (Lz, see below) (Fossett *et al*. 2003; Waltzer *et al.* 2003) (Fig. 1B). Similarly, in mouse, GATA-3 can either promote or inhibit T helper 2 cells development depending respectively on the absence or presence of FOG-1 (Zhou *et al*. 2001; Kurata *et al.* 2002). Of note, Srp expression is lower in differentiating crystal cells than in plasmatocytes or blood cell progenitors (Lebestky *et al.* 2003), and maintaining high level of Srp in the crystal cells inhibits their differentiation (independently of *ush*) (Waltzer *et al.* 2002). Likewise, in mouse, GATA-2 is expressed at high level in haematopoietic progenitors cells but has to be down-regulated for normal erythrocytic differentiation to occur (Persons *et al.* 1999; Kumano *et al.* 2001). Thus, GATA level of expression is critical for proper blood cell differentiation.

In the larval lymph gland, Srp is expressed in all blood cells (Lebestky *et al.* 2003; Jung *et al.* 2005). Its requirement there for plasmatocyte differentiation has not been studied but several lines of evidence suggest that, as in the embryo, Srp cooperates with Lz to induce larval crystal cell fate (Fossett *et al.* 2003; Ferjoux *et al.* 2007). In addition, SrpNC associated to Ush might inhibit lamellocyte differentiation (Sorrentino *et al.* 2007). Indeed, reducing *ush* level causes lymph gland hypertrophy and overt lamellocyte differentiation, whereas its overexpression suppresses $Hop^{Tum\text{-}l}$-induced lamellocyte formation (see below). In the lymph gland, Ush is expressed in the plasmatocytes but not in the crystal cells or the prohemocytes. This led to the proposal that Ush might restraint the differentiation of mature plasmatocytes into lamellocytes. However, a lineage relationship between these two cells types has not been demonstrated. Surprisingly, Ush is also strongly expressed in differentiated lamellocytes, but its function here remains unknown (Sorrentino *et al.* 2007). Hence, Ush might regulate several steps of lammellocyte formation.

All together, it is striking that a single GATA factor perform so many functions at multiple steps of blood cell development. The fine tuning of its expression, the existence of functionally distinct protein isoforms and the presence of lineage-specific partners, are crucial to endow *srp* with such a broad range of functions but other levels of regulation likely participate in its mutlitasking activity.

The RUNX transcription factor Lozenge

lozenge, which encodes a transcription factor of the RUNX family, is required for crystal cell differentiation both in the embryo and in the larva (Rizki *et al.* 1985; Lebestky *et al.* 2000). RUNX proteins harbour a conserved 128-amino-acid long DNA binding domain, the Runt domain, as well as a C-terminal WRPY motif that is capable of recruiting transcriptional corepressors of the Groucho/TLE class (Wheeler *et al*. 2000). In mammals, all three RUNX factors participate in one or more stages of haematopoiesis (Blyth *et al*. 2005). In particular, *RUNX1* (also known as *AML1*), one of the most frequently mutated gene in human acute myeloid or lymphoid leukaemia, is required for definitive haematopoietic stem cell formation as well as for megakaryocytic maturation and lymphocytic differentiation (*North et al.* 1999; Ichikawa *et al.* 2004). Beside *lz*, there are three other RUNX genes in *Drosophila*: *runt*, *RunxA* and *RunxB* (Rennert *et al*. 2003). *runt* is not implicated in blood cell development while the expression patterns and functions of *RunxA/CG1379* and *RunxB/CG15455* are not known. Interestingly, a genome-wide dsRNA screen showed that *RunxB* (and *srp*) is required for growth and viability of two embryonic hemocyte lines in culture (Boutros *et al.* 2004). Whether *RunxB* controls blood cell survival *in vivo* remains to be established.

Binding of RUNX factors to the RACCRCA consensus DNA sequence is enhanced by dimerisation with members of the CBFβ family, encoded by *brother* (*bro*) and *big brother* (*bgb*) in *Drosophila* (Wheeler *et al.* 2000; Tahirov *et al.* 2001). In addition, CBFβ protects RUNX proteins from degradation by the proteasome (Huang *et al*. 2001). In mammals, *CBFβ* loss of function phenocopies *RUNX* mutations in the haematopoietic system (Talebian *et al*. 2007), and translocation affecting CBFβ are associated to the development of acute myeloid leukaemia in human (Blyth *et al.* 2005). The functions of *bro* and *bgb* in *Drosophila* blood cells have not been analysed. However, it is worth mentioning that RUNX factors display also CBFβ-independent function (Bollerot *et al*. 2005; Yokomizo *et al*. 2008), thus Lz might not require Bro or Bgb to promote crystal cell fate.

In the embryo, *lz* expression is first detected in the anterior-most raw of prohemocytes slightly after the onset of *srp* expres-

sion (Bataille *et al.* 2005) (Fig. 1A). Only a fraction of these cells maintains *lz* transcription and differentiates into crystal cells whereas the remaining progenitors give rise to plasmatocytes (Lebestky *et al*. 2000; Bataille *et al*. 2005). Furthermore, using a temperature-sensitive allele of *lz*, it was shown that *lz* is continuously required to maintain crystal cell fate (Lebestky *et al*. 2000). How exactly *lz* is switched on is not known but its expression depends on *srp* and could be maintained in the crystal cell lineage by an autoregulatory loop (Bataille *et al*. 2005; Ferjoux *et al*. 2007; Muratoglu *et al*. 2007). Contrary to the embryo (Bataille *et al*. 2005), *lz* expression in the larval lymph gland is activated in scattered cells by Serrate/Notch signalling, which plays an instructive role for crystal cell differentiation both in the lymph gland and in circulating larval hemocytes (Duvic *et al*. 2002; Lebestky *et al*. 2003).

At the molecular level, Lz synergizes with Srp to induce crystal cell fate (Fossett *et al*. 2003; Waltzer *et al*. 2003). This functional cooperation is mediated in part by a direct interaction between both isoforms of Srp and Lz (Waltzer *et al*. 2003) and in part at the level of several crystal cell specific genes (including *lz* itself) that harbour a particular *cis*-regulatory module composed of at least one GATA and one RUNX binding site in close association (Ferjoux *et al*. 2007; Gajewski *et al*. 2007; Muratoglu *et al*. 2007) (Fig. 1B). Both type of binding sites are required for Srp/Lz-mediated transactivation *in vivo,* suggesting that Srp and Lz simultaneously bind their targets (Ferjoux *et al*. 2007). The synergy between Srp and Lz might rely on cooperative DNA binding and/or on the formation of a transactivating platform (Levine and Tjian, 2003). Alternatively, Srp may already bind these enhancers in the prohemocytes and prime them for activation by Lz. It is striking that the same complex composed of a pan-haematopoietic (Srp) and a lineage-specific (Lz) transcription factor is directly involved in maintaining the expression of the lineage-specific partner and in coordinating the expression of a wide array of differentiation markers (Ferjoux *et al*. 2007). This probably ensures a tight coupling between crystal cell fate choice and differentiation. Interestingly, the interaction between GATA and RUNX transcription factors has been conserved through evolution (Waltzer *et al*. 2003). Therefore, Srp/Lz cooperation might be used as a paradigm to study how GATA/RUNX complexes regulate transcription and blood cell development from *Drosophila* to vertebrates. In human, GATA1 and RUNX1 were shown to cooperate during megakaryopoiesis *ex vivo* (Elagib *et al*. 2003; Xu *et al*. 2006) and deregulation of the GATA1/RUNX1 complex activity might be implicated in the development of blood cells disorders such as familial platelet disorders and acute megakaryoblastic leukaemia (Elagib and Goldfarb, 2007).

Further studies indicated that crystal cell fate choice relies on the exquisite balance and cross-regulatory interactions between GATA and its two partners RUNX and FOG. Indeed, Ush, which antagonises crystal cell development, is initially detected in all the prohemocytes including the procrystal cells but its expression in this lineage vanishes by the end of embryogenesis (Fossett *et al*. 2001) (Fig. 1A). *ush* is directly activated by Srp (Fossett *et al*. 2001; Waltzer *et al*. 2002; Muratoglu *et al*. 2006) and, unexpectedly, it is further upregulated by Srp/Lz during the initial step of crystal cell commitment (Muratoglu *et al*. 2006, 2007) (Fig. 1B). Given that Ush, by competing with Lz, interferes with SrpNC/Lz–induced activation of *lz* and *ush* (Waltzer *et al*. 2003; Muratoglu *et al*. 2007), it was proposed that the $Lz^+/Srp^+/Ush^+$ population is in a dynamic, bi-potential, regulatory state which resolves into two populations: Srp^+/Lz^+ (crystal cells) and Srp^+/Ush^+ (plasmatocytes) (Muratoglu *et al*. 2006, 2007) (Fig. 1A). Yet, how *ush* is turned off in the crystal cells is not resolved.

The Glial Cells Missing transcription factors

Two additional key regulators of plasmatocyte *versus* crystal cell fate in the embryo are *glial cells missing* (*gcm*) and *gcm2* (also known as *glide* and *glide2*). *gcm* and *gcm2* are the primary determinant of glial cell fate in *Drosophila* (Jones *et al*. 1995; Vincent *et al*. 1996; Kammerer and Giangrande, 2001; Alfonso and Jones, 2002). They encode two related transcription factors with a conserved Gcm-type zinc finger DNA-binding domain that recognizes the DNA sequence 5'-ATGCGGGR-3' (Hashemolhosseini and Wegner, 2004). In the embryo, *gcm* is initially expressed throughout the haematopoietic anlage but its expression is rapidly turned off in the Lz+ progenitors (Bataille *et al.,* 2005), while it is transiently maintained in the differentiating plasmatocytes (Bernardoni *et al*. 1997; Lebestky *et al*. 2000; Kammerer and Giangrande, 2001; Alfonso and Jones, 2002) (Fig. 1A).

In the absence of both *gcm*/*gcm2*, plasmatocyte differentiation is severely impaired and their number is strongly reduced (Bernardoni *et al*. 1997; Kammerer and Giangrande, 2001; Alfonso and Jones, 2002). Conversely, the ectopic expression of *gcm* is sufficient to induce the expression of several plasmatocytes markers (Bernardoni *et al*. 1997; Freeman *et al*. 2003) and its misexpression in the crystal cells turn them into plasmatocytes (Lebestky *et al.* 2000), suggesting that *gcm/gcm2* play an instructive role during plasmatocyte specification similar to their role in gliogenesis (Fig. 1B). Consistent with this idea, several genes expressed in the plasmatocytes contain clusters of Gcm binding sites and depend on *gcm/gcm2* (Freeman *et al*. 2003).

In addition, *gcm/gcm2* mutant embryos display an increase in the number of Lz+ progenitors and all these progenitors differentiate into crystal cells (as compared to 60% in the wildtype situation) (Bataille *et al*. 2005). Thus *gcm/gcm2* restrict *lz* induction in the prohemocytes and interfere with the maintenance of *lz* expression in the early Lz+ progenitors. There are no consensus Gcm binding site in *lz* hemocyte-specific enhancer and *gcm/gcm2* might impair *lz* maintenance indirectly, for instance by promoting *ush* expression. As mentioned above, *gcm* can inhibit *lz* expression and reprogram crystal cells into plasmatocytes (Lebestky *et al*. 2000; Bataille *et al*. 2005). On the opposite, *lz* is unable to repress *gcm* expression and/or plasmatocyte differentiation (Waltzer *et al*. 2003). Hence crystal cell *versus* plasmatocyte fate does not rely on reciprocal antagonism between two lineage-specific transcription factors, as it is often observed for alternate blood cell fate choice in vertebrates (Galloway *et al*. 2005; Rhodes *et al*. 2005), but it requires additional input to turn-off the priming of the prohemocytes toward the plasmatocyte fate (Bataille *et al*. 2005).

Of note, neither *gcm* nor *gcm2* appear to be expressed in the lymph gland, and which transcription factor controls larval plasmatocyte differentiation is still unknown. Finally, although it was shown that the zebrafish *gcmb* is expressed in macrophages (Hanaoka *et al*. 2004), the putative functions of the *gcm* homo-

logues during haematopoiesis in vertebrates have not been studied.

The transcriptional network controlling embryonic blood cell development

Our current knowledge of the transcriptional network controlling embryonic blood cell development is summarised Fig. 1. *srp* expression in the head mesoderm initiates at the zygotic transition (stage 5) and is required for blood cell fate specification. In its absence, neither *gcm*/*gcm2*, *ush* or *lz* transcription is detected in stage 7 embryos. Overexpressing *srp* throughout the mesoderm is sufficient to induce ectopic expression of *ush* (which was shown to be a direct target gene) and *gcm* but not *lz*. The majority of the prohemocytes maintains *srp*, *gcm/gcm2* and *ush* expression until stage 10 and differentiates into plasmatocytes. Gcm/Gcm2 directly activate the expression of a number of plasmatocyte markers, while Ush associated to SrpNC antagonises SrpC-mediated activation on some *srp* target genes such as *crq* to fine tune plasmatocyte differentiation. In the Lz^+ progenitors, Lz associated to Srp promotes its own expression, whereas Ush and Gcm impair *lz* autoregulatory loop. We don't know what triggers *lz* induction and *gcm* or *ush* downregulation in these cells, but ultimately only a fraction of them maintain *lz* expression. There, Srp and Lz cooperate to activate crystal cell differentiation markers such as the three prophenoloxidase genes.

All together, the resolution of *Drosophila* embryonic blood cell fate choice relies on the balance and intricate relationships between several transcription factors. Strikingly, it appears that depending on its partners, the GATA factor Srp can promote the two alternate fates. A similar situation has been described during erythro/megakaryocytic differentiation: GATA-1 is expressed in their common progenitor and it is required to promote the two fates (Goldfarb, 2007). It induces megakaryocyte differentiation by associating with Fli-1 and RUNX1, whereas it activates the erythroid program by associating with EKLF. Obviously, several gaps persist in this model. It is expected that future experiments aiming at deciphering the molecular mechanisms of action of these different transcription factors acting either alone or in combination will help bridging these gaps.

Transcription factors controlling larval blood cell development

Embryonic haematopoiesis proceeds swiftly with the subdivision of a small mesodermal territory whose progenitors differentiate in a highly stereotypical manner within 14 hours. On the contrary, larval haematopoiesis is a step-wise process that takes place over several days and in a specialised organ that is immune responsive (*i.e.* blood cell number and differentiation can be regulated by immune challenge). Consequently, this stepwise process implicates several additional signalling pathways and regulatory transcription factors as compared to embryonic haematopoiesis. Yet, as discussed above, Srp and its partners Ush and Lz participate both in embryonic and larval blood cell development. It is tempting to speculate that these transcription factors are a part of an ancestral molecular core underlying the developmment of haematopoietic cell types from invertebrates to vertebrates (Rothenberg and Pant, 2004). Other transcription factors required specifically for larval haematopoiesis might then interact with or regulate this core group of factors to enable the development of a more complex haematopoietic system.

That is the case notably for those controlling the first steps of lymph gland specification and regionalisation. Lymph gland precursors are specified in the lateral mesoderm during mid-embryogenesis. The TALE-class homeodomain transcription factor Homothorax (Hth) is initially expressed ubiquitously in the lymph gland but its expression is subsequently downregulated in the posterior cells as they start to express the HOX factor Antennapedia (Antp) and Collier (Col), the orthologue of mammalian Early B-cell Factor (EBF) (Crozatier *et al.* 2004; Mandal *et al*. 2007). These posterior cells prefigure the Posterior Signalling Center (PSC), which plays a key role in maintaining the cells of the medullary zone into a progenitor state in the larval lymph gland (Krzemien *et al*. 2007; Mandal *et al*. 2007). *col* is required for PSC cells identity and thus for progenitor blood cell maintenance (Crozatier *et al*. 2004; Krzemien *et al*. 2007; Mandal *et al*. 2007). Its initial expression in the PSC precursors requires *antp* (Mandal *et al*. 2007), while its maintenance requires Serrate/Notch signalling (Krzemien *et al*. 2007). It was proposed that Antp and Hth cross inhibit each other to specify the PSC and the rest of the lymph gland, respectively (Mandal *et al*. 2007), but the molecular basis for this antagonism remains to be explored. In mammals, Meis1, the homologue of Hth, is also required for definitive haematopoiesis (Hisa *et al*. 2004; Azcoitia *et al*. 2005) and it plays a crucial role in leukaemogenesis, notably as a cofactor for Hoxa9 (Zeisig *et al*. 2004; Wong *et al*. 2007).

In addition, some transcription factors involved in regulating larval blood cell homeostasis have been identified. First, the STAT DNA binding protein STAT92E and the Gli transcription factor Cubitus interruptus (Ci), which respectively mediate the transcriptional response to JAK/STAT and Hedgehog signalling, are both required to maintain cells of the medullary zone in a progenitor state in response to the corresponding signals coming from the PSC (Krzemien *et al.* 2007; Mandal *et al.* 2007). How these two signalling cascade are integrated at the molecular level to prevent blood cell differentiation has not been investigated yet. Second, the *Drosophila* Myb transcription factor homologue is required for larval hemocyte proliferation (Davidson *et al.* 2005). Interestingly, mouse B-Myb rescued blood cell proliferation and differentiation defects of *myb* mutant larvae, indicating that B-Myb and *Drosophila* Myb share essential conserved functions. In mammals, *B-myb* plays an ubiquitous role in controlling G2/M cell cycle progression and genome stability (Ramsay and Gonda, 2008) but its role in haematopoiesis has not been thoroughly studied. On the other hand, *c-myb* is involved in leukaemia and it is critical for the expansion of the definitive haematopoietic stem cells as well as for the generation of several blood cell lineages. However, these function of *c-myb* might not be conserved in *Drosophila myb* (Davidson *et al*. 2005). Third, the Rel/NF-kB transcription factors Dif and Dorsal, which mediate the response to the Toll pathway, also control larval blood cell homeostasis. It has been known for a long time that constitutive activation of the Toll pathway activates blood cell proliferation and induces lamellocyte differentiation (Gerttula *et al*. 1988; Qiu *et al*. 1998). More recently, it was shown that Dif and Dorsal are required cell autonomously in circulating hemocytes for efficient phagocytosis and to prevent apoptosis (Matova and Anderson, 2006). Thus the

Toll/NF-κB pathway plays an important role in maintaining larval blood cell number. However, the induction of lamellocyte differentiation by constitutively activated Toll/NF-κB signalling might be a secondary event as it is suppressed both in *STAT92E* and *myb* mutants (Remillieux-Leschelle *et al.* 2002; Sorrentino *et al.* 2004; Davidson *et al.* 2005). Dif and Dorsal cooperate with Srp and dGATAe to induce antimicrobial peptide expression in the fat body and in the midgut, respectively (Senger *et al.* 2004, 2006). It is thus tempting to speculate that Dif/Dorsal and Srp might also cooperate to regulate transcription in larval hemocytes.

Finally, some transcription factors affecting larval blood cell differentiation have been identified. As mentioned above, crystal cell differentiation in the larva also depends on *lz* (Lebestky *et al*. 2000) but there its expression is induced in response to Serrate/Notch signalling (Duvic *et al*. 2002; Lebestky *et al.* 2003). Accordingly, Suppressor of Hairless, the transcription factor of the CSL family that regulates Notch target genes, is active in the procrystal cells and required for *lz* expression. It is worth noting that crystal cells were absent in larvae lacking *dmyb*, but this phenotype was attributed to a profound defect in proliferation rather than differentiation (Davidson *et al.* 2005). Mutations in *Dif/dorsal* affect circulating plasmatocyte function but are not sufficient to suppress their formation (Matova and Anderson, 2006). Similarly, clonal analysis indicated that *myb*$^{-/-}$ plasmatocytes exhibit cell autonomous defect in phagocytosis (Davidson *et al*. 2005). Actually, it was shown that the differentiation of this lineage requires the receptor tyrosine kinase Pvr, but the transcription factor mediating its effect remains to be discovered. Similarly, the transcriptional network controlling lamellocyte lineage formation is poorly understood. The misexpression in the larval blood cells of several transcription factors such Yan/Aop (Zettervall *et al*. 2004), Dref (Yoshida *et al*. 2001), Collier (Crozatier *et al*. 2004) or Caudal (Hwang *et al*. 2002) can induce their formation but these gain of function experiments have to be considered with caution. Along the same line, the misexpression of a dominant negative form of the transcription factor Pangolin/dTCF, which mediates Wg/Wnt signalling, or that of an inhibitor of Wg signalling, Shaggy, induces lamellocyte differentiation, suggesting that this particular pathway might be involved in controlling lamellocyte formation (Zettervall *et al*. 2004). However, no genetic experiments have confirmed this hypothesis so far. Beside *ush* (see above) (Sorrentino *et al*. 2007), zygotic mutation in several transcriptional coactivators, corepressor or chromatin remodelling factors also induce lamellocyte formation (Garzino *et al*. 1992; Kodjabachian *et al*. 1998; Badenhorst *et al*. 2002; Bantignies *et al*. 2002; Remillieux-Leschelle *et al*. 2002; Minakhina and Steward, 2006). Yet, whether these mutations promote lamellocyte formation in a cell-autonomous manner or reveal a more complex innate immune response is not clear. Indeed, it is known that lamellocyte differentiation can be induced as part of an immune response to "aberrant" self tissues (Dearolf, 1998). Reassessing the function of these genes specifically in the blood cells might shed new light on the control of *Drosophila* innate immune response.

Concluding remarks

Although our knowledge of the transcriptional network controlling embryonic and larval blood cell development in *Drosophila* is still rudimentary, it is striking that most pieces of this network have been conserved in mammals. The relative simplicity of the *Drosophila* haematopoietic system and the reduced level of gene redundancy facilitate the functional characterisation of each transcription factor controlling the multiple steps of haematopoiesis, from mesodermal progenitors to fully differentiated blood cells. One challenge will be to decipher the combinatorial code that permits blood cell type specific gene expression. *Drosophila* is a model system amenable both to genetic and biochemical approaches. Their combination should allow to unravel the epistatic and molecular relationships between the members of this network and to discover new factors participating in haematopoiesis from *Drosophila* to mammals.

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